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Katedra agroenvironmentální chemie a výživy rostlin

Fosfor a síra – limitující prvky ve výživě rostlin

Habilitační práce

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Poděkování

Poděkování patří z mého pohledu možná k tomu nejtěžšímu z celé práce, protože ač bych nerad na někoho zapomněl, vím, že se to stane. Začít musím od rodiny. Díky prarodičům jsem se naučil s mnohým si poradit a nevnímat svět pouze černobíle. Rodičům děkuji za trpělivost a všestrannou podporu. Jsou celoživotně mými vzory a doufám, že je budu nadále důstojně následovat.

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Již před více než rokem jsem od katedry dostal darem cyklistický dres s vizionářským mottem „Jedeme v tom všichni“. Tím bych chtěl zakončit mé skromné poděkování, protože bez týmové práce by tato habilitace nemohla být ani zahájena.

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Souhrn

Fosfor a síra patří k nezastupitelným prvkům ve výživě rostlin. Donedávna však těmto prvkům nebyla z různých důvodů věnována patřičná pozornost.

Je zřejmé, že zdroje kvalitních fosfátů pro minerální hnojiva jsou silně limitovány a odhadované zásoby vystačí na 50-100 let. Proto jsou testovány nové způsoby hnojení fosforem, popřípadě podpory jeho využitelnosti. Souborným cílem této práce bylo ověřit některé z těchto strategií.

Po celé období závěrečné práce byly průběžně vyhodnocovány výsledky dlouhodobých pokusů. Zde byly sledovány jednak různé frakce fosforu a jejich přeměny, jednak dlouhodobé systémy hnojení. V těchto pokusech byla mj. vyhodnocována dlouhodobá aplikace čistírenských kalů, které jako odpadní materiál představují jednu z perspektiv šetření neobnovitelných zdrojů fosforu. Ukazuje se, že aplikace čistírenských kalů zajišťuje přísun zejména mobilních forem fosforu. V navazujícím výzkumu je však nutno zohlednit i negativní vlastnosti tohoto materiálu, jako jsou rizikové prvky a organické polutanty.

V práci byla rovněž intenzivně zkoumána možnost využití dalších odpadních materiálů (popel, separovaný digestát, torefikované kaly, …). Zde se jako problematický ukazuje buď malý podíl P (digestát), nebo fosfor v obtížně přístupné formě (torefikované kaly, popel). Proto byla dílčím cílem výzkumu i aplikace tzv. biostimulantů podporujících mobilizaci P z půdy, popř. aplikovaných hnojiv. Výsledky jsou však dosud rozporuplné. I přes náznaky pozitivního působení biostimulantů v nádobových pokusech se nepodařilo potvrdit jejich účinnost v provozních podmínkách.

V dlouhodobých pokusech bylo rovněž sledováno chování síry. Zde byl potvrzen výrazný pokles obsahu přístupné síry v půdě po roce 1989, zejména díky omezení vstupů S v atmosférických depozicích v ČR. Přirozené vstupy síry do půdy tak aktuálně nepřesahují odběr S hlavními plodinami, a proto se síra stává z nežádoucího prvku klíčovou živinou. V návaznosti na toto zjištění byl realizován intenzivní výzkum různých možností hnojení sírou. Jako výhodná cesta se ukazuje použití především chlévského hnoje a/nebo minerálních hnojiv s obsahem S (např. ledek amonný se sírou).

V případě fosforu i síry byly potvrzeny vzájemné vztahy s ostatními živinami v půdě (např. s Fe, Al, Zn, N), a proto se jako jeden z perspektivních diagnostických kroků k optimalizaci výživy rostlin jeví využití tzv. výživových indexů.

Předložená práce přináší více nových otázek než odpovědí, a je tak třeba navazující výzkum v uvedených oblastech.

Summary

Phosphorus and sulfur belongs to necessary elements in plant nutrition. But, due to different reasons, only little attention was passed to these elements in former times.

It seems to be obvious that the sources of high-quality phosphates for mineral fertilizers production are limited and their overall stocks are estimated on 50-100 years. Because of that, new strategies for phosphorus fertilizing and/or improving its acquisition are tested. The general aim of this work was to test some of these strategies.

During the whole time of these thesis, the results of long-term field experiments were continuously evaluated. Here, the different phosphorus fractions and their transformations as well as different fertilizing systems were studied. One of the investigated treatments was sewage sludge, which was proven as a source of bioavailable phosphorus forms. On the other hand, the potentially negative properties of this fertilizer (trace elements, organic pollutants) have to be included in following research.

In the frame of this thesis, the possibilities of use another waste materials (ashes, digestate, torrefied sludge,…) were intensively studied. Here, the low rate of phosphorus (digestate) or non-bioavailable P forms (ashes, torrefied sludge) seems to be problematic in practical use of these fertilizers. As a secondary objective, the application of biostimulants supporting the mobilization of phosphorus from soil was also investigated. But, the results are inconsistent. Although the indications of positive biostimulants influence in pot experiments, their effect in field conditions was not proven.

The behavior of sulfur was investigated in long-term field experiments as well. Here, the significant decrease of soil bioavailable S forms was proven after the year 1989. It was caused mainly due to limitation of sulfur inputs in soil with the atmospheric deposition in the Czech Republic. Natural sulfur inputs into the soil are also actually not overreaching the amounts taken up by plants. It is the reason, why sulfur became a crucial element for plant nutrition. Following this finding, intensive research of different S fertilizing strategies was started. As a promising way seems to be application of farmyard manure and/or mineral fertilizers containing S (e.g., ammonium nitrate with calcium sulfate)

Strong relationships were found among phosphorus or sulfur and other nutrients (e.g., Fe, Al, Zn, N). Therefore, one of the perspective steps to the optimization of plant nutrition seems to be using of so called “nutrient indexes”

Submitted work brings more new questions than answers and is also only background for following research in above mentioned areas.

Klíčová slova: Koloběh fosforu a síry v zemědělství; Dlouhodobé polní pokusy; Odpadní materiály jako hnojiva; Biostimulanty; Výživa rostlin P a S

Keywords: Cycle of phosphorus and sulfur in agriculture; Long-term field experiments; Waste materials as a fertilizers; Biostimulants; P and S fertilizing

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1. Úvod

„Ustavičnými sklizněmi chudnou pole“. Tento citát Publiuse Ovidiuse Nasa (43 př. n. l. – 18 n. l.) svědčí o tom, že už ve starém Římě, a možná mnohem dříve, byl znám význam výživy rostlin v oblasti dosažení výnosů a kvality produkce. Už tehdy tak existovalo povědomí o zákonu zachování hmotnosti, později definovaným Lomonosovem v r. 1758. Tento zákon pak dále z hlediska výživy rostlin rozšířili K. Sprengel (1787 – 1859) a J. von Liebig (1803 – 1873). Ti vyzdvihli význam vyrovnané výživy rostlin, a naopak zbytečnost aplikace prvků přítomných v dostatečném množství. Tento historický výňatek jen poukazuje na to, že výživa rostlin je již dlouhodobě studovaným tématem a za každým dalším pokrokem je nyní třeba vidět mnohem více práce. Zatímco v historii bylo možno některými revolučními změnami dosáhnout nárůstu výnosů v oblasti desítek procent, nyní je úspěchem změna v rámci jednotek. Cílem by tak mělo být především lepší využití stávajících zákonitostí, jejich šíření a především zavádění do praxe. O tom, že je zde stále obrovský prostor pro zlepšení, svědčí aktuální situace v ČR i ve světě. Velmi často se hnojení provádí pouze s jedním záměrem: dosáhnout nejrentabilnějšího výnosu za každou cenu bez ohledu na budoucnost a kvalitu. Dalšími strategiemi je nehnojit vůbec, což na základě výše zmíněného citátu vede pouze k drancování půdní zásoby a dlouhodobé neudržitelnosti tohoto systému.

Předložená práce je zaměřena na dva makroprvky, které, každý z různých důvodů, představují ve většině světových oblastí ve vyrovnané výživě rostlin ty nejkritičtější body. Jedním z nich je fosfor, jehož omezené zásoby pro výrobu hnojiv znamenají rozmach výzkumu zaměřeného na řešení této situace, ať již se jedná o využití odpadů, biostimulantů nebo o šlechtění rostlin. Druhou studovanou živinou je síra. Zde donedávna nebylo situaci třeba řešit. Naopak až škodlivé množství S vstupovalo do půdy v atmosférických depozicích. Síra je tak často dosud chápána jako prvek balastní až nežádoucí. Současné výzkumy však ukazují na sílící nedostatek síry ve výživě rostlin. Tento prvek je tak už na mnoha stanovištích limitující živinou z hlediska výnosu i kvality.

Habilitační práce je souborem poznatků zabývajícím se výzkumem výživy fosforem a sírou. Ještě jednou děkuji všem kolegům, kteří přijali mou nabídku spolupráce nejen v autorském kolektivu a kolegům, kteří mě do kolektivu přizvali. Dovoluji si úvodní část zakončit dalším citátem. „Kniha, která má odpověď na všechny otázky, je špatná“. Tímto citátem F.L. Heka (1769 – 1847) jsem se snažil řídit, a proto níže hledejte spíše náměty na další výzkum a navazující studie.

2. Hypotézy

- Dlouhodobé hnojení různými organickými i minerálními hnojivy povede k průkazným změnám v obsahu mobilních frakcí fosforu v půdě. Naopak obsah stabilních fosforečnanů zůstane pravděpodobně bez výrazných změn.
- Aplikací čistírenských kalů bude ve srovnání s chlévským hnojem dosaženo vyšší krátkodobé akumulace mobilních frakcí fosforu. Dlouhodobou aplikací hnoje bude naopak dosaženo stabilních výsledků v delším časovém horizontu.
- Aplikace různých odpadních materiálů (čistírenské kaly, popel, digestát) povede ke zvýšení obsahů přístupných forem P v půdě, avšak efekt bude nižší ve srovnání s běžně užívanými minerálními hnojivy.
- Aplikace biostimulantů umožní mobilizovat hůře přístupné formy P z půdy.
- Biostimulanty při společné aplikaci s odpadními materiály obsahujícími P (popel, digestát) podpoří uvolňování fosforu z těchto materiálů a následně i příjem P rostlinami.

- Z důvodu omezení vstupu síry v atmosférických depozicích dojde v dlouhodobém časovém horizontu k výraznému poklesu obsahu přístupné síry v půdě.
- Vstupy S do půdy (kromě cíleného hnojení) nebudou přesahovat odběry síry rostlinami, a tak bude postupně docházet k častějším projevům nedostatků síry, zejména u náročných plodin.
- Zatímco minerální hnojiva doplní síru do půdy pouze krátkodobě, hnojení organickými hnojivy bude představovat dlouhodobý zdroj přístupné síry pro rostliny.
- Metoda Mehlich 3, používaná v současnosti jako standartní pro stanovení obsahu přijatelných živin v půdách ČR, pravděpodobně nebude vhodnou metodou pro stanovení obsahu přístupné síry.

3. Cíle práce

- Sledování změn obsahů různých frakcí fosforu v půdě v dlouhodobých přesných polních pokusech zaměřených na vyhodnocení vlivu rozdílných systémů hnojení.
- V přesných polních pokusech vyhodnotit vliv dlouhodobé aplikace čistírenských kalů na obsah různých frakcí fosforu v půdě.
- Posouzení vlivu aplikace různých odpadních materiálů (popel, kaly, digestát) na změny obsahu mobilních fosforečnanů v půdě.
- Vyhodnocení účinnosti různých biostimulantů na mobilizaci hůře přístupného fosforu z půdních zásob.
- Testování efektivity různých biostimulanů z hlediska uvolňování fosforu z odpadních materiálů s hůře přístupným fosforem.

- Hodnocení vývoje změn obsahů různých frakcí síry v dlouhodobých přesných polních pokusech.
- Srovnání různých systémů hnojení z hlediska dlouhodobé udržitelnosti vyhovujícící zásoby přístupné síry v půdě.
- Na základě srovnávací analýzy (s ostatními extrakčními postupy a obsahy S v rostlině) určit vhodnost metody Mehlich 3 ke stanovení přístupných forem síry v půdě.

4. Fosfor a síra – limitující prvky ve výživě rostlin (literární přehled)

4.1 Význam fosforu ve výživě rostlin

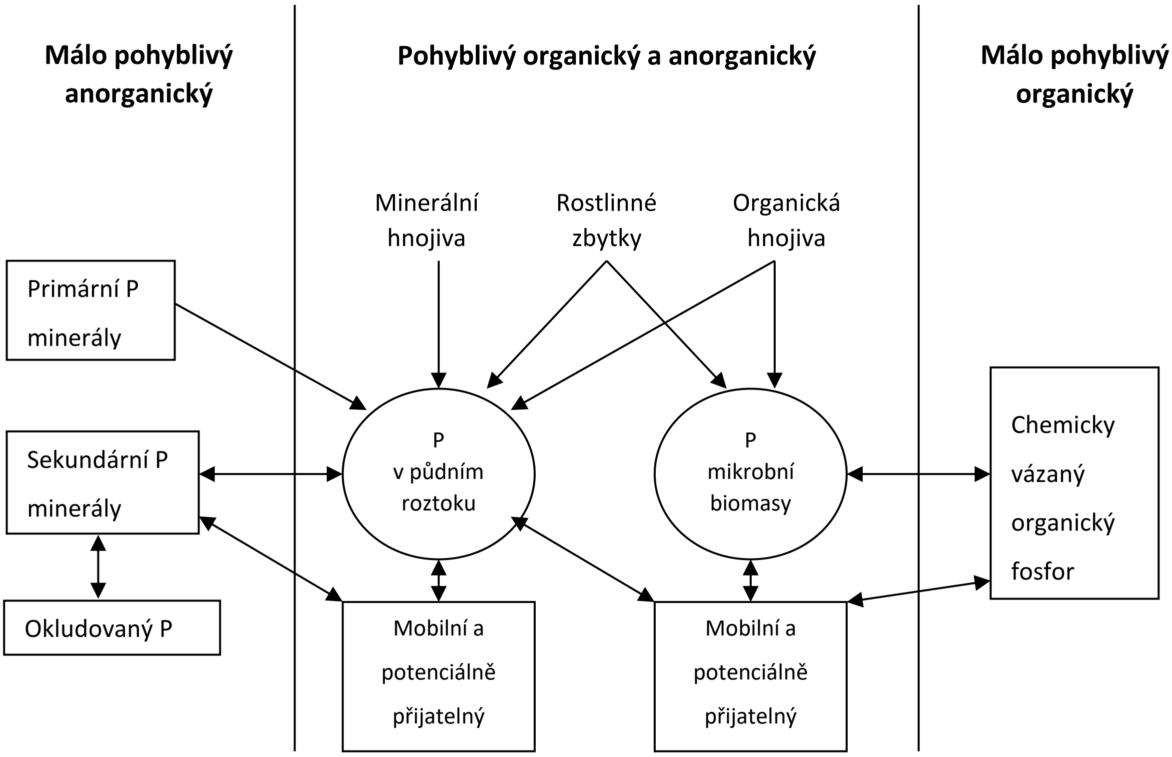
Poté, co před 350-ti lety (v r. 1669) Henning Brandt při výrobě kamene mudrců omylem objevil fosfor, dostává se dnes tento prvek do centra pozornosti v oblasti udržitelného rozvoje, kvality vody a bezpečnosti potravin (Withers et al. 2015). Je to dáno nesčetnými faktory, začínaje omezenými zdroji fosforečných minerálů, přes jejich způsoby aplikace na zemědělskou půdu, až po hledání možností využití odpadních materiálů jako zdroje fosforu (Leinweber et al. 2018, Sharpley et al. 2018). Vzhledem k tomu, že více než 90 % fosforu je společností využíváno k produkci potravin, měla by být prvořadým cílem podpora reutilizace fosforu z odpadních materiálů a snaha o dosažení centrálního systému, sloužícího k dlouhodobě udržitelnému využívání P (van Dijk et al. 2016). V rámci produkce potravin je hospodář většinou tím prvním, který denně rozhoduje o aplikaci fosforu. Jeho cílem by mělo být zajištění využití P pěstovanou rostlinou, aniž by došlo k úniku do povrchových vod a k jejich eutrofizaci (Withers et al. 2014). Z hlediska ochrany kvality vody by mělo být hlavním záměrem zamezit nadbytečným vstupům fosforu. Tento cíl však může být obtížné naplnit z důvodu nedostatečně prozkoumaných vztahů v rámci koloběhu P v prostředí a vztahů mezi půdou a rostlinou, nedostatečným legislativními opatřeními (Sharpley et al. 2018), a v neposlední řadě také nezájmem či neinformovaností v uvedené oblasti (Kleinman et al. 2015). Z výše uvedeného vyplývá, že je v současné době třeba fosforu věnovat mimořádnou pozornost a zejména předcházet jeho nevratným ztrátám (Drohan et al. 2019).

4.2. Koloběh fosforu v zemědělství

Fosfor je nezastupitelným prvkem ve výživě rostlin. V přírodě se vyskytuje vždy ve svém nejvyšším oxidačním stupni ve formě aniontu kyseliny trihydrogenfosforečné PO_4^{3-}, ve sloučeninách pak téměř výhradně tvoří orthofosforečnany nebo v menším množství pyrofosfáty (Mengel 1991). Jeho přístupná forma však v celkovém koloběhu představuje jen nepatrný podíl (Marschner 2012, Blume et al. 2002, Vaněk et al. 2016, aj.). Podle Marschner (2012) se celkový obsah fosforu v závislosti textuře a typu půd pohybuje v rozmezí 0,01 až 0,20 %, přístupný podíl však dle Schilling et al. (2000) nepřekračuje 10 % z celkového. Vyšší obsah fosforu se u většiny půd vyskytuje v povrchových vrstvách díky zvýšené biologické aktivitě způsobující akumulaci organického materiálu, popř. i díky aplikaci hnojiv. Obsah půdního fosforu je dále závislý na mateční hornině, textuře a způsobu hospodaření (zpracování půdy, pěstované plodině). Uvedené vlivy pak působí i na relativní obsahy organických a minerálních forem fosforu (Vaněk et al. 2012, Sharpley et al. 1995). Nejvýznamnějším přirozeným zdrojem fosforu v půdě je primární minerál apatit, který se ve všech magmatických horninách vyskytuje rozptýlený v různých formách (fluorapatit, chlorapatit a hydroxylapatit), přičemž jeho podíl v bazických horninách je vyšší než v kyselých (Mehmood et al. 2018). V menší míře se nacházejí v půdách jako primární minerály fosforečnany železa s příměsí Mn – tripity nebo vodnaté fosforečnany hliníku – wawelity. V málo provzdušněných zamokřených půdách se může vyskytovat fosforečnan železnatý – vivianit (Ivanič et al. 1984). Zvětráváním těchto primárních fosfátových minerálů se uvolňují anionty kyseliny orthofosforečné, které v důsledku sorpční schopnosti půdy přecházejí do jiných, tzv. sekundárních minerálů, velmi rozmanitých forem minerální nebo organické povahy (Mehmood et al. 2018).

Z hlediska vazeb P v půdě můžeme vytvořit tyto tři základní skupiny: anorganické sloučeniny fosforu, organicky vázaný fosfor a výměnně sorbovaný fosfor (Balík et al. 2002). Sharpley a Menzel (1987) uvádějí schéma s jednotlivými skupinami fosforu a možnosti jejich přeměny (obr. 1). Podle McGechan a Lewis (2002) závisí množství fosforu vázané v jednotlivých frakcích především na době aplikace hnojiva včetně vlivu historických zásahů.

Obr. 1: Cyklus P v půdě: komponenty a měřitelné frakce (upr. dle Sharpley a Menzel 1987)



4.3. Stanovení obsahu P v půdě

Volba vhodné dávky fosforečných hnojiv je velmi závislá na půdních vlastnostech, a to zejména aktuálního obsahu přístupného fosforu a hodnotě pH. Proto je před samotným hnojením důležité mít přehled o obsahu P v půdě. Správně provedená analýza půdy včetně vhodné interpretace výsledků je nutností pro pozdější úspěšné hospodaření s fosforem. Za účelem analýz půdního fosforu byla vyvinuta řada (>100) extrakčních metod. Většina z nich je využívána zejména pro agronomické využití – poskytují informaci o aktuální zásobě přístupného fosforu. Další metody jsou pak určené především k výzkumným účelům (obsah hůře dostupných forem P, dynamika P v půdě). Extrakční postupy pro stanovení fosforu lze rozdělit na základní 2 skupiny: **statické** a **dynamické** (kinetické).

4.3.1 Statické metody pro stanovení P v půdě

Tyto metody spočívají ve stanovení aktuální formy P v půdě. Jedná se tak pouze o jeden výsledek udávající obsah dané formy P. Do této skupiny je možné zařadit metody pro stanovení okamžitě a potenciálně přístupného (mobilního) P, stanovení obtížně dostupných forem P, reziduálního a celkového fosforu. Na pomezí statických a dynamických metod se vyskytují různé sorpční indexy (Wang et al. 2016, Pose et Zamuner 2016) a indexy využití P rostlinou (Sedlář et al. 2018), které sice udávají jeden údaj, avšak je podle nich možné do značné míry odhadnout i dynamiku P.

4.3.1.1. Okamžitě přístupný a mobilní fosfor

Tato skupina představuje nejširší škálu metod, neboť zahrnuje extrakční postupy běžně využívané pro praktické účely i výzkum. Zejména z důvodu praktického využití by tyto metody měly dle Bray (1948) splňovat následující parametry:

- Použité činidlo by mělo z půdy extrahovat všechen (nebo odpovídající podíl) rostlinám přístupných forem P z půd lišících se chemickými i mineralogickými vlastnostmi
- Půdní test by měl být rychlý a přesný
- Výsledky extrakce by měly korelovat s obsahem P v rostlinách (včetně reakce rostlin na aplikované fosforečné hnojivo)
- Pomocí půdního testu by mělo být možné detekovat rozdíly v obsahu P způsobené hnojením organickými či minerálními hnojivy obsahujícími fosfor.

Přehled nejběžnějších metod pro stanovení okamžitě přístupného i mobilního fosforu je uveden v tabulce 1 a přehled jejich účinnosti v grafu 1. Pro stanovení okamžitě přístupného fosforu je nejčastěji využíván vodný výluh (např. Luscombe 1979), popř. výluhy ve slabých solích, zejména 0,01 mol/l $CaCl_2$ (Houba et al. 2000). Výhodou těchto metod je stanovení obsahu P, který přibližně odpovídá podílu v půdním roztoku a je tedy rostlinám okamžitě přístupný (Kulhánek et al. 2009). Uvedené metody však vykazují i některé nevýhody. Naměřené hodnoty jsou často velmi nízké, a tak zejména při kolorimetrickém měření dochází k problémům způsobeným detekčními limity fotometru (Wuenscher et al. 2015, Kulhánek et al. 2009). Kulhánek et al. (2009) naměřili nejnižší hodnoty při použití 0,01 mol/l $CaCl_2$, což vysvětlují tvorbou Ca-P komplexů, Wuenscher et al. (2014) a Zehetner et al. (2018) dosáhli opačných tendencí. To vysvětlují jiným použitým poměrem půdy a vyluhovadla.

Tabulka 1. Přehled nejběžněji používaných metod pro stanovení přístupných forem fosforu v půdě.

Metoda	Extrakční činidlo	pH roztoku	Poměr w/v*	Doba extrakce	Zdroj
$CaCl_2$	0,01 mol/l $CaCl_2$	-	1:10	2 hod.	Houba et al. (2000)
H_2O	Destilovaná H_2O	-	1:10	1 hod.	Luscombe et al. (1979)
AEM	Destilovaná H_2O	-	1:10	16 hod.	Tiessen a Moir (1993)
Bray 2	0,03 mol/l NH_4F, 0,1 mol/l HCl,	1,0	1:7	40 s	Sims (2000)
CAL	0,3 mol/l CH_3COOH, 0,05 mol/l $C_6H_{10}CaO_6$, 0,05 mol/l $(CH_3COO)_2Ca$	4,0	1:20	2 hod.	Schüller (1969)
Olsen	0,5 mol/l $NaHCO_3$	8,5	1:20	0,5 hod.	Olsen (1954)
Egner (DL)	0,01 mol/l (CH3COO)2Ca, 0,01 mol/l HCl	3,6	1:50	1,5 hod.	Egner (1960)
Mehlich 3	0,2 mol/l CH_3COOH, 0,25 mol/l NH4NO3, 0,015 mol/l NH_4F, 0,013 mol/l HNO_3, 0,001 mol/l EDTA	2,5	1:10	5 min.	Mehlich (2008) (reprint z r. 1984)

* w/v – poměr navážky (w) a vyluhovadla (v); AEM –iontovýměnné membrány

Mobilní (potenciálně přístupné) fosforečnany představují P zastoupený v půdním roztoku spolu s výměnně sorbovaným fosforem. Níže je uveden stručný popis některých metod běžně užívaných k jejich stanovení. Extrakce CAL je doposud používaná především v Německu (Zbíral 2001). Extrakční roztok vyvinutý Schüllerem (1969) je díky vyšší koncentraci reagencií, vyšší pufrační schopnosti extrakčního činidla a menším poměru navážky půdy k činidlu méně ovlivňována přítomností karbonátů, a proto v alkalických půdách extrahuje více fosforu než Mehlich 3 (Zbíral 2001, Kulhánek et al. 2009).

V Evropě je rovněž využívána extrakce dle Egner et al. (1960). Je založena na podobném principu jako metoda CAL. Nevýhodou této metody je, že selhává na karbonátových půdách (Zbíral 2001).

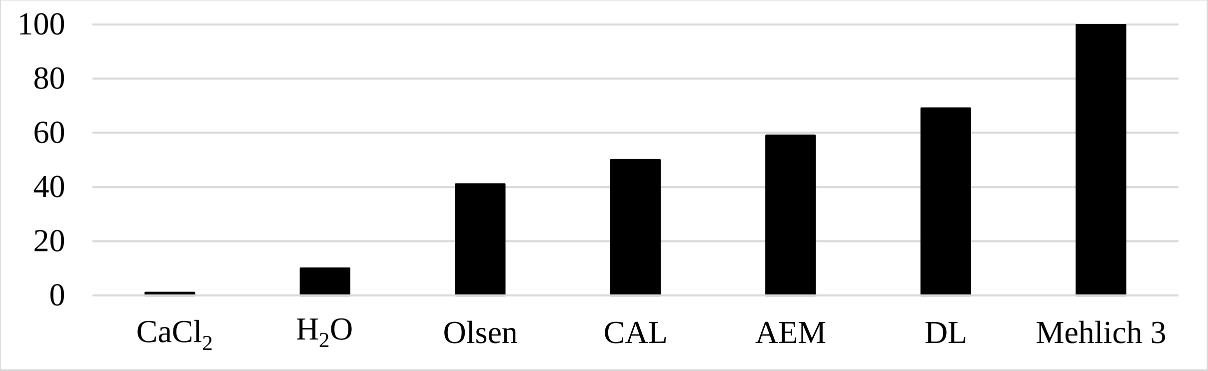
Extrakce Bray 2 je stále hojně používanou metodou (Sims 2000). Určitou nevýhodu představuje krátká doba extrakce. Běžně extrahovaná jemnozem (< 2 mm) se může značně lišit ve velikosti agregátů, popř. jílovitých částic. Při extrakci trvající pouze 40 sekund tak nedochází k dostatečnému uvolnění přístupného P a dochází tak často k výkyvům hodnot v rámci jednoho půdního vzorku. Proto někteří autoři (Hylander et al. 1999) doporučují prodloužení extrakčního času na 15 min. Stejní autoři dále poukazují na důležitost kontroly času filtrace při této extrakci.

Velmi rozšířenou metodou je Mehlich 3 (Mehlich 1984). Jedná se o univerzální roztok, kterým lze kromě fosforu stanovit také draslík, vápník, hořčík, sodík, bór, měď, železo, mangan a zinek ve všech kyselých půdách i substrátech (Benton Jones 1990, Rao et Sharma 1997, Monterosso et al. 1999, Zbíral 2016). Toto činidlo je běžně užíváno ve Spojených Státech i v některých evropských, jihoamerických, australských i asijských regionech, a to pro karbonátové i nekarbonátové půdy (Gartley et al. 2002, Ring et al. 2004, Kulhánek et al., 2009, Bortolon et al. 2011).

Extrakt dle Olsen et al. (1954) je úspěšně používán na širokém spektru kyselých i alkalických půd (McDowell et al. 2003, Carmo Horta et Torrent 2007). Oblast využití spadá hlavně do anglicky mluvících zemí (Macháček 1999). Nespornou výhodou této metody je podle Zbíral (2001), Macháček (1999), McDowell et al. (2003) její využitelnost právě na karbonátových půdách a podle Buondomo (1992) i velmi dobré korelace s metodou Mehlich 3. Naopak jako nevýhody jsou uváděny vysoké požadavky na přesnost při zhotovování výluhů, existence značných rozdílů mezi měřením vzorků odebraných v jarním, resp. podzimním termínu (Zbíral 2001) a menší schopnost extrahovat fosfor z labilních organických vazeb (Bowman et Cole 1978).

Jako další poměrně perspektivní metoda se jeví využití iontovýměnných membrán (AEM). Jedná se v podstatě o vodný výluh, kdy jsou ovšem vodou extrahovatelné fosforečnany sorbovány na membránu a nedochází tak k jejich zpětné desorpci na půdní částice. Membrány tak v podstatě simulují kořeny rostlin a korelace s odběry P rostlinou by měly být vyšší než u ostatních metod. Výhodou AEM je i možnost opakované použitelnosti membrán a celkem jednoduché provedení celé metody bez nutnosti odstřeďování vzorků (Tiessen et Moir 1993, Schoenau et Huang 1991). Kuono et al. (1995) tuto metodu využili i pro stanovení mikrobiálního fosforu s tím, že je provedení jednodušší než extrakce chloroformem a výsledky jsou na určitých půdních typech (Andosoly) přesnější.

Graf 1.: Porovnání extrakční účinnosti metod pro stanovení okamžitě přístupných a mobilních fosforečnanů (v %) na nekarbonátových půdách (Kulhánek et al. 2009)

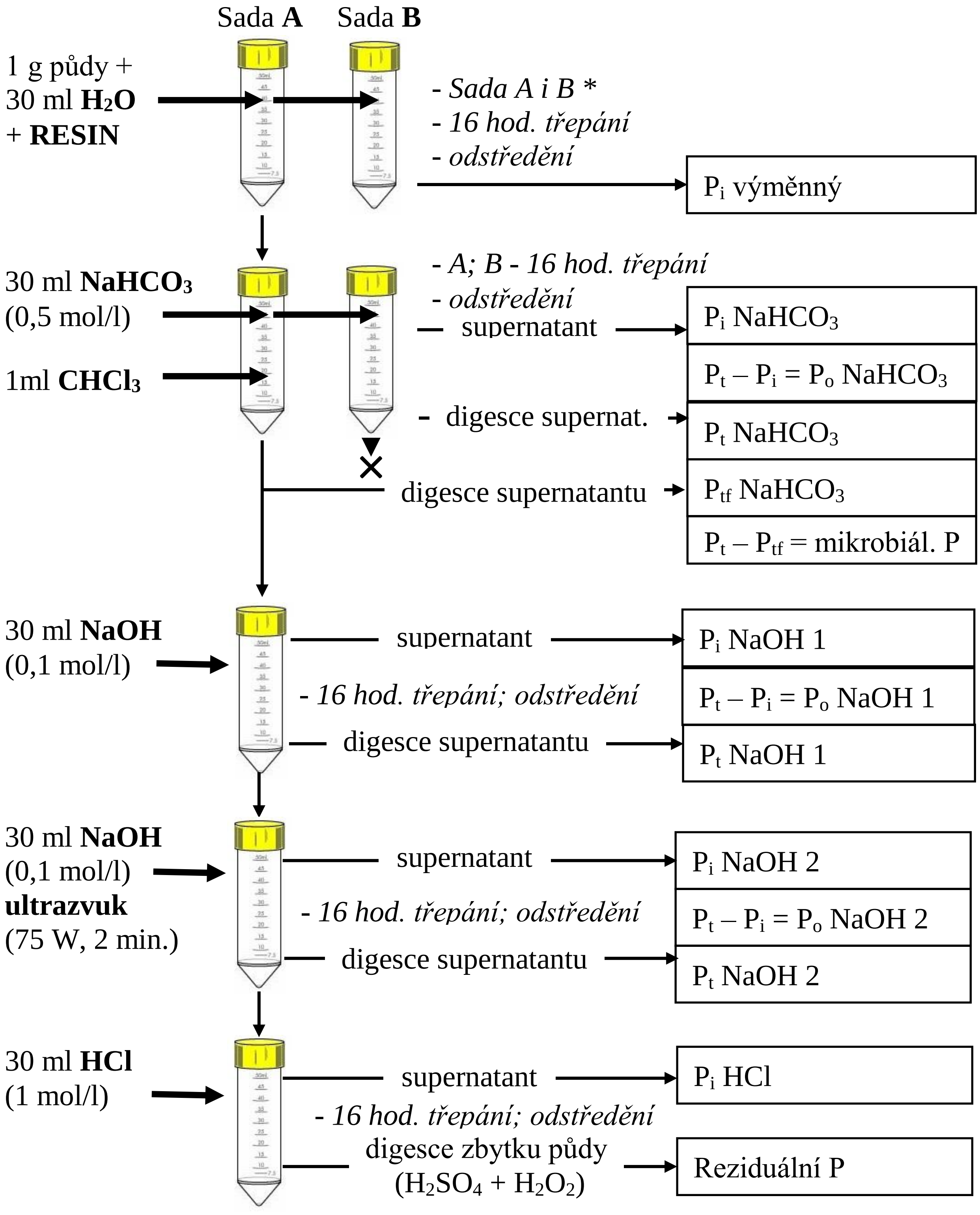


4.3.1.2. Hůře přístupné formy fosforu

Hůře přístupné formy fosforu nejsou zpravidla stanoveny individuálně, ale v rámci různých frakcionačních analýz. Důvodem je nutnost stanovení přístupných forem P, které jsou pak od následujících frakcí odečítány. Frakcionačních analýz existuje celá řada. Jednou z nejstarších, dosud hojně užívaných, je metoda dle Chang et Jackson (1957). Výhodou této metody je relativní jednoduchost, rychlost a možnost srovnání výsledků s řadou publikací. Jako hlavní nevýhoda je naopak uváděno nezahrnutí organických forem P. Již ze složení a koncentrace přidávaných extraktantů je zřejmé, že se jedná o stanovení snadněji mobilizovatelných forem P a nejsou tedy extrahovány stabilní fosforečnany, jako např. z primárních minerálů.

V současné době je nejpoužívanějším frakcionačním postupem metoda dle Hedley et al. (1982). Schéma je znázorněno na obrázku 2. Tento postup představuje řadu vylepšení předchozích metod. Fosfor stanovený pomocí resinu vykazuje úzké korelace s obsahy a odběry rostlinami a jedná se tedy o skutečný podíl přístupného P. Dále je zde zahrnuto i stanovení mikrobiálního P. Ten v půdě zpravidla představuje jen nepatrný podíl, ale je rozhodující při mineralizaci a biologické sorpci. Celý extrakční postup zahrnuje širokou škálu organických i minerálních forem P od přístupných až po pevně vázané a reziduální. Další nespornou výhodou je časté využívání, a proto i možnost porovnání výsledků s literaturou. Jako hlavní nevýhody této metody jsou uváděny vysoká časová i pracovní náročnost. Po zavedení postupu dle Hedley et al. (1982) již prakticky nebyly vyvíjeny zcela nové frakcionační metody. Byla však vytvořena řada modifikací výše uvedených typů analýz.

Obr. 2: Schéma frakcionační analýzy dle Hedley et al. (1982)



Vysvětlivky:
P_i – anorganický fosfor, P_o – organický fosfor, P_t – Celkový fosfor, P_{tf} – celkový P získaný fumigací, P_{mb} – fosfor mikrobiální biomasy

4.3.1.3. Stanovení celkového fosforu

Některé formy fosforu jsou v půdě vázány velmi pevně. Proto je nutno použít razantní procedury umožňující převedení veškerého P do roztoku. Jako příklady lze uvést digesci v prostředí kyseliny chloristé dle Sommers et Nelson (1972), alkalické tavení uhličitanem sodným (Burhan 1995 in Kara et al. 1997), digesce vzorku se směsí kyseliny chloristé a fluorovodíkové (Syers et al. 1968), popř. digesci s kyselinou sírovou, fluoridem amonným a peroxidem vodíku (Bowman 1988). Hojně využívaným postupem je extrakce lučavkou královskou. Zde je však stanoven pouze reziduální fosfor a nikoliv celkový. Přesto Crossland et al. (1995) uvádějí vysoké korelace této metody s alkalickým tavením.

4.3.2 Principy stanovení dynamiky P v půdě

Stanovení dynamiky fosforu v půdě slouží k určení dynamické rovnováhy fosforu v půdě v závislosti na jejích vlastnostech a je stále hojněji využíváno k pochopení přeměn P v půdě. Jednotlivé metody mohou sloužit i k dlouhodobým prognózám uvolňování fosforečnanů z půdní matrix do roztoku (McGechan 2002).

Při realizaci těchto metod se zpravidla vychází ze čtyř základních parametrů:

- ***Intenzita – I*** (úroveň okamžité přístupnosti fosforu rostlinám). Určuje se jako Schofieldův fosfátový potenciál – obdoba chemického potenciálu. Nejvíce se používá extrakce 0,001 – 0,01 M $CaCl_2$ (KCl, NaCl, K_2SO_4) a získané výsledky se vyjadřují v µmol P na dané množství půdy (hmotnost nebo objem). V různých modelech je intenzita pouze vypočtena z pufrační kapacity (McGechan et Lewis 2002, Kulhánek et al. 2008).
- ***Rychlost uvolňování fosforu z půdního komplexu do roztoku – R.*** Zde je sledována časová závislost uvolňování fosforu za určitou dobu (McGechan a Lewis 2002). Měření se provádí pomocí anexu a katexu a je možné využít i elektroultrafiltraci. Výpočet se pak provádí po dosazení do rovnice $P = R.\sqrt{t} + b$, kde P je množství fosforu extrahovaného v čase t a b je množství fosforu v čase t = 0 (Macháček 2002).
- ***Adsorpční charakteristika půdy*** – zde se stanovuje sorpční maximum pomocí různých izoterm - Langmuirova, Freundlichova (Pal 2011), Elovichova (Cheung et Venkitachalam 2006) aj., popř. sorpčního indexu (Wang et al. 2016). Sorpční index (PSI) je jedním z bodů sorpční izotermy, a dá se vyjádřit rovnicí $PSI = x/\log c$, kde x je množství adsorbovaného fosforu a c je koncentrace P po ukončení reakce (Bache a

Williams 1971). Nově je možno využít metody infračervené spektroskopie půdy (MIR DRIFT), která vykazuje dobrý potenciál ke stanovení sorpce P v půdě (Dunne et al. 2020). Ke stanovení adsorpčních charakteristik půdy je možno využít i některé multifunkční statické metody, např. Mehlich 3. Princip spočívá v zahrnutí P, Fe a Al stanovených touto metodou a výpočtu PSI na základě předpokládaných vazeb P s těmito prvky (Szara et al. 2018). Tak je možno vypočítat i stupeň nasycení půdy fosforem (Jalali et Jalali 2017)

- ***Pufrační kapacita – BC*** se vyskytuje i pod názvy diferenciální, fosfátová a rovnovážná BC (Macháček 2002). Dá se definovat jako schopnost půdy vyrovnávat změny koncentrací jednotlivých forem P (Hartikainen 1991). Je obecně používána jako indikace množství fosforu, který je rychle desorbován. Nepatří sem tedy fosfor uložený pomalými procesy (McGechan et Lewis 2002). Výpočet se provádí jako celková pufrační kapacita z adsorpčně-desorpční závislosti jako podíl změny množství (ΔQ) a intenzity (ΔI). Výhodou použití těchto vztahů je umožňění předpověďi zadržování a uvolňování P v půdách (Morel et al. 1996), a oproti standardním půdním testům technicky lepší přístup ke stanovení přijatelnosti půdního P (Hartikainen 1991).

Pro hlubší popsání dynamiky v půdě je možno využít pokročilejší modely. Ty dosud nejsou široce využívány, neboť jejich hlavními nevýhodami je 1) značné množství potřebných vstupních dat, 2) komplikované správné hodnocení výsledků a 3) dosud neexistující modely použitelné univerzálně pro větší skupiny druhů a typů půd. Hlavní výhodou je naproti tomu možnost predikce chování fosforu v nejbližším období, včetně odhadu vlivu různých opatření. Přes uváděné množství nevýhod existuje nepřeberné množství modelů, od relativně jednoduchých po velmi složité. Přehled některých typů modelů uvádějí např. Lewis et McGechan (2002), Kruse et al. (2015), Morelli et al. (2018).

4.4. Nové perspektivy hnojení fosforem

Zejména omezené zdroje fosforečných minerálů podněcují výzkum různých alternativ. První zmínky o hnojení fosfáty spadají do 20. let 19. století, avšak prudký nárůst poptávky byl zaznamenán až po roce 1940. Odhad z roku 2011 udává, že bylo vytěženo přibližně 20 Mt fosforu ve formě apatitů (tj. přes 200 Mt těchto minerálů) (Jasinski 2013). Se stoupající populací však prudce stoupají i požadavky na potraviny, a tím pádem na P-hnojiva. Z toho je možno vyvodit, že se zdroje kvalitních fosfátů tenčí (Cordell et Neset 2014), a to i přes některé nověji objevené zdroje (Midgley 2012 in Torri et al. 2017). Fosforečné minerály jsou tak považovány za neobnovitelné zdroje. Jak již bylo uvedeno výše, podle různých studií budou kvalitní fosfáty vyčerpány přibližně za 50-100 let (Cordell et al. 2009). V této souvislosti se intenzivně rozvíjejí převážně následující odvětví:

- Využití odpadních materiálů jako zdroje fosforu.
- Šetření minerálních P hnojiv pomocí jejich lokální aplikace, popř. udržení rozpustné formy P v půdě.
- Využití biostimulantů k podpoře příjmu fosforu
- Šlechtění odrůd s nižším odběrem P nebo (a) s vyšší výkoností jeho příjmu

4.4.1. Využití odpadních materiálů jako zdroje fosforu

Odpadní materiály biologického i průmyslového původu mohou být aplikovány přímo, nebo přetvořeny na hnojiva dalšími způsoby, jako např. vysrážením fosforečných solí, spalováním, popřípadě pyrolýzou. Podmínkou je, aby získaný fosfor byl rostlinám přístupný, a tak použitelný jako hnojivo pro dosažení vyššího výnosu a kvality produkce (Huygens et Saveyn 2018).

4.4.1.1. Čistírenské kaly

Jako nejjednodušší cesta se jeví přímá aplikace odpadů na zemědělskou půdu. Celosvětově se jedná již o desetiletí praktikovanou strategii, která je obecně akceptována (Lu et al. 2012). Velmi často jsou takto aplikovány čistírenské kaly, které jsou zdrojem nejen fosforu, ale i organické hmoty, dusíku a dalších živin. Aplikace kalů tak má potenciál pro využití na zemědělské, popř. degradované půdě i z hlediska zachování koloběhu uhlíku a živin (Kowaljow et al. 2010, García-Orenes et al. 2005, Kulhánek et al. 2014). Další výhodou jsou nízké investiční náklady a možnost zvýšení kvality kompostováním. Nevýhodu naopak představuje poměrně dlouhá doba stabilizace v nízkoteplotních provozech (Cieślik et al. 2014). Dalším negativním parametrem je hrozba kontaminace prostředí rizikovými prvky, organickými polutanty, parazity a patogenními mikroorganismy (Dean et Suess 1985). Velký objem kalů rovněž komplikuje manipulaci a přepravu.

Zpětné získání fosforu z čistírenských kalů pomocí vysrážení je jedna z nejrozšířenějších technologií na světě (Cieślik et Konieczka 2016). Při tomto procesu vznikají struvity, hydoxyapatity, popř. fosforečnany vápenaté. Tato technologie je již zavedena do mnoha provozů (Yuan et al. 2012). Za největší výhodu je považován fakt, že se jedná o velmi kvalitní fosforečné minerály s nízkým obsahem rizikových prvků, které jsou přímo použitelné v zemědělství (Nakakubo et al. 2012). Další výhodu představuje jejich horší rozpustnost. Při jejich nadměrné aplikaci tak nehrozí únik fosforu do prostředí a následná eutrofizace vod (Kataki et al. 2016). Huygens et Saveyn (2018) ve své meta-analýze dále prokázali, že právě struvity se svou kvalitou téměř rovnají minerálním P-hnojivům, a to s poměrně konzistentními výsledky nezávislými na pěstované plodině a půdním vlastnostem.

V některých provozech dochází dokonce ke spontánnímu vzniku struvitu. Týká se to zejména čistíren odpadních vod s přidruženou výrobou bioplynu, kde dochází ke zvýšení koncentrací Mg^{2+}, NH_4^+ a PO_4^{3-} při vysokém pH (7,0-11,0). Vzniklý struvit pak může způsobit problémy v podobě ucpaného potrubí. Jako vhodná prevence se jeví řízené srážení struvitu (před vstupem odpadních vod do reaktoru), který je následně možno využít jako hnojivo (Jaffer et al. 2002). Při vhodné technologii je možné do formy struvitu vysrážet až 54 % fosforu z odpadních vod (Worwag 2018). Nevýhodou jsou vyšší investiční náklady a podobně jako u aplikace surových kalů i možnost vstupu organických polutantů a patogenů (Kataki et al. 2016).

Spalování čistírenských kalů a výroba hnojiv ze vzniklého popelu je nejnákladnější z uvedených procedur. Výhodou je výrazné snížení obejmu při spalování, a eliminace patogenních činitelů. Celkově je aplikace popelu z čistírenských kalů ke hnojení nevýhodná z důvodu zvýšení koncentrace rizikových prvků, malé přístupnosti P pro rostliny, vzniku polycyklických aromatických uhlovodíků a obtížné aplikace. Popel je tak třeba náročně upravovat za účelem odstranění rizikových prvků a zvýšení mobility P. Aplikaci lze vyřešit granulováním či peletizací popele, což ovšem zvyšuje už tak vysoké náklady. Proto je popel z čistírenských kalů častěji používán ve stavebnictví jako přídavek do asfaltu nebo cementových směsí (Havukainen et al. 2016, Shafii et al. 2019, Jeon et Kim 2018).

4.4.1.2. Další odpadní materiály jako zdroje P

V posledních desetiletích došlo k výraznému nárůstu agronomického využití organických matriálů a vedlejších produktů, dříve odpadů vyvážených na skládky. Prostřednictvím anaerobní fermentace, pyrolýzy, spalování nebo kompostování komunálního odpadu tak vznikají hnojiva (Cordell et al. 2011). Takto vzniklé materiály obsahují často významný podíl organické hmoty i živin (včetně fosforu), které spolu mohou zvyšovat půdní úrodnost i výnosy a kvalitu sklizených produktů. Výzkum těchto produktů z hlediska dlouhodobé účinnosti aplikovaného fosforu je stále zatím v počátcích, přesto často vykazují dobrý potenciál jako zdroj P. Z novějších výzkumů např. Wang et al. (2014) poukazují na potenciál biocharu vyrobeného z chlévského hnoje jako hnojiva s pomalu se uvolňujícím fosforem. Výsledky s produkty pyrolýzy jsou však zatím dosti nejednotné, a proto je třeba další výzkum (Huygens et Saveyn 2018).

Popel z odpadů upravených spalováním může být rovněž kvalitní náhradou minerálních hnojiv, avšak zde je kvalita silně ovlivněna zdrojovým materiálem, procesem spalování, následnými opatřeními, a rovněž i délkou vegetační doby pěstovaných rostlin (Huygens et Saveyn 2018).

Hlavními pěti faktory limitujícími širší využití fosforu z odpadních materiálů jsou dle Withers et al. (2014) následující:

- Nízká kvalita materiálů (i při nižším podílu kontaminantů)
- Riziko kontaminace životního prostředí (včetně eutrofizace)
- Sociologické aspekty (např. přímá aplikace kalů)
- Dopady na biochemii půdy (zejména na organickou hmotu)
- Obtížný transport na delší vzdálenosti

4.4.2. Lokální aplikace fosforečných hnojiv a jejich modifikace

Hnojení fosforem je nezbytné pro současné moderní zemědělství. Zároveň ale představuje jednu z nejpodstatnějších hrozeb pro eutrofizaci vod. Hnojiva jsou často aplikována s cílem dosažení co nejvyššího výnosu. Proto je často realizována plošná aplikace fosforečných hnojiv přesahující mnohonásobně množství požadované rostlinami. Využití živin rostlinami z hnojiv dodaných plošnou aplikací je totiž zvláště u fosforu minimální. V dlouhodobých pokusech bylo prokázáno, že dokonce 85 % fosforu dodaného minerálními hnojivy je imobilizováno různými chemickými a biologickými procesy. Tento fosfor následně není detekovatelný běžnými půdními testy pro stanovení přístupného P, a proto dochází k nadměrnému hnojení. Dochází tak k akumulaci fosforu v půdě a ke zvýšenému riziku jeho uvolnění do prostředí (MacDonald et al. 2011). Aby se tedy předešlo hromadění P v intenzivně obhospodařovaných oblastech, nabízí se 2 následující možnosti:

- Modifikace fosforečných hnojiv na formu s omezenou tvorbou komplexů v půdě
- Modifikace metod aplikace umožňující dopravit fosforečné hnojivo blíže ke kořenům

Je možné očekávat, že obě uvedené možnosti povedou k zvýšení využitelnosti P rostlinou, a tím pádem i ke snížení vstupu fosforečných hnojiv (Withers et al. 2014). Bylo již vyvinuto mnoho postupů vedoucích k lepšímu využití fosforu, od hnojiv stimulujících mikrobiální aktivitu, mobilizujících půdní zásobu P přes aplikaci hnojiv „pod patu" nebo na list, obalování osiv až po obalování tekutých a organominreálních hnojiv polymery. Tyto techniky sice většinou neřeší vynechání minerálních hnojiv na bázi fosforečných minerálů z výživy rostlin, ale mohou vést k jejich výraznému omezení. Např. Sekiya et Yano (2010) zjistili, že při obalování osiva pšenice P-hnojivem stačí pouze 60 % běžné dávky fosforu k optimálnímu růstu rostlin. Wager et al. (1996) dosáhli na kanadských černozemích prakticky stejných výsledků při aplikaci 10 kg P/ha pod patu, jako při plošné aplikaci 40 kg P/ha. Dobré výsledky jsou rovněž dosahovány pásovou aplikací fosforečných hnojiv, zejména pokud je pásová aplikace realizována jindy než setí rostlin (Lal et Stewart 2015). Další možnosti aplikace fosforu a jejich efektivita jsou přehledně shrnuty v publikaci Hopkins et Hansen (2019).

4.4.3. Využití biostimulantů

V současné době je intenzivně studována možnost využití biostimulantů (též bioefektorů) ke zlepšení příjmu P rostlinami s cílem dosáhnout vyšších výnosů rostlin a kvality produkce. Jedná se nejčastěji o mikrobiální preparáty na bázi hub a bakterií. Jsou však využívány i výtažky z rostlin a řas. I přesto, že jsou podobné preparáty s úspěchem aplikovány v ochraně rostlin (shrnuto v přiložené studii Holečková et al. 2018a), jejich role ve výživě rostlin je zatím nejistá.

Ačkoliv již bylo dosaženo pozitivních výsledků při laboratorních testech a nádobových pokusech, výsledky polních pokusů zatím nepoukazují na efektivitu těchto preparátů (Withers et al. 2014, Holečková et al. 2018b et c). Dále je třeba dodat, že v prestižních časopisech jsou mnohem snáze publikovány průkazné výsledky. Proto se může zdát, že biostimulanty skutečně fungují téměř za všech podmínek. Je však velmi pravděpodobné, že vliv biostimulantů je prozatím silně nadhodnocen (Lekfeldt et al. 2018). Přesto se zde skrývá velký potenciál a je třeba další intenzivní výzkum. V tabulce 2. jsou shrnuty výsledky některých výzkumů s různými druhy biostimulantů použitých z hlediska výživy rostlin.

Tabulka 2. Přehled vlivu biostimulantů zlepšujících příjem živin na produkci rostlin

Houby	**Podmínky pokusu**	**Efekt na rostlinu**	**Zdroj**
Trichoderma sp.	Laboratorní	Zlepšení růstu a výnosu sóji	Paradiso et al. (2017)
	Laboratorní	Zlepšení růstu Vigna unguiculata	Chagas et al. (2016)
Trichoderma harzianum	Nádobový	Zlepšení klíčení a vzcházení pšenice	El-Gremi et al. (2017)
	Nádobový	Neprůkazný vliv na odběr živin a výnos kukuřice	Holečková et al. (2018b)
	Nádobový	Vyšší délka kořenů a nadzemní hmoty, výnos zrna a sušiny pelušky	Gupta et al. (2016)
	Nádobový	Vyšší aktivita kyselé fosfatázy v půdách s pěstovanou kukuřicí.	Mercl et al. (2020)
	Nádobový	Lepší růst brukve sítinovité	Ahmad et al. (2015)
	Nádobový	Vyšší délka kořenů a nadzemní hmoty, výnos melounů	Galletti et al. (2015)
	Skleníky	Zvýšení výnosu brambor	Buysens et al. (2016)
Penicilium Bilalii	Rhizoboxy	Delší kořeny kukuřice	Gomez Munoz et al. (2017)
	Nádobový	Vyšší výnos vojtěšky	Beckie et al. (1998)
	Polní	Vyšší výnos zrna pšenice	Ram et al. (2015)
	Polní	Delší kořeny a vyšší obsah P v kořenech hrachu	Vessey et Hiesinger (2001)
Bakterie	**Podmínky pokusu**	**Efekt na rostlinu**	**Zdroj**
Pseudomonas sp.	Laboratorní	Stimulace růstu rajčat	Gravel et al. (2007)
	Nádobové a polní	Neprůkazný vliv na odběr živin a výnos kukuřice	Holečková et al. (2018b, c)
	Polní	vyšší výnos zrna a slámy ječmene	Fröhlich et al. (2012)
	Nádobové a polní	Zlepšení klíčení, růstových a výnosových parametrů kukuřice	Gholami et al. (2009) Nezarat et Gholami (2009)
	Laboratorní, skleníky a polní	Zlepšení klíčení, délky kořenů a nadzemní biomasy kukuřice	Kifle a Laing (2016)
Pseudomonas jesenii	Skleníky	Zlepšení růstu rajčat	Valverde et al. (2006)
	Skleníky a polní	Zvýšení výnosů nadzemní hmoty a semene cizrny	
Bacillus amyloliquefaciens	Laboratoř	Lepší růst kořenů a nadzemní hmoty rýže	He et al. (2013)
	Nádobové	Neprůkazný vliv na odběr živin a výnos kukuřice	Holečková et al. (2018b)
Bacillus subtilis	Polní	Zlepšení příjmu makro a mikroprvků u rajčat	Altuhaish et Tjahjoleksono (2014)
	Polní	Vyšší výnos nadzemní hmoty a kořenů zelí	Turan et al. (2014)
Paenibacillus mucilaginosus	Nádobový	Lepší vzcházení citronečníku trojlistého	Wang et al. (2016)
	Nádobový	V kombinaci s popelem mobilizoval P a naopak imobilizoval NO_3^- u pokusů s kukuřicí	Mercl et al. (2018)
Rhizophagus intraradices	Skleníky	Zlepšení výnosových parametrů čaje	Sharma et Kayang (2017)
	Polní	Lepší růst rajčat	Mohamed et al. (2016)

Z tabulky 2 dále vyplývá, že většinou byly jako hlavní parametry hodnoceny výnosy, klíčení, vzcházení, popř. délka nadzemní hmoty a kořenů. Proto až na výjimky zatím nelze jednoznačně potvrdit vliv biostimulantů na mobilizaci P a jiných živin z hůře dostupných forem.

4.4.4. Šlechtění rostlin

Fosfor se v rostlinách vyskytuje v mnoha formách. Jako příklady lze uvést cukry, tuky i bílkoviny, ale jeho primární úlohou je zajištění přenosů energie pomocí adenosintrifosfátu (ATP) a podíl na stavbě nukleových kyselin (DNA a RNA). Požadavky rostliny na fosfor jsou tak dány zejména potřebou energie k fotosyntéze (Ghannoum et al. 2008). Přesto je množství fosforu potřebného k tvorbě ATP a RNA relativně nízké. Odhaduje se, že na 1 m^2 listové plochy stačí pouze 0,12 mg fosforu k zajištění maximální konverze uhlíku (Bingham 1966). Optimálně zapojené porosty většiny plodin představují přibližně 6 m^2 listové plochy na 1 m^2 pozemku (Sylvester-Bradley et al. 2008), z čehož lze odvodit přibližné množství fosforu v nadzemní biomase potřebného k metabolismu, tj. přibližně 7,5 kg P/ha (Withers et al. 2014). To je mnohem méně než běžný odběr P rostlinami pohybující se mezi 15-40 kg P/ha, což ukazuje na fakt, že značný podíl fosforu je uložen ve vakuolách ve formě orthofosforečnanů, fosfolipidů, esterů a fytátů (Veneklaas et al. 2012). Část takto uložených orthofosforečnanů je potřebná k průběžnému doplňování P do cytoplazmy pro syntézu RNA (Reich et al. 2010). Část fytátů je dále potřebná na tvoru fytinu v semenech pro jejich klíčení (Nadeem et al. 2011). Rostliny však vytvářejí vyšší zásobu P z preventivních důvodů jako pojistku proti vyčerpání zdroje P nebo přerušení jeho dodávání (Withers et al. 2014). Proto určitá část zůstává nevyužita, a tak se objevují snahy vyšlechtit rostliny, které odeberou méně fosforu, aniž by to následně ovlivnilo jejich výnos a životaschopnost (Landoni et al. 2013, White et Veneklaas 2012, Wang et al. 2010). Fytáty jsou navíc pro monogastry (tedy i člověka) obtížně využitelným zdrojem P, a zároveň zhoršují příjem některých esenciálních prvků (Fe, Zn) a kationtů (Ca, Mg) (White et Broadley 2009). Redukce jejich nadbytečné zásoby ve vakuolách a později v semenech tak paradoxně zvýší nutriční hodnotu sklizených rostlin.

Dalším cílem šlechtění je podpora následujících pěti mechanismů zajišťujících rostlině příjem fosforu při jeho nedostatku v půdě:

- Změny v růstu kořenů (podpora růstu kořenového vlášení) (Lynch 2007).
- Podpora symbiotické mykorhizy (Parihar et al. 2019).
- Uvolňování různých exsudátů (protony, uhlíkaté substráty, enzymy), které mobilizují organický i anorganický P (Jones et al. 2009).
- Fyziologické změny minimalizující požadavky na P při metabolismu. Změna v činnosti přenašečů P (Postma et Lynch 2010).

- Kořeny zprostředkovaný transport vody z hlubších vrstev půd do suché ornice. Voda pak v ornici zvyšuje rozpustnost P (Shen et al. 2011)

Mechanismy pro příjem fosforu jsou přitom mnohem lépe vyvinuty u planých, než u kulturních rostlin. Je to dáno tím, že kulturní rostliny byly šlechtěny především na výnos v podmínkách s dostatečným množstvím P. Plané rostliny sice dokážou lépe přijímat fosfor, ale na druhé straně je to stojí energii potřebnou k zajištění dostatečného výnosu (Zhu et al. 2001).

4.5. Význam síry ve výživě rostlin

Síra se podobně jako fosfor stává limitujícím prvkem ve výživě rostlin v celosvětovém měřítku (Fowler et al. 2005). Důvody jsou zde ovšem jiné. Je známo, že v posledních desetiletích došlo významnému k poklesu obsahu síry v půdách (Balík et al. 2009, Kulhánek et al. 2016, Scherer 2001, Zbíral et al. 2018), a to příčinou:

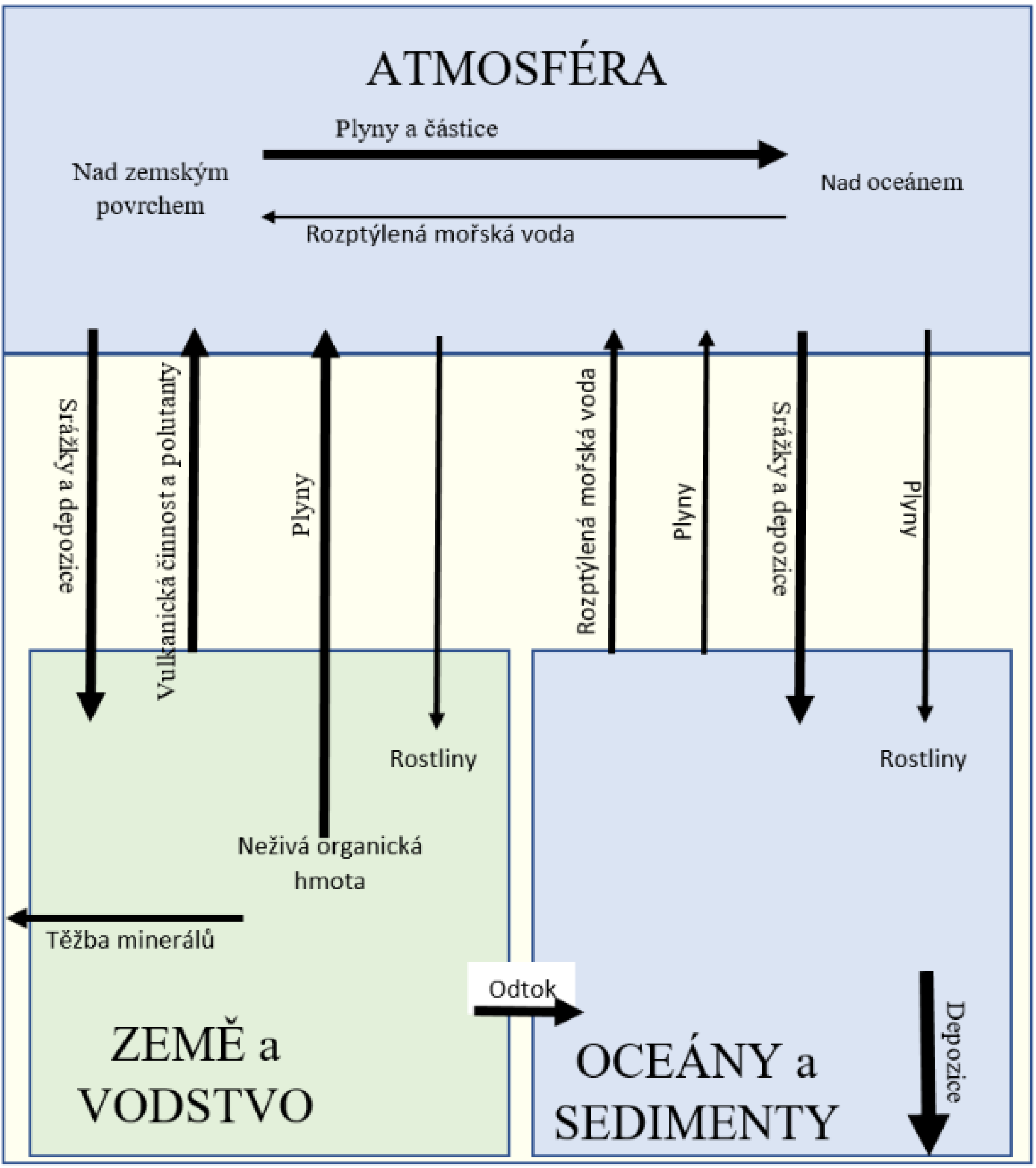
- Poklesu vstupů síry v atmosférických depozicích
- Menší míře aplikace hnojiv s balastní sírou
- Pěstování výkonnějších odrůd rostlin s vyšším odběrem S

Uvedené příčiny způsobily pokles obsahů přístupných forem síry v půdě za posledních 40 let často o více než 70 % (Balík et al. 2009, Fowler et al. 2005). Na rozdíl od fosforu tak není síra limitována nedostatkem hnojiv, ale spíše podceněním aktuální situace. Donedávna byl v půdě dostatek až nadbytek tohoto prvku, a proto síře zatím není věnována dostatečná pozornost.

4.5.1. Koloběh síry v přírodě

Stručný nástin koloběhu síry v přírodě je znázorněn na obr. 3. Půdní síra se vyskytuje v četných formách a její dynamika hraje důležitou roli ve vztahu k příjmu rostlinami. Organická síra se dělí na široké spektrum sloučenin odrážejících historické zásahy, avšak má pouze malou vypovídací hodnotu při určování dávek hnojiv (Marschner 2012). Je proto několik důvodů: 1) organická síra sestává z mnoha frakcí s různou dobou mineralizace, 2) asociace organické síry s půdními částicemi představuje fyzikální ochranu před rozkladem a 3) ročně je mineralizováno pouze 0,5 – 3 % z podílu organické síry. Přechodná povaha rostlinám přístupné síry představuje značné komplikace při interpretaci výsledků běžných půdních testů a přesnější informace nám tak často poskytuje pouhé vypočtení bilance síry na základě odběru S rostlinami. Ve srovnání s jinými makroprvky je využitelnost síry rostlinami nízká a pohybuje se okolo 25 %. K optimalizaci hnojení sírou je nutné zahrnout hnojení organickými i minerálními hnojivy. Minerální hnojiva mohou být částečně nahrazena organickými. Při nadbytečné aplikaci síry zejména chlévským hnojem hrozí únik přebytečné S do ovzduší v plynných formách. Dále hrají důležitou roli i podmínky stanoviště a osevní postup, kdy např. zařazení meziplodiny může výrazně omezit ztráty vyplavováním (Eriksen 2009).

Obr. 3 Základní složky koloběhu síry v přírodě



4.5.2. Síra v půdě

Síra je v geosféře hojným nekovovým prvkem a v přírodě se vyskytuje v různých formách. Jako jeden z mála prvků se v přírodě nachází i v elementární podobě (Lucheta et Lambais 2012). Zemská kůra obsahuje mezi 0,06 a 0,10 % S (Havlin et al. 2005). Síra se vyskytuje také jako sloučenina s dalšími prvky ve formě sulfidů, síranů nebo organických forem, v sedimentárních, metamorfovaných a vyvřelých horninách nebo fosilních palivech (Albuquerque et al. 2008). Globální zdroje síry jsou odhadovány na 5 mld. tun v zemním plynu, ropě, kovových sulfidech, ložiskách soli a vulkanických depozicích, dále přibližně 600 mld. tun v uhlí a břidlicích a prakticky nevyčerpatelné zdroje síranů ve formě sádrovce nebo anhydritu (Lucheta et Lambais 2012).

Celkový obsah síry v půdě dobře koreluje s obsahem organické S, což ukazuje na fakt, že celková síra je tvořena zejména organickou frakcí S (Biederbeck 1978). Celkový obsah síry je dále silně ovlivněn i různými typy organických i minerálních hnojiv, přičemž zásadní vliv na akumulaci síry mají zejména organická hnojiva (Yang et al. 2007, Balík et al. 2009, Förster et al. 2012).

Síra se v půdě vyskytuje v organických i anorganických formách, mezi kterými se přeměňuje pomocí mobilizace, mineralizace, imobilizace, oxidace a redukce. Organický podíl je zpravidla málo pohyblivý, zatímco nejpohyblivější formu tvoří síranový aniont (SO_4^{2-}) (Scherer 2001). Transport síry v půdě je zpomalován adsorpcí, která je spolu s desorpcí zásadně ovlivněna aktuální koncentrací síranových aniontů v půdním roztoku, hodnotou pH, charakterem povrchu koloidů a dalších aniontů v půdním roztoku. Organická síra se vyskytuje zejména ve dvou formách: estersulfátové (C-O-S) a přímo vázané na uhlík (C-S) (Scherer 2009).

4.5.2.1. Organický podíl síry v půdě

Jak již bylo dříve uvedeno, převážný podíl síry v půdě tvoří její organická forma (Yang et al. 2007, Balík et al. 2009, Kulhánek et al. 2011 et 2018a, b). Její podíl dosahuje zpravidla 95-98 % (Kertezs et Mirleau 2004, Bloem 1998). Organická síra se vyskytuje v mikroorganismech, rostlinách a makroedafonu, ať již v živé či neživé formě. Jedná se tak o velmi heterogenní směs různých forem síry, které dosud nebyly podrobně popsány (Kertezs et Mirleau 2004, Scherer 2009). Vzhledem k tomu, že uhlík, dusík a síra jsou základními komponenty organických sloučenin, vyskytují se zpravidla těsné vzájemné korelace mezi obsahem organické síry, organického uhlíku a celkového dusíku (Wang et al. 2006). I přes značnou diverzitu jednotlivých forem organické síry v půdě je možné vyčlenit dvě základní skupiny, tj. síra nepřímo vázaná na uhlík ve formě estersulfátů (C-O-S) a síra přímo vázaná na uhlík (C-S). Existuje i několik dalších možností organických vazeb síry, jako jsou sulfonáty či heterocyklické formy S, avšak ty v půdě zpravidla nenabývají vyššího významu (Edwards 1998). Obsahy estersulfátové síry tvoří běžně 30 – 70% z organické S (Neptune et al. 1975). Při stanovení pomocí redukce kyselinou jodovodíkovou jsou zde však zahrnuty i sulfamáty (C-N-S), polysacharidy obsahující S a síra vázaná v cholinu (Nguyen et Goh 1992, Edwards 1998). Proto se jedná o sloučeniny s velmi variabilní rozpustností (Eriksen et al. 1998). Již ve své publikaci z roku 1981 McGill et Cole poukazují na fakt, že estersulfátová síra je mnohem pohyblivější, než síra přímo vázaná na uhlík. Rok poté doplnili toto zjištění Fitzgerald et al. (1982) o fakt, že estersulfátová síra navíc dosahuje rovnovážného stavu dříve než C-S, avšak v některých půdách tento stav (zabudování SO_4^{2-} do C-O-S) nastává po několika týdnech až měsících.

Síru přímo vázanou na uhlík představují převážně aminokyseliny cystein a methionin. Ty úzce korelují s podílem síry půdní mikrobiální biomasy (Castellano et Dick 1991). Korelace C-S frakce s celkovým obsahem C, N a S jsou navíc těsnější, než v případě estersulfátové síry (Scott et Anderson 1976). Z toho se dá usuzovat, že síra přímo vázaná na uhlík je stabilnějším a zároveň integrálním komponentem půdní organické hmoty. Förster et al. (2012) stanovili ve dlouhodobých pokusech (Bonn, Německo) 19 – 43 % C-S, přičemž na organicky hnojených parcelkách byly obsahy signifikantně vyšší. V návazné studii Scherer et al. (2012) udávají, že se C-S i C-O-S frakce mají tendenci se akumulovat především na nejjemnějších půdních částicích (<0,002 mm). Díky zpravidla malému výskytu těchto částic v půdě, a tím pádem i menšímu celkovému podílu S, se však dá usuzovat, že významnější roli v příjmu S rostlinami hrají větší půdní částice.

4.5.2.2. Minerální podíl síry v půdě

Sírany, které jsou nejvýznamnějším zdrojem S pro rostliny, představují obecně pouze méně než 5 % celkové půdní síry. Tyto je dále možné rozdělit na síru v půdním roztoku a adsorbovanou S (Barber 1995). V karbonátových půdách však přibývá ještě frakce síry vysrážená spolu s vápníkem nebo hořčíkem (Tisdale et al. 1993).

Síra v půdním roztoku představuje velmi malý podíl, pohybující se často řádově v mg/l. Tato složka je však velmi dynamická a mění se v závislosti na sezóně, odběru rostlinami, mineralizaci a imobilizaci. Vyšší obsahy se přitom častěji vyskytují ve svrchnějších vrstvách půdy. To je dáno jednak vyšší intenzitou minerelizace (McLaren et Cameron 1996), jednak aplikací hnojiv obsahujících S (Eriksen 1996), jednak pravděpodobně i přímým vstupem

S z atmosférických depozic (Balík et al. 2009). Nejnižší obsahy síry v půdním roztoku se vyskytují zpravidla v zimě nebo na jaře. Důvodem je především vyšší intenzita vyplavování a naopak nižší mineralizace (Castellano et Dick 1991).

Ačkoliv je **adsorbovaná síra** považována za labilní frakci S uvolňující se snadno do půdního roztoku, představuje rovněž účinný nástroj k zabránění vyplavování. Její obsah může být poměrně variabilní. Např. Schnug (1988) stanovil ve 300 půdách Německa méně než 1 mg/kg adsorbované S, Kulhánek et al. (2018) stanovili v českých půdách 6,15 mg S/kg a v půdách Skotska bylo zjištěno dokonce 48 mg S/kg (Scott et Anderson 1976). Adsorbovaná síra je v půdě vázána různými způsoby a je silně ovlivněna především podílem jílových minerálů a hodnotou pH. Ve studii Prietzel et al. (2001) bylo prokázáno, že s klesající hodnotou pH stoupá podíl adsorbované síry v půdě. Maximální podíl síry je adsorbován při pH 3 a s nárůstem pH podíl adsorbované S prudce klesá. Při hodnotě pH > 6.5 je už podíl adsorbované síry zanedbatelný a většina síranů už se vyskytuje v půdním roztoku (Curtin et Syers 1990). Dá se tedy předpokládat, že vápněním dojde k poklesu obsahu adsorbované S, způsobeném kompeticí mezi anionty OH^- a SO_4^{2-} (Korentajer et al. 1983). Adsorpce síry je rovněž podpořena přítomností hydratovaných oxidů železa a hliníku, které často obalují povrch jílových minerálů a volných oxidů, a rovněž jsou součástí rozhraní hlinitokřemičitanových jílových částic (Bohn et al. 1986). Adsorpce síranů na vodíkem saturované jíly je dána pořadím: smektit < illit < kaolinit (Delfosse et al. 2006). Z výsledků Tisdale et al. (1993) vyplývá, že adsorpce síry je rovněž ovlivněna dalšími anionty, a to v pořadí fosforečnany > dusičnany = chloridy. Nárůst obsahu fosforečnanů a síranů v půdě vede ke snížení adsorpce S (Bohn et al. 1986), a protože jsou sírany vázány výrazně slabší vazbou než fosforečnany, vede hnojení fosforem spolu s vápněním k zvýšení přístupnosti S (Scherer 2009). Role síry obsažené v organické hmotě je nesporná, avšak studie o významu její adsorpce na organické látky se rozcházejí. Singh et Johnson (1986) stanovili negativní korelace mezi obsahem adsorbované síry a podílem organické hmoty. To je ve studii Johnson et Todd (1983) vysvětleno blokováním sorpčních míst pro síru jinými anionty. Naproti tomu Harrison et al. (2009) stanovili pozitivní korelace mezi adsorbovanou S a organickou hmotou, čímž ukazují na fakt, že i síra může být adsorbována na organickou hmotu. Podle většiny výzkumů dochází spíše ke kompetici síranů a organických aniontů o sorpční místa a proto je adsorpce S v půdách bohatých na organickou hmotu nižší (např. Martinez et al. 1998).

Podíl **okludované S** může dosahovat desítek procent a stoupá úměrně s podílem karbonátů (Hu et al. 2005). V australských karbonátových půdách bylo zjištěno dokonce 93 % takto okludované síry (Williams et Steinbergs 1962). Výše uvedená data byla zjištěna extrakcí

1 mol/l HCl. Novější studie ukazují, že tato metoda je vhodná pouze pro karbonátové půdy s obsahem uhličitanu vápenatého přesahujícím 1 %. Pro půdy s obsahem $CaCO_3$ nižším než 1 % je totiž v HCl stanoven signifikantní podíl organické S (Morche 2008, Kulhánek et al. 2016, 2018a). Proto se zde při stanovení skutečně okludovaných síranů doporučuje použít iontovou chromatogtrafii (Hanousek et al. 2017). Totéž platí i pro adsorbovanou síru a S v půdním roztoku, kde iontová chromatografie stanoví pouze sírany (Förster et al. 2012, Scherer et al. 2012). Zde však podíl organické síry nebývá signifikantní.

4.5.2.3. Přeměny půdní síry

Půdní síra se nepřetržitě přeměňuje z anorganické síry na organickou a naopak. Sloučeniny organické síry jsou rostlinám nepřístupné a musí jim být zpřístupněny biochemickou nebo mikrobiologickou mineralizací za vzniku anorganického síranového iontu (Castellano et Dick 1991). Tohoto procesu se účastní enzymy i mikroorganismy a je značně ovlivněn vnějšími podmínkami. Ačkoliv mikrobiálně vázaná síra představuje pouze malou frakci celkové organické síry v půdě a množství síry v mikrobní biomase se pohybuje mezi 0,81 - 13,4 mg/kg půdy, s mediánem 6,15 mg/kg (Chowdhury et al. 1999), toto malé množství může v půdě rychle cirkulovat a má tak zásadní význam (Randlett et al. 1992). Mineralizace sloučenin půdní organické síry (při vyloučení elementární S) a imobilizace aplikované elementární síry do organických sloučenin ukazuje na význam půdní mikrobiální biomasy jako zdroje S v dlouhodobém hospodaření se sírou (Jaggi et al. 1999). Rychlost obratu síry v mikrobiální biomase je důležitým faktorem v dynamice sloučenin organické síry v půdě (Banerjee et Chapman 1996). Studií zabývajících se přeměnami síry v mikrobiální biomase v průběhu vegetačního období je však stále velmi málo.

Zatímco mikrobiální mineralizace síry vázané přímo na uhlík závisí na aktivitě půdních mikroorganismů (ty potřebují organický uhlík k pokrytí své potřeby energie a síra se uvolňuje jako vedlejší produkt oxidace uhlíku na CO_2), biochemická mineralizace představuje enzymatickou hydrolýzu sulfátových esterů sulfatázami (aryl-, alkyl-, steroid-, gluko-, chondro- a mykosulfatázy) (Eriksen et al. 1998).

Estersulfáty jsou považovány za nejlabilnější formu půdní organické síry a podléhají více vlivům ročníku než síra přímo vázaná na uhlík (Schnitzer 1991, McLaren et al. 1985), avšak Klose et al. (1999) nalezli během dvou let jen malé odchylky v obsahu estersufátů. Je proto pravděpodobné, že ne všechny skupiny estersulfátů v půdní organické hmotě jsou stejně labilní. Nannipieri et al. (1990) uvádějí, že biochemická stabilita estersulfátů pravděpodobně závisí na jejich poloze ve struktuře huminových polymerů. Zatímco estersulfátové skupiny umístěné na povrchu těchto polymerů mohou být přístupné sulfatázám a snadno mineralizovatelné, sulfátové skupiny nacházející se v jejich vnitřní struktuře jsou dostupné méně. Proto Eriksen et al. (1998) předpokládají, že estersulfátová frakce je součástí organických sloučenin s velmi odlišnou přístupností rostlinám - od rozpustných sloučenin k frakcím fyzikálně chráněným vazbami s jílovými minerály.

Hlavní podíl půdních estersulfátů je hydrolyzován pravděpodobně pomocí mikrobiálních enzymů. Výsledný minerální síran je pak rostlinám přístupný. Půdní enzymy jsou považovány za známku existence specifických biochemických reakcí celé půdní mikroflory (Nannipieri et al. 1990) a celková aktivita enzymů v půdách je odvozena od aktivity akumulovaných enzymů a živých mikroorganismů (Tabatabai a Fu 1992). To platí i ve vztahu k přeměnám estersulfátů, významně přispívajících k příjmu síry rostlinami (Shan et al. 1997). V přírodě se vyskytuje několik typů sulfatáz, avšak většina prací se soustřeďuje na arylsulfatázu, která byla prvním zjištěným enzymem zpracovávajícím síru v přírodě a která katalyzuje hydrolýzu estersulfátů na anorganické sírany rozštěpením O-S vazby (Fitzgerald, 1978, Siwik-Ziomek et Koper 2018, Godlewska 2018, Kotková et al. 2008, Saviozzi et al. 2006 aj.). Obecně platí, že kolem 45% veškeré aktivity arylsulfatázy se odehrává vně buňky, zatímco cca 55% aktivity je přisuzováno mikrobiální biomase (Klose et Tabatabai 1999). Knauff et Scherer (1998) spojují aktivitu arylsulfatázy s obsahem půdního humusu. Její aktivita dále klesá s přibývající hloubkou půdního profilu (Tabatabai et Bremner 1970). Roli regulátorů aktivity arylsulfatázy hrají také patrně půdní vlhkost a teplota. Dle výzkumu Castellano a Dick (1991) byla nejvyšší aktivita enzymu zaznamenána na osetých pozemcích počátkem léta, což ukazuje na zvýšený potenciál mineralizace organických sloučenin síry způsobený jednak kořenovými exsudáty, jednak rozkladem odumřelých kořenů a listů. Klose et al. (1999) dále zjistili, že aktivita arylsulfatázy je významně ovlivněna střídáním plodin a typem rostlinného pokryvu.

Jak již bylo zmíněno, v půdě probíhá rovněž degradace síry přímo vázané na uhlík, přičemž hlavním cílovým prvkem mikroorganismů je zde uhlík a síra se uvolňuje jako vedlejší produkt. Redukované sloučeniny síry jsou převážně přítomny v bílkovinách a v aminokyselinách obsahujících síru. U bílkovin je prvním krokem jejich přeměny aerobní hydrolýza za vzniku sirných aminokyselin. Dalším krokem tohoto procesu je tvorba sulfanu (H_2S), který za aerobních podmínek rychle podléhá autooxidaci na síranový aniont (Mengel a Kirkby 2001, Marschner 2012). Za anaerobních podmínek je sulfan oxidován na elementární síru chemotrofními sirnými bakteriemi rodu *Thiothrix*. Tyto bakterie mohou také za aerobních podmínek oxidovat síru na kyselinu sírovou, což má za následek pokles půdního pH. Přeměnu H_2S a elementární síru v půdě, tzv. sulfurifikaci, provádějí fotosystetizující a chemolitotrofní bakterie. Proces se odehrává buď působením sirných bakterií (v půdě rod *Thiobacillus*) anebo anaerobně fotosyntetizujícími bakteriemi (čeleď *Thiorhodaceae, Chlorobacteraceae*). Hlavním zástupcem bakterií účastnících se redukčních pochodů - desulfurikace za vzniku sirovodíku je *Desulfovibrio* (Mengel et Kirkby 2001). Tyto bakterie v redukčních podmínkách využívají síranový kyslík jako konečný akceptor elektronu v dýchacím řetězci (Scherer 2001) Výskyt anorganických sloučenin síry nižšího oxidačního stupně (S^{2-}) v půdě je spojen s anaerobními podmínkami. Signalizuje vysoký stupeň redukčních pochodů v půdě, kterému běžně předchází vznik potenciálně toxických produktů (Mn^{2+}, Fe^{2+} a alkoholů vzniklých z organických kyselin) (Matula 2007). Na podmáčených půdách je za redukčních podmínek sulfan nejdůležitějším konečným produktem anaerobní degradace S (Mengel et Kirkby 2001).

Mikrobiální mineralizace sloučenin organické síry je pochopitelně ovlivněna celou řadou faktorů, zejména však teplotou půdy. Jaggi et al. (1999) studovali vliv tří různých teplotních režimů na mineralizaci sloučenin půdní organické síry a prokázali její nejvyšší intenzitu při 36° C. Matula (2007) se pro české půdy zabýval problematikou, do jaké míry může mineralizace organické hmoty přispět k výživě porostů sírou, a to zvláště v období intenzivního jarního růstu řepky. Došel k závěru, že v kritickém jarním období nelze počítat s dostatečným doplňováním síranů v půdě prostřednictvím mineralizace půdní organické hmoty. Během 60-i dnů na přirozeně úrodných půdách nepřevyšovalo množství takto produkovaných síranů hodnotu 4 kg S/ha, zatímco u ostatních půd (s četností zastoupení v souboru kolem 50 %) byl registrován spíše úbytek, tj. imobilizace minerální síry. Dalším významným poznatkem této studie je fakt, že hnojení dusíkem snižovalo produkci síranů z mineralizace organické složky půdy a u půd s imobilizační tendencí bylo poutání síry ještě intenzivnější. Výsledky řádově odpovídají hodnotám rychlosti mineralizace, ke kterým dospěl Preuschoff (1995) na německých luvisolích na spraších. Podle studie Bloem (1998) bylo na třech různých stanovištích v severním Německu mineralizací během jednoho vegetačního období uvolněno 7 - 49 kg S/ha. Eriksen et al. (1995) zjišťovali rozsah mineralizace síry v pěti dánských půdách v průběhu dvou po sobě jdoucích vegetačních obdobích a zjistili, že čistá mineralizace se pohybovala mezi 3,30 - 6,70 mg S/kg půdy za rok, což představuje 1,70 - 3,10 % celkové půdní organické síry. Podle Williams (1967) je mineralizace zpočátku vegetačního období výrazně intenzivnější a po 1 – 4 týdnech v závislosti na druhu půdy postupně klesá.

Značná část síry, přijaté rostlinami, se může vrátit do půdy ve formě reziduí. U některých plodin se znovu zužitkuje až 85 % síry z nadzemních částí rostliny (Nelson 1973). Uvolnění přístupné síry z rozkládajících se zbytků závisí především na obsahu síry. Analogicky s mineralizací dusíku probíhá čistá mineralizace síry tehdy, pokud je zapravena do půdy hmota bohatá na síru, např. mladé listy řepky. Naproti tomu zapravení organické hmoty chudé na síru (např. sláma) má za následek imobilizaci síry (Mengel 1996). Eriksen (1997) v této souvislosti zjistil, že přítomnost pohotově přístupného zdroje uhlíku způsobila rychlé snižování množství anorganických síranů a redukci příjmu síry. V mnohých půdách dostupnost síry pro rostliny vysoce koreluje s obsahem půdní organické hmoty a poměr C:S v půdní organické hmotě poskytuje orientační přehled o schopnosti uvolňování síranů. Zatímco se sírany akumulují při poměru C:S < 200:1, k imobilizaci dochází při poměru C:S > 400:1 (Barrow 1960).

V inkubačních pokusech Williams (1967) zaznamenal, že aplikace $CaCO_3$ způsobila zvýšení koncentrace rozpustných síranů a mineralizace síry probíhala zcela proporcionálně až do pH 7,5. Nad tuto hodnotu koncentrace síranů rostla rychleji, což dokazuje, že chemické procesy mají dopad na mobilizaci síranů. Protože je část uvolněné síry též použita pro syntézu nových buněčných stěn půdních mikrobů, probíhá mineralizace a imobilizace současně (Maynard et al. 1983). Chapman (1997) dokázal, že v regionech s deficiencí síry může mít zapravení rostlinných zbytků chudých na obsah síry za následek omezený růst rostlin a zpomalenou dekompozici. To jen potvrzuje zjištění, že přídavek uhlíku (Saggar et al. 1981) nebo přítomnost metabolizovatelné organické hmoty (Goh et Gregg 1982, Eriksen 1997) přispívá ke zvýšení množství síranů imobilizovaných do organických forem.

4.5.2.4. Analýzy síry v půdě

K vyhodnocení obsahu síry v půdě byly vyvinuty metody zahrnující chemickou extrakci různě vázaných forem síry a stanovení síry uvolněné v inkubačních pokusech nebo působením mikrobů (Jones 1986). Koncentrace rostlinám přístupné minerální síry se během roku mění (Ghani et al. 1991) a je výsledkem změn rovnováhy mezi aktivitou mikrobiální biomasy, ztrátami vyplavením do hlubších vrstev půdy a povrchovým odtokem, hnojením, atmosférickými vstupy a příjmem plodinou. Proto se každá analytická hodnota koncentrace síranů ve vzorku půdy vztahuje v podstatě jen k té době, během které byl vzorek odebírán (Schnug a Haneklaus 1998).

Ke stanovení různých kategorií síry v půdě byla vyvinuta řada extrakčních činidel. Jejich největší skupinu představují činidla pro extrakci rostlinám přístupné síry (Shan et al. 1997). Běžně je používán vodný výluh (Walker et Doornenbal 1972, Kulhánek et al. 2016, aj.) nebo slabé roztoky solí: $CaCl_2$, NaCl, LiCl, $BaCl_2$, KH_2PO_4 (Tabatabai 1982, Saalbach et Aigner 1987, Ketterings et al. 2011). Vzhledem k problémům s disperzitou v případě extrakcí vodou se často dává přednost solným roztokům. Matula (1999) naopak zjistil, že hlavní překážkou, omezující užití roztoku $CaCl_2$ ke stanovení S v půdním roztoku je nebezpečí srážení síranů vápenatých při extrakci, což má za následek snížení koncentrace síry ve výluhu. Potvrdil naopak výborně fungující korelaci mezi obsahem síry v rostlině a jejím obsahem získaným extrakcí vodou. Link (1997) stanovoval v časném jarním období přístupnou S v různých vrstvách půdy extrakcí jednak 0,05 mol/l $CaCl_2$, jednak směsí 1 mol/l NaCl a 0,2 mol/l $CaCl_2$. Pokusné pozemky s řepkou vykazovaly 11 - 238 kg síranů/ha se střední hodnotou 68 kg/ha. Na základě výsledků určil, že hodnota 60 kg síranů/ha ve vrstvě půdy 0 - 60 cm je dostatečná k pokrytí požadavků řepky. Timmermann et al. (1995) extrahovali půdu různými extrakčními činidly (H_2O, 0,03 mol/l H_3PO_4, směsí 0,01 mol/l $CaCl_2$ a 0,02 mol/l DTPA nebo směsí $Ca(H_2PO_4)_2$ a CH_3COOH). Průkazné vztahy mezi takto stanovenou sírou v půdě a S v rostlině však nenašli. Proto publikovali domněnku, že mnohem významnější než stanovení samotné síry je její vztah s jinými prvky, zejména dusíkem.

Pro kombinovanou extrakci rozpustných a adsorbovaných síranů v půdě byly vyvinuty extrakce pomocí roztoků $CaCO_3$ (Williams et Steinbergs 1959), $NaHCO_3$ (Kilmer et Nearpass 1960) a octanu amonného (NH_4OAc) (Bardsley et Lancaster 1960) nebo roztoků obsahujících fosfor jako $Ca(H_2PO_4)_2$ (Fox et al. 1964) nebo KH_2PO_4 (Ensminger 1954). Poslední dva zmiňované roztoky obsahují dostatek fosfátových skupin pro výměnu většiny adsorbované síry. Množství adsorbovaných síranů extrahovaných $Ca(H_2PO_4)_2$ přitom může dosahovat hodnot <1 až po několik tisíc mg/kg. Vzájemná srovnání obou uvedených extrakčních činidel s obsahem fosforečnanů ukazují na jejich srovnatelný extrakční potenciál (Link 1997). Výsledky pokusů s jetelem, jílkem a pšenicí dále ukazují, že roztok $Ca(H_2PO_4)_2$ je vhodným extrakčním činidlem pro stanovení rostlinám skutečně přístupné síry (Warman et Sampson 1992), avšak je nutno podotknout, že se jednalo o nádobové pokusy za řízených podmínek. Výsledky z polních pokusů mohou vykazovat zcela jiné tendence. Např. při stanovování požadavků pastvin na síru zjistili Anderson et al. (1998) neprůkazné vztahy mezi půdními testy a odběrem S rostlinami, protože extrakční činidla buď nadhodnotila, nebo podhodnotila přístupnost sloučenin organické síry. Tito autoři došli k závěru, že výslednou schopnost půdy poskytovat síru dle potřeb plodiny je třeba dále ověřovat s přihlédnutím k procesu mineralizace a vyplavování síry. Právě mineralizovatelná organická síra by měla být chápána jako potenciální zdroj přístupné S, zejména v půdách bohatých na lehce mineralizovatelnou organickou hmotu. Proto byla navržena některá alkalicky působící činidla ($NaHCO_3$ a $CaCO_3$) extrahující i část organické síry, přičemž $NaHCO_3$ extrahuje více organické síry než $CaCO_3$ (Tabatabai 1982). Poslední dobou se ukazuje dobrý potenciál metody Mehlich 3 stanovení minerální frakce S (Kulhánek et al. 2018a, b, Zbíral et al. 2018, Ketterings et al. 2011). Ze studií Kulhánek et al. (2018a, b) vyplývá, že výsledky metody Mehlich 3 úzce korelují s obsahy minerální S v půdě a naopak korelace s podílem organické síry jsou velmi slabé. Velmi těsné korelace metody Mehlich 3 ($r > 0.85$) byly navíc zjištěny s obsahem přístupných forem síry v půdě (adsorbovaná a okludovaná).

Dříve zmiňované AEM membrány ke stanovení přístupného fosforu použili Schoenau et al. (1993) rovněž ke stanovení přístupné síry. Využili však jiný technický postup, kdy proužky membrán aplikovali přímo do půdy. Výsledky pak srovnávali s běžnou extrakcí 0,01 mol/l $CaCl_2$. Z vyhodnocení vyplývají dva základní poznatky: 1) těsné vzájemné korelace mezi oběma použitými metodami, 2) výrazně lepší vztah výsledků AEM/odběr S řepkou než $CaCl_2$/odběr S řepkou. Proto autoři AEM metodu doporučují jako jednoduchý test pro stanovení skutečně přístupné síry.

Další možností analýz je stanovení mineralizačního potenciálu půd, kterou představuje inkubace půdních vzorků za aerobních podmínek a stanovení množství síry uvolněné během pokusu. Nicméně tyto metody k vyhodnocení přístupné síry včetně její lehce mineralizovatelné formy se (na rozdíl od stanovení dusíku) nesetkaly s úspěchem. Pravděpodobnou příčinou byla absence rostlin (Tabatabai et Bremner 1972). Předpokládá se, že v přítomnosti rostlinných kořenů je mineralizace vyšší, protože organická síra z kořenových exsudátů a odumřelých částí kořenů je snadno mineralizována na sírany (Castellano et Dick 1991). Přesnost procesu stanovení mineralizace dále závisí na metodě inkubace. Množství síranů mineralizovaných za laboratorních podmínek je zpravidla nízké. V aerobním inkubačním pokusu s ornicí z Iowy zjistili Tabatabai et Bremner (1972), že během 10 týdnů bylo mineralizováno 2,8 mg S kg^{-1}, což představuje kolem 1,3% celkové půdní síry. Podle jejich výsledků množství mineralizovatelné síry nekorelovalo s obsahy celkové půdní síry, síranů, celkového dusíku ani celkového uhlíku. Na tyto výsledky navazuje i publikace Eriksen (1994) který zjistil, že za rok je mineralizováno zpravidla pouze 1,7 – 3,1 % zásoby půdní organické síry.

Nesourodost výsledků půdních analýz s obsahem S v rostlinách a jejím odběrem je zřejmá. Vlivy počasí a vymývání síranů limitují použitelnost výsledků analýz půd ke stanovení odezvy rostlin na hnojení sírou. Proto lze půdní testy ve většině případů brát jako orientační záležitost (Robson et al. 1995), a je třeba se více zaměřit na sledování vztahů síry s dalšími živinami, zejména dusíkem, a to v půdě i v rostlině (Sedlář et al. 2019, 2020).

Méně používanými analytickými metodami jsou ty zaměřené na organické složky síry. Zde existují buď tradiční metody umožňující stanovit síru pomocí redukce kyselinou jodovodíkovou (Johnson et Nishita 1952, Shan et Chen 1995, Hanousek et al. 2017) nebo novější techniky umožňující využití rentgenové absorpce v blízkosti absorpční hrany (NEXAFS nebo XANES). Pomocí této techniky lze sledovat různé frakce organické síry v neporušeném půdním vzorku. Jako první tuto metodu pro stanovení síry použili Prietzel et al.

(2003). Tito autoři rovněž potvrdili úzké vztahy XANES s C-S a se sírany stanovenými tradičními extrakčními postupy. Zatímco tradiční extrakční postupy umožňují stanovení pouze přibližné množství dané frakce síry, pomocí XANES je možné oddělit síru v různých oxidačních stupních. Solomon et al. (2005) popisují XANES jako účinný nástroj umožňující detekovat organickou síru ve všech oxidačních stupních. Nevýhodou při těchto stanoveních je však podle Prietzel et al. (2007) nutnost využití synchrotronu, což prakticky znemožňuje širší využití této metody při rutinním stanovení S.

4.5.3. Síra v rostlině

4.5.3.1. Příjem/vylučování síry rostlinami

Ačkoliv jsou vyšší rostliny schopny využít také atmosférický oxid siřičitý, nejdůležitějším zdrojem síry pro ně zůstávají sírany přijímané kořeny (Marschner 2012). Síra je vesměs rostlinou přijímána z půdního roztoku jako dvoumocný aniont SO_4^{2-}. Hlavním místem příjmu síranů je oblast kořenového vlášení (Cacco et al. 1980). Aktuálnější výzkumy potvrdily předpoklad, že síranový aniont je přijímán spolu s vodíkovým kationtem (H^+/SO_4^{2-}) (Leusteck et Saito 1999). Mobilita SO_4^{2-} je nejvyšší při pH 4,0 a klesá s jeho rostoucí hodnotou (Leggett et Epstein 1956).

V rozmezí fyziologického pH je aniont SO_4^{2-} přijímán v relativně malém množství a na velké vzdálenosti je veden převážně xylémem. V určitých ohledech je příjem S obdobný příjmu dusíku, například tím, že k zabudování síry do aminokyselin, proteinů a koenzymů je nutná její redukce a redukčním činidlem síranů je ferredoxin v zelených částech rostliny. Na rozdíl od nitrátového dusíku však může být také síran rostlinou zužitkován, aniž by byl redukován, a může být zabudován do základních organických struktur, jako jsou sulfolipidy v membránách nebo polysacharidy (Marschner 2012). Redukovaná síra může být také, na rozdíl od dusíku v rostlinách reoxidována. Při této reakci se redukovaná síra v cysteinu oxiduje na síran, který představuje „nejbezpečnější“ skladovatelnou formu síry v rostlinách (Sekiya et al. 1982).

Rostliny jsou schopny využívat i SO_2 z ovzduší, ovšem jen určitou část celkové potřeby (asi do 30 %) (Vaněk et al. 2012). Ukládání atmosférických depozic SO_2 do rostlinného pokryvu představuje významný zdroj síry pro rostliny (Baldocchi 1993). Některé studie ukazují, že oxid siřičitý vstupuje do rostliny hlavně průduchy (Taylor et Tingey 1983, Olszyk et Tingey 1985). Touto cestou může být v listech uloženo 75 – 90 % oxidu siřičitého (Garsed 1985). Příjem SO_2 převažuje v denní fázi, ale vegetační pokryv absorbuje SO_2 i v noci díky jeho vysoké rozpustnosti ve vodě. Po vstupu do průduchů je oxid siřičitý distribuován v rostlině a zabudován do různých sirných sloučenin (Marschner 2012). Vaněk et al. (2012) dále uvádějí, že zatímco při malé koncentraci SO_2 v ovzduší a při nedostatku síranů v půdě je využití SO_2 vyšší a působí příznivě, od koncentrací 1,0 - 1,5 mg SO_2/m^3 vzduchu působí již poškození rostlin. Vliv mají ovšem i další složky emisí, které se do ovzduší dostávají. Pak může být pro rostliny nebezpečná i podstatně nižší koncentrace.

Příjem sulfanu (sirovodíku, H_2S) listy také převažuje v denní fázi vegetace při otevírání a zavírání průduchů (Marschner 2012). Pro citlivé rostliny (špenát) je však sulfan toxický už při koncentracích pod 0,7 mg H_2S/m^3 (De Kok 1989). Naopak ztráty síry emisemi sulfanu a ostatních těkavých sloučenin síry z listů mohou dosáhnout značných hodnot. Předpokládá se, že se jedná o jeden způsob detoxikačního mechanismu poté, co byla rostlina vystavena vysokým koncentracím atmosférického oxidu siřičitého (Sekiya et al. 1982). Podle výsledků Grundon et Asher (1986) jsou emise těchto sloučenin síry výrazně spojené s denním rytmem, s maximem v poledne. Spektrum vylučovaných sloučenin a jejich množství záleží na druhu rostliny a během vegetační doby nabývá hodnot od cca 100 g do několika kilogramů na hektar za veetační sezónu. Rostliny pěstované v neznečistěném ovzduší za dodávání síry jen v podobě síranů do půdy rovněž uvolňují průduchy podstatná množství těkavých sloučenin síry, konkrétně až 3 kg/ha/rok (De Kok et al. 1993).

Ačkoliv sloučeniny organické síry mohou být syntetizovány už v kořenech, je síra převážně translokována do nadzemní části rostliny jako síranový aniont (De Kok et al. 1993). Transpirační tok je přitom hybnou silou translokace síranů do listů (Rennenberg et al. 1979).

4.5.3.2 Asimilace síry

Síra je v rostlinách relativně málo mobilní. Při limitovaném obsahu S v půdě tak odběr na počátku vegetace stačí pokrýt pouze produkci mladých listů. Tím je ovšem indukován deficit síry v dalších částech rostlin a jsou omezeny přírůstky (Moore 2001). Redukce síry v rostlině probíhá hlavně enzymatickou cestou. Ve vyšších rostlinách jsou enzymy redukující sírany lokalizovány hlavně v chloroplastech zelených listů. Redukce je silně závislá na intenzitě světla (Schmutz et Brunold 1984).

U vyšších rostlin a u zelených řas je prvním krokem asimilace síry aktivace síranového iontu pomocí ATP. Asimilace síry tak začíná tvorbou adenosinfosfosulfátu. Reakce je katalyzována ATP-sulfurylázou (ATP-sulfátadenyltransferázou). Tento enzym, který se nachází u mnoha druhů vyšších rostlin je zde považován za hlavní sulfotransferázu. Je regulován různými vnějšími (např. světlo) a vnitřními (např. redukované sloučeniny síry) vlivy.

Reakce je dána výměnou sulfurylové skupiny, přenášené ze síranu na ATP, za dvě fosfátové (fosforečnanové) skupiny, přičemž vzniká adenosinfosfosulfát (APS) a pyrofosfát ($P_2O_7^{4-}$). APS se může fosforylovat pomocí ATP za vzniku fosfoadenosinfosfosulfátu (PAPS). PAPS je považován za univerzální donor sulfurylovaných skupin tvorby sulfoesterů (De Kok et al. 1993). APS jako donor sulfurylované skupiny působí v následující posloupnosti: 1) APS přenáší svou sulfurylovanou skupinu na enzym za tvorby sulfurylovaného enzymu (E-SH), 2) sulfurylová skupina je přenesena na dosud neznámý nosič, na němž je vodík thiolové skupiny (·SH) substituován za sulfurylovou skupinu, čímž se vytvoří disulfidický můstek, 3) sulfurylovaná skupina nosiče je redukována elektronem z ferredoxinu, čímž se vytvoří ·SH skupina vázaná na atom síry komplexu nosiče a 4) thiolová skupina (·SH) je přenesena na acetylserin a cystein za vzniku kyseliny octové. Reakce vyžaduje dva elektrony, které poskytuje ferredoxin (Marschner 2012). Hawkesford et al. (1995) zjistili, že rostliny pěstované za naprostého nedostatku síry vykazovaly 5x vyšší aktivitu ATP-sulfurylázy a také zvýšenou aktivitu nosičů síry na plasmatické membráně. Volné sulfidové anionty (S^{2-}), katalyzované cysteinsyntázou, mohou reagovat s acetylserinem za tvorby cysteinu.

Cystein, první stabilní produkt asimilační redukce sulfátů, se chová jako prekurzor syntézy všech ostatních sloučenin obsahujících redukovanou síru (Marschner 2012). Síra tak v rostlinách představuje především nezbytnou součást aminokyselin methioninu, cysteinu a cystinu, které dále tvoří nepostradatelné bílkoviny. Nedostatek síry tak logicky omezuje i tvorbu bílkovin (MacRitchie et Gupta 1993).

4.5.3.3. Analýzy síry v rostlině

Převažuje názor, že analýzou síry v rostlinném materiálu získáme přesnější informace o výživném stavu. Proto byla navržena řada diagnostických ukazatelů, avšak zatím bez obecné shody na tom, který z nich poskytuje nejpřesnější výsledky. Proto by měly být části rostliny a časy vzorkování standardizovány z důvodu zamezení vzniku nejasností v interpretaci analytických dat (Jones 1986). Analýzy rostlin jsou oproti analýzám půdy založeny na principu, že každý esenciální prvek je přítomen v rostlině v takovém množství, které je dostatečné pro její neomezený růst (Ensminger et Freney 1966). K posouzení stavu cekového obsahu síry v rostlině je často používáno stanovení obsahu S v celé nadzemní části rostlin nebo v jejich specifických částech (např. Ryant et Skládanka 2009, Jones 1986). Obecně platí, že kritický obsah síry závisí hlavně na druhu rostliny, ale také na na odebrané části rostliny, stadiu vývoje a úrovni výnosu (Haneklaus et Schnug 1994, Hahtonen et Saarela 1995). K předpovědi výživného stavu rostliny sírou doporučují právě (Haneklaus et Schnug 1994) odebírat k analýze mladší části rostlin a to v období nejvyšších požadavků na síru, tzn. od období dlouživého růstu do začátku květu. Generativní materiál je dle (Gupta 1976) pro diagnostické účely méně vhodný. U jednoděložných je nejvhodnější praporcový list nebo celá rostlina před objevením druhého kolénka (Zorn et al. 2016), protože S rostoucím stářím rostliny klesají obsahy síry a analýzy už nejsou tak spolehlivé (Robson et al. 1995). U dvouděložných rostlin jsou k analýze doporučovány mladé, plně rozvinuté listy, ve kterých je obsah síry největší (Haneklaus et Schnug 1996).

Někteří autoři upřednostňují stanovení pouze síranové síry v rostlinách, kde byl prokázán vztah ke stavu síry v půdách u leguminóz (Jones et Martin 1964), jílku vytrvalého a řepky olejné (Maynard et al. 1983). Toto platí obecně pro všechny analýzy rostlinných tkání. Koncentrace síranů ve vakuole je považována za spolehlivý indikátor výživového stavu sírou podobně jako je tomu u fosfátů (Bollons et Barraclough 1997).

Analýzy rostlin však rovněž představují určitá úskalí. Při nedostatku síry jsou sírany z kořenů a nadzemních částí během několika dnů mobilizovány. Někdy se může dokonce zvýšit obsah síranů v dospělých listech, zatímco mladé listy trpí jejich nedostatkem (Cram 1990). Pokud odebrané vzorky obsahují více glukosinolátů, může dokonce po jejich odebrání vzrůst koncentrace síranů vlivem jejich enzymatického štěpení (Schnug 1988). Je velmi pravděpodobné, že vztah mezi sírou v rostlinách a přístupnou sírou stanovenou v půdě bude možno potvrdit jen u některých plodin. Např. Kulhánek et al. (2018a) potvrdili průkazný vztah mezi zmíněnými obsahy u pšenice ozimé a u řepky ozimé v raných fázích jarní vegetace, zatímco u kukuřice se tento vztah prokázat nepodařilo. Ukazuje se, že stanovení samotné síry může vést ke zkresleným informacím o výživném stavu porostu. Proto jsou stále více využívány i vztahy mezi sírou a dalšími prvky, zejména dusíkem. Na význam těchto vztahů ukazují i zjištění Sedlář et al. (2019, 2020), kteří se zabývali srovnáním různých indexů výživy rostlin, zahrnujících obsahy dusíku i síry u pšenice ozimé. Jako nejvýhodnější je podle těchto autorů využití indexu výživy sírou dle Reussi et al. (2012). Jeden z problémů použití poměru N:S však je, že přebytek jednoho z těchto prvků může být interpretován jako nedostatek druhého. Dalším problémem je, že síra je v rostlinách značně nepohyblivá a starší listy mívají vyšší podíl síry než mladé, zatímco u mobilního dusíku je tomu naopak (Pasricha et Fox 1993).

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6. Výsledky a diskuze k vybraným recenzovaným článkům vztaženým k tématu (publikované v angličtině; seřazené chronologicky od nejnovějších)

1) Improved phosphorus fertilisation efficiency of wood ash by fungal strains *Penicillium sp.* PK112 and *Trichoderma harzianum* OMG08 on acidic soil

Využití kmenů hub *Penicillium sp.* PK112 and *Trichoderma harzianum* OMG08 na kyselých půdách za účelem zvýšení efektivity fosforu dodaného v dřevěném popelu.

Tento článek je zaměřen na kombinaci dvou cest potenciálního řešení hrozícího nedostatku fosforu. Jedná se o společné využití houbových biostimulantů a dřevěného popela. Proto byla vybrána půda se silným nedostatkem fosforu, a to dle výživového indexu DRIS. Nedostatek P byl později potvrzen i na základě rozborů rostlin, kde byl obsah P hluboko pod běžnou hladinou uváděnou Jones et al. (1990). Ukázalo se, že samotná aplikace dřevěného popele nevadla ve srovnání s kontrolou k signifikantnímu nárůstu výnosu kukuřice ani ke zvýšení odběru fosforu. Inokulace oběma houbovými biostimulanty však vedla ke statisticky průkaznému ($p<0,05$) zvýšení výnosu nadzemní biomasy i vyššímu odběru fosforu. Naproti tomu inokulace samotnými *Penicillium sp.* a *Trichoderma harzianum* nevedla k průkazným změnám ve srovnání s kontrolou, a to jak z hlediska výnosu, tak z hlediska odběru P a obsahu přístupného P v půdě. To je v souladu s výzkumem Vinci et al. (2018), kteří v dosáhli pozitivních výsledků pouze při kombinaci *T. harzianum* OMG08 s koňským a kravským hnojem, avšak samotná aplikace uvedeného biostimulantu vedla dokonce k negativnímu efektu. Dále byl zkoumán vliv aplikace obou biostimulantů na enzymatickou aktivitu v půdě. Zde bylo u kontrolních variant i variant s popelem dosaženo srovnatelných výsledků, přičemž inokulace oběma mikroorganismy dokonce vedla ke snížení aktivity dehydrogenázy. To lze pravděpodobně vysvětlit potlačením mikrobiální aktivity v důsledku aplikace biostimulantů. To dokládají i výsledky Cunningham et Kuiack (1992) a Yang et al. (2011), kteří u *Penicillium bilaiae* a *Trichoderma harzianum* prokázali produkci metabolitů potlačujících činnost ostatních mikroorganismů. Z dosažených výsledků lze souhrnně konstatovat, že aplikace dřevěného popela spolu s biostimulanty je perspektivní cestou k mobilizaci aplikovaného P do přístupných forem. Pravděpodobným principem mobilizace P je acidifikace popela a omezení imobilizace uvolněného P mikroorganismy.

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Improved phosphorus fertilisation efficiency of wood ash by fungal strains *Penicillium* sp. PK112 and *Trichoderma harzianum* OMG08 on acidic soil

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ABSTRACT

Effective recycling of phosphorus (P) remains a critical issue in sustainable agriculture. Wood ash represents valuable soil amendment and potential source of P for agriculture, but its solubility and subsequent P-fertilisation efficiency is extremely low. This study tested fungal inoculants (*Penicillium* sp. PK112 and *Trichoderma harzianum* OMG08) applied alone and in combination with wood ash on P-limiting acidic soil to determine if they can improve P-nutrition in maize. Wood ash alone did not have any significant P-fertilising effect. Application of both inoculants, when combined with wood ash, led to significant increment of plant-available P content in soil, increased P uptake by maize plants and consequently to higher production of maize shoot biomass. Both inoculants suppressed overall microbial activity in soil as determined by the activity of dehydrogenase, alkaline phosphatase and microbial P content. Only *T. harzianum* led to higher activity of soil acid phosphatase. This study demonstrated that tested strains may be co-applied with wood ash to improve its P-fertilisation efficiency. The positive influence of inoculants on P availability was mainly due to stronger acidification of rhizosphere and decreased content of microbial P. However, both effects seemed to be hindered by the P sorption capacity of the soil in the case of inoculation without wood ash. Such findings may lead to development of novel formulations of recycled fertiliser products and boost nutrient recycling in agriculture.

1. Introduction

Effective recycling of P is one of the main challenges of sustainable agriculture. Wood ash represents an important source of P and valuable soil amendment. Wood fuel provides 40% of today's global renewable energy supply and thanks to its net carbon emissions being zero; its production is expected to grow in future. Wood ash is also one of the cheapest and most available soil amendments in developing countries (FAO, 2018). Soil application of wood ash supplies mineral nutrients, especially Ca, K, Mg and P (Ochecová et al., 2017) and increases soil pH with concomitant benefits such as element leaching reduction, mitigation of possible Mn- and Al-toxicities (Demeyer et al., 2001) or reduction of the heavy metal uptake by crops (Ochecová et al., 2014). However, the solubility, and therefore the plant-availability of wood ash-bearing P is generally very low (Demeyer et al., 2001; Pesonen et al., 2017). The predominant P-minerals present in wood ash are apatite ($Ca_5(PO_4)_3(F,Cl,OH)$) and whitlockite ($Ca_9(MgFe)(PO_4)_6PO_3OH$) (Boström et al., 2011; Vassilev et al., 2013). Due to the very low water solubility of these minerals, the P fertilisation efficiency of wood ash is very low, incomparably lower than that of soluble commercial fertilisers (Demeyer et al., 2001; Nkana et al., 1998; Ohno and Erich, 1990; Park et al., 2012). One strategy for improving the P availability from wood ash may be the application of P-solubilising microorganisms. Such approach represents environmentally friendly, easy-to-use and low-cost strategy, especially when compared with industrial leaching or thermochemical P-recovery methods used for ashes (Ohtake and Tsuneda, 2019).

Some soil fungi species are well known for their strong P-solubilisation potential and/or mobilisation of organic P in soils (Jones and Oburger, 2011; Richardson and Simpson, 2011). Their general involvement in P cycling in soils is therefore well recognised. *Penicillium* and *Trichoderma* have been studied extensively in recent decades regarding to their plant-growth promotion abilities (Harman et al., 2004; Harman, 2006; Chaudhary et al., 2018). Many successful applications were demonstrated mostly in their use for biological control of plant diseases (Howell, 2003). However, their use in terms of P-solubilisation still remains challenging. A perfect example is the work of Karamanos et al. (2010) demonstrating the positive effect of *Penicillium bilaiae* application only in 5 out of 47 field experiments. Although some recent studies (Gómez-Muñoz et al., 2017; Lekfeldt et al., 2016; Raymond

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et al., 2019) reported very limited capacity of the inoculants to influence P uptake of crops, many authors rather report strong influence of soil type and P-source on the outcome of fungal inoculants (Leggett et al., 2015; Mpanga et al., 2018; Sánchez-Esteva et al., 2016; Thonar et al., 2017). Poor understanding of the actual mechanisms involved in plant-growth promotion of P-solubilizing strains is clearly among the reasons of poor reproducibility of their applications.

The objectives of this study were (1) to test commercially available P-solubilising strains of *Penicillium* and *Trichoderma* whether they can be co-applied with wood ash for improving its P-fertilisation efficiency, and (2) to identify key mechanisms responsible for their influence on P transformations in soil-plant system.

2. Materials and methods

2.1. Soil and wood ash characteristics

Experimental soil (Haplic Cambisol) was collected from a field (plow depth, 0–20 cm) near the city of Žamberk, Czech Republic. The soils textural class was silt loam and soil had following properties: cation exchange capacity (CEC) 74.9 $\text{mmol}_+\ \text{kg}^{-1}$; pH 5.2 in 0.01 M $CaCl_2$ 1:2.5 (w/v); C_{tot} 1.9%; total P 551 mg kg^{-1}; organic P 244 mg kg^{-1}; $P_{\text{Mehlich III}}$ 22 mg kg^{-1}. For more detailed characterisation and P fractionation of the soil we refer to Mercl et al. (2018). The soil was air dried and passed through a 2 mm mesh stainless sieve prior to using. Wood ash originated from industrial power plant from Czech Republic combusting exclusively wood chips in a fluidised bed reactor (15 MWt). Tested wood ash was of following properties: CEC 125 $\text{mmol}_+\ \text{kg}^{-1}$; pH_{H2O} 11.2; C_{tot} 8%; total P 10,195 mg kg^{-1} and $P_{CH3COOH}$ 315 mg kg^{-1}. Detailed characterisation of the wood ash can be found in work by Mercl et al. (2016). Wood ash was also air dried and passed through 2 mm sieve prior to use. The ash was then thoroughly mixed with soil in the amount corresponding to addition of 24 mg P per kg of dry soil. This dose corresponded to theoretical ash application rate of 7 t/ha. Treatments without ash application (plain soil) are denoted as CON and treatments where ash was applied are denoted WA.

2.2. Tested inoculants

Two fungal inoculants were tested in this study, namely *Penicillium* sp. PK112, and *Trichoderma harzianum* OMG08. The *Penicillium* sp. PK112 was provided by Bayer Crop Science Biologics GmbH as a product Biological Fertilizer OD (BFOD) containing concentrated liquid spore culture. The *T. harzianum* (OMG) was provided as a dry spore powder by Institute of Bioanalytical Sciences at Anhalt University of Applied Sciences in Germany. Both fungi, commercially available, were selected for the study based on their potential to solubilise Ca_3PO_4 in vitro. Inoculants were dissolved (OMG) or diluted (BFOD) in tap water (free of chlorine) and applied on the soil surface after sowing and immediately before first watering. The amount of applied solution (only tap water in the control treatments) was always 20 mL and the final concentration of inoculants was 1×10^9 CFU kg^{-1} soil (dw; dry weight). Treatments where *Penicillium* was inoculated are denoted as + BFOD and treatments inoculated with *T. harzianum* are denoted as + OMG.

2.3. Experimental setup

The experiment was established as a 2 × 3 full factorial design where each treatment was replicated four times. Maize was selected as a test plant as it is the most grown cereal worldwide (FAOSTAT, 2019) with relatively high yields. Due to this fact, even small improvement in its yield may result in a significant nutritional and financial benefit. Plants of maize (*Zea mays* L. var. Colisee; KWS Saat, Germany) were grown in polyethylene pots placed in a greenhouse with natural light conditions under controlled temperature (22/18 ± 2 °C day/night). Each pot contained soil in an amount corresponding to 0.5 kg of dry soil. All pots received basal fertilisation of 100 mg N kg^{-1} in the form of NH_4NO_3 (aqueous solution) prior to sowing. Five untreated seeds were sown into each pot and plant numbers were thinned to two after germination. Immediately after sowing, pots were inoculated with tested microorganisms. During the experiment, pots were irrigated using demineralised water to reach 60% of maximum soil water holding capacity (gravimetrically monitored twice per week). Position of pots was fully randomised with three re-randomisations during the experiment. After 45 days from emergence, the above-ground biomass of maize plants was harvested, dried at 60 °C, milled to fine powder and analysed. Soil was passed through the 2 mm mesh stainless sieve in order to remove roots, and divided into two parts. One part was air dried and used for determination of pH, available P concentrations and P sorption capacity, whereas the second part was stored at 4 °C and used for determination of enzymatic activities and microbial P biomass (P_{mic}).

2.4. Analytical procedures

2.4.1. Soil enzymatic activities

The activity of dehydrogenase (DHA) (EC 1.1) was assayed following the methodology described by García-Sánchez et al. (2018). Briefly, 1 g of fresh soil was incubated with triphenyltetrazolium chloride (TTC) dissolved in 0.1 M Tris–HCl buffer (pH 7.6) for 24 h at 30 °C. After incubation, the triphenylformazan (TPF) produced was extracted with acetone in a ratio of 1:4 (extract/acetone; v/v) and measured spectrophotometrically at 490 nm. The results are expressed as µg TPF g^{-1} dry soil day^{-1}. Activities of acid (EC 3.1.3.2) and alkaline (EC 3.1.3.1) phosphatases were determined according to Eivazi and Tabatabai (1977). In brief, the sample (1 g of fresh soil) was mixed with a modified universal buffer (pH 6.5 for acid and 11 for assays of alkaline phosphatase) and incubated for 1 h at 37 °C. The p-nitrophenyl-phosphate was used as a substrate and the concentration of produced p-nitrophenol was determined spectrophotometrically at 400 nm after addition of 0.5 M NaOH and 0.5 M $CaCl_2$.

2.4.2. Determination of nutrients

In dried plant biomass, total concentrations of P, K, Mg and Ca were determined by inductive coupled plasma-optical emission spectrometry (ICP-OES; Agilent 720, Agilent Technologies Inc., Santa Clara, CA, USA) after the dry-ashing procedure according to Mader et al. (1998). Nitrogen concentration in plant biomass was determined by the Kjeldahl method using the automatic distillation system Vapodest 50 s (Gerhardt, Germany). The pH of soil samples was measured after extraction with 0.01 M $CaCl_2$ (VDLUFA Methods, 1991). The plant-available portion of inorganic P in the soil after harvesting (P_{AEM}) was assayed by a 16-h extraction of soil with demineralised water (1:60; w/v) with two anion-exchange membrane strips (AEM-PES membrane; FumaTech GmbH, Bietigheim-Bissingen, Germany). The P adsorbed by the membranes (P_{AEM}) was then extracted using 0.5 M HCl (Tiessen and Moir, 2007). This method provides more accurate information about the real amount of plant-available P in the soil compared to conventional soil-liquid extractions because only inorganic phosphate ions (Pi) are continuously taken up by the membrane from solution. The adsorbed P is not desorbed back into the solution; the membrane therefore, mimics continuous uptake of P by the plant root. The amount of microbial biomass P (P_{mic}) in the soil was determined according to the method of Brookes et al. (1982) as a difference in Pi (determined according to Murphy and Riley (1962) extractable by 0.5 M $NaHCO_3$ between fumigated ($CHCl_3$) and unfumigated samples. A conversion factor (K_p) of 0.4 was used for calculation. The P sorption capacity of the soil and soil + ash mixture was determined by shaking 1 g of air dried soil with 20 ml of 75 mg P L^{-1} solution (KH_2PO_4) for 24 h at 4 °C. The amount of sorbed P was determined by ICP-OES after centrifugation for 10 min at 4500 × g. Water soluble organic carbon (WSOC) was determined in the supernatant by the Walkley-Black method (Walkley and Black, 1934) after extraction of soil with demineralised water (1:10; w/v).

2.5. Statistics and data analyses

STATISTICA 12 (StatSoft, Inc., Tulsa, OK) was used for statistical analyses. Figures were prepared using SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA). Statistically significant differences (post-hoc Fisher's Least Significant Difference - LSD) are shown at the 95.0% confidence level.

Diagnosis and Recommendation Integrated System (DRIS) indices (Beaufils, 1973) were used to determine the most plant-growth limiting nutrient in individual treatments. For the calculation, biomass concentrations of N, P, K, Mg and Ca were used and data by Elwali et al. (1985) were used as a reference.

3. Results and discussion

3.1. Nutritional status and biomass production of maize

Phosphorus was the most growth-limiting macronutrient in all variants of the experiment. This is indicated by calculated DRIS index values (Table 1) and also by P concentrations in biomass ranging from 1.3 to 2 g kg^{-1} (data not shown) which are considered far below sufficiency range (Jones et al., 1990). The sole application of wood ash (WA) (~ 7 t ha^{-1}) did not result in higher production of maize shoot biomass or P uptake as compared to CON (Fig. 1a) indicating very low plant-availability of P in this material. This is contrary to our previous experiment (Mercl et al., 2018) where the same soil, ash and maize were used likely because of the N-fertilizer application. Similar results to the current study were recently published by Cruz-Parades et al. (2017) who observed a P-fertilising effect of wood ash only at the application rate of 22 t ha^{-1}.

Inoculation of wood ash-treated soil with *Penicillium* (WA + BFOD) as well as *T. harzianum* (WA + OMG) led to significantly ($p < 0.05$) higher shoot biomass production (Fig. 1a) and higher plant uptake of P (Fig. 1b) as compared to WA treatment. This leads to the suggestion that the better growth performance was caused by higher availability of P in soil. The results of P_{AEM} in soil after harvest confirmed this hypothesis as both treatments (WA + BFOD and WA + OMG) showed significantly higher concentrations of this readily plant-available inorganic P in soil (Fig. 2). Contrarily, the inoculation of *Penicillium* or *T. harzianum* to plain, non-treated soil (CON + BFOD; CON + OMG) did not result in any significant difference in shoot biomass production, P uptake or the concentration of P_{AEM} as compared to corresponding non-inoculated treatments (CON). Similar results have been reported by Vinci et al. (2018), who found positive effect of *T. harzianum* OMG08 on maize growth and P nutrition when combined with horse and cow manure composts on pH neutral, low P soil. Authors (Vinci et al., 2018) concomitantly reported negative impact of the strain on maize P uptake when applied on the plain, non-amended soil and suggested a possibility of competition for P between maize and *T. harzianum* OMG08 in case of very low available P environments. Such competition was not observed in our study as there was no significant difference in P uptake between CON and CON + OMG treatments.

Table 1
Nutrient DRIS index values.

Treatment	N_{index}	P_{index}	K_{index}	Mg_{index}	Ca_{index}
CON	−2,0	**−31**	−20	21	32
CON + BFOD	−10	**−27**	−13	19	31
CON + OMG	−7,9	**−23**	−9,4	16	24
WA	−6,9	**−27**	−15	24	24
WA + BFOD	−17	**−21**	−0,4	17	21
WA + OMG	−13	**−23**	−1,4	18	20

Values in bold represent the most limiting nutrient.

3.2. Microbial activity in the rhizosphere of maize

In order to elucidate the reason why maize plants exhibited positive and significant response to tested inoculants only in the case of the wood ash-treated soil, a set of microbial analyses was performed on soil samples after the harvest. Application of wood ash alone did not influence the overall activity of DHA, acid phosphatase or the concentration of WSOC in the soil (Table 2). A slight, but significant decrease in WA treatment was found in the activity of alkaline phosphatase. Phosphomonoesterases are generally known to be very sensitive to pH. In the long term, alkaline phosphatases are more present in alkaline soils (Ekenler and Tabatabai, 2003), but application of ash may result in a short-term disturbance of autochthonous microbial communities and lead to decreased activity of these enzymes as demonstrated by several works (Noyce et al., 2017; Perucci et al., 2006). Concomitantly, a significantly higher amount of P_{mic} was determined in WA compared to CON treatment. This may point to the fact that potentially plant-available P from wood ash was preferably immobilised into organic forms by the autochthonous soil microorganisms and therefore, not directly available to the plants.

The effect of *Penicillium* and *T. harzianum* inoculation on soil enzymatic activities was surprisingly the same in CON and WA treatments. In both cases, both inoculants significantly decreased the activity of DHA (Table 2). Activity of DHA represents cumulative activities of many microbial dehydrogenases involved in the multiple oxidation reactions during respiration processes, and it is considered that all determined activity is intracellular (Prosser et al., 2011). The lowered activity of these enzymes in inoculated treatments can be therefore considered as a suppression of overall microbial activity in soil as induced by two tested fungi. Some strains of *P. bilaiae* and *T. harzianum* had been reported to produce secondary metabolites with antibacterial as well as antifungal activity (Cunningham and Kuiack, 1992; Yang et al., 2011). However, it is difficult to distinguish whether the inhibition of activity was caused by production of such secondary metabolites or by competition for available carbon sources between inoculated fungi and autochthonous microbes. Results of WSOC in the soil (Table 2) likely confirms the latter hypothesis because significantly lower concentrations of WSOC were always found in inoculated compared to non-inoculated treatments. The inhibiting effect of tested inoculants on autochthonous microbes is further confirmed by significantly decreased activities of alkaline phosphatases (Table 2). As the soil alkaline phosphatases are not reported to be produced by higher plants but solely by soil microorganisms (Nannipieri et al., 2011; Spohn and Kuzyakov, 2013), the activities of this enzyme confirm the overall inhibition of microbial activity in treatments inoculated by both, *Penicillium* and *T. harzianum*. Likewise, the amount of P_{mic} in inoculated variants was lower in all cases compared to non-inoculated treatments (Table 2).

Inoculation by *T. harzianum* led to significantly higher activities of acid phosphatase in soil. The effect was the same for CON and WA treatments (Table 2). Acid phosphatases (namely phosphomonoesterases) are responsible for hydrolysis of phosphomonoesters in soil. Soil acid phosphatases may be produced by soil microorganisms as well as by plant roots (Spohn and Kuzyakov, 2013). Therefore, it is impossible to distinguish whether the higher activity in + OMG treatments is caused by direct production of this enzyme by the inoculated fungi, other autochthonous microorganisms or by maize roots. Furthermore, all commonly used assays for determination of phosphatases in soil do not distinguish between extracellular and intracellular phosphatases, but likely measure their potential maximal activity at optimal conditions. Therefore, higher rates of phosphatase activity cannot be considered as a higher rate of organic P transformations but should be interpreted a higher potential to transform organic P (Nannipieri et al., 2012). It is noteworthy that Vinci et al. (2018) successfully combined the strain (*T. harzianum* OMG08) with composts providing better P supply for maize. Taking into consideration higher

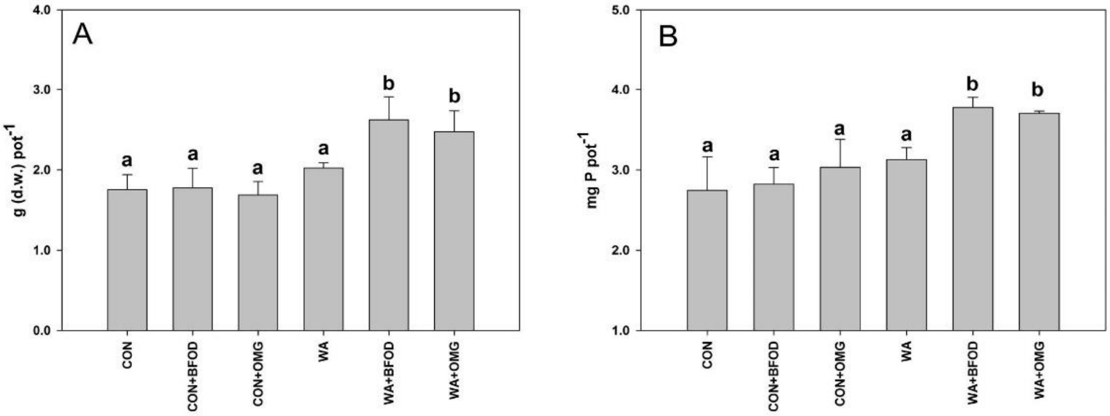

Fig. 1. Maize shoot biomass and P uptake.
Dry weight of maize shoot biomass at a harvest (A) and P content in the shoot biomass at a harvest (B); data shown represent arithmetic mean ($n = 4$); error bars indicate standard deviation; different letters above bars indicate significant differences (Fisher's LSD; $p < 0.05$) between treatments; CON control; CON + BFOD control soil inoculated with *Penicillium* sp. PK112; CON + OMG control soil inoculated with *T. harzianum* OMG08; WA control soil amended by wood ash; WA + BFOD control soil amended by wood ash and inoculated with *Penicillium* sp. PK112; WA + OMG control soil amended by wood ash and inoculated with *T. harzianum* OMG08

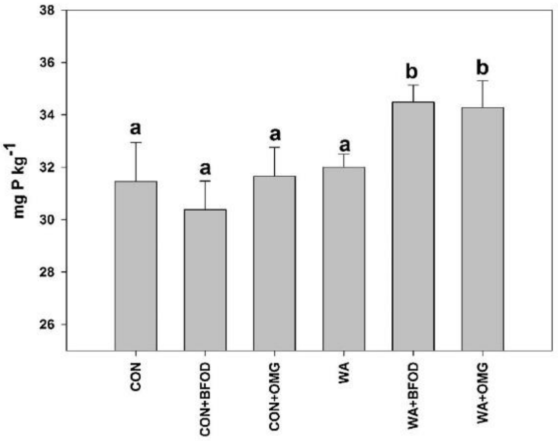

Fig. 2. Soil concentration of P_{AEM} after harvest.
Data shown represent arithmetic mean ($n = 4$); error bars indicate standard deviation; different letters above bars indicate significant differences (Fisher's LSD; $p < 0.05$) between treatments; CON control; CON + BFOD control soil inoculated with *Penicillium* sp. PK112; CON + OMG control soil inoculated with *T. harzianum* OMG08; WA control soil amended by wood ash; WA + BFOD control soil amended by wood ash and inoculated with *Penicillium* sp. PK112; WA + OMG control soil amended by wood ash and inoculated with *T. harzianum* OMG08

activities of acid phosphatase in + OMG treatments of our study, it well demonstrates a great potential of the strain for organic P mobilization.

3.3. Mobilisation of P in the maize rhizosphere

The application of wood ash alone did not influence the concentration of plant-available P in soil, but it increased the amount of soil P_{mic}. The amount of added ash-bearing P extractable in acetic acid, usually considered as exchangeable or bioaccessible, was 0.7 mg P kg^{-1} of soil, but the content of P_{mic} in the WA treatment increased by 24.2 mg P kg^{-1} of soil after application of wood ash compared to the control (Table 2). Such significant disproportion indicates that autochthonous microbes utilized, besides readily available ash-bearing P, also P of soil origin, which probably became available due to chemical changes caused by wood ash. The soil used in this experiment contained approximately 34% of total P in organic forms while more than 40% of total soil P was associated with Fe/Al minerals (whether present as precipitated Fe- and Al-phosphates, or sorbed on Fe- and Al- oxides) (Hinsinger, 2001; Mercl et al., 2018). McLaren et al. (2015) reported that the majority of organic P in soil is present as phosphomonoesters in supramolecular structures of humic substances. As the solubility of Fe/Al-phosphates increases with increasing pH (Lindsay, 1979); the same goes for humic molecules (Piccolo, 2001), only a slight increment in pH, as induced by wood ash, could mobilise a certain amount of P. This P was probably rapidly utilised by soil microbes.

Contrarily, both applied inoculants inhibited the soil autochthonous microbial communities and caused a significant decrease in amount of

Table 2
Soil physicochemical and microbial characteristics at harvest.

Treatment	pH -log c(H^+)	WSOC mg C kg^{-1} (d.w.)	Dehydrogenase U_D	Acid phosphatase U_P	Alkaline phosphatase U_P	Microbial P U_{PMIC}
CON	4.95 ± 0.07d	46.6 ± 8.54d	66.8 ± 6.22c	234 ± 21.4a	92.1 ± 1.45d	47.1 ± 4.64b
CON + BFOD	4.61 ± 0.13b	18.6 ± 4.33bc	39.1 ± 4.41b	223 ± 11.4a	89.6 ± 4.36 cd	37.2 ± 0.76a
CON + OMG	4.41 ± 0.11a	21.1 ± 7.28c	25.7 ± 3.94a	331 ± 36.4b	76.5 ± 1.14b	43.9 ± 2.18ab
WA	5.16 ± 0.05e	48.7 ± 4.54d	77.5 ± 4.55c	221 ± 15.3a	85.4 ± 1.91c	71.3 ± 6.64d
WA + BFOD	4.74 ± 0.10c	8.66 ± 4.37a	45.9 ± 3.14b	251 ± 10.1a	74.2 ± 2.98b	47.3 ± 7.95b
WA + OMG	4.83 ± 0.03 cd	9.92 ± 3.35ab	43.7 ± 7.09b	317 ± 22.7b	66.0 ± 0.75a	55.7 ± 5.86c

Data shown represent arithmetic mean ($n = 4$) ± standard deviation; different letters indicate significant differences (Fisher's LSD; $p < 0.05$) between treatments. Units: U_D µg TPF g^{-1} (dw) day^{-1}, U_P µg pNP g^{-1}(dw) h^{-1} and U_{PMIC} mg P_{mic} kg^{-1} (dw).

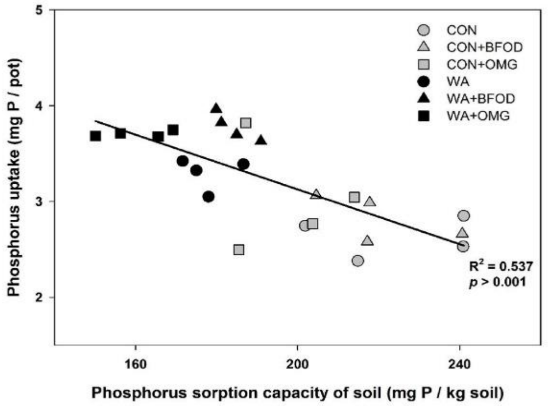

Fig. 3. Regression of P uptake by P sorption capacity of soil at the harvest. CON control; CON + BFOD control soil inoculated with *Penicillium* sp. PK112; CON + OMG control soil inoculated with *T. harzianum* OMG08; WA control soil amended by wood ash; WA + BFOD control soil amended by wood ash and inoculated with *Penicillium* sp. PK112; WA + OMG control soil amended by wood ash and inoculated with *T. harzianum* OMG08

P_{mic}. This effect was same for plain soil as well as ash-treated treatments. One would expect that such a decrease in P_{mic} will lead to a higher amount of plant-available P; at least in treatments with a higher potential of phosphatase activity because the phosphatases catalyse the hydrolysis of phosphomonoesters leading to the release of orthophosphate. However, the decreased content of P_{mic} in plain soil treatments (CON + OMG; CON + BFOD) led neither to increased P_{AEM} nor to higher P uptake. Interestingly, even in CON + OMG treatment with significantly higher activity of acid phosphatase (Table 2), there was no detectable increment in plant-available P (Fig. 2). Such a result can be explained either that P released from microbial cells was rapidly sorbed and immobilised by soil particles or that the organic P (mainly nucleic acids and phospholipids) was not mineralised. However, these sources of P are usually mineralised within a few days (Spohn and Widdig, 2017; Van Veen et al., 1987) so the preferential sorption of released orthophosphate is more likely.

Both inoculants caused significant acidification of the maize rhizosphere (Table 2). Acidification, as caused by a production of low-molecular-mass organic anions and/or protons, has been frequently reported to be an adaptive trait for many *Trichoderma* and *Penicillium* species (Pelagio-Flores et al., 2017; Li et al., 2016; Watkinson, 2016). In the case of inoculated wood ash treatments (WA + OMG; WA + BFOD), it is difficult to distinguish the origin of extra P taken up by maize plants compared to the WA variant because two main possible modes of action were detectable in these treatments, namely a decrease in P_{mic} and acidification (Table 2). Even though the wood ash-treated variants contained 0.7 mg kg^{-1} more of exchangeable P (extractable in 0.11 M CH$_3$COOH supplied by wood ash) compared to plain soil treatments, sole acidification caused by inoculants cannot account for all of the excess P_{AEM} and excess P taken up by plants (sum of differences over WA variant in P_{AEM} and P uptake were 3.46 and 3.12 mg kg^{-1} in WA + BFOD and WA + OMG, respectively). Similarly, the decrease in P_{mic} solely is insufficient to explain why the overall equilibrium of the soil-plant system shifted to more plant-favourable conditions because the same trend was observable in inoculated treatments without wood ash. As the desorption rates of P in soil are much slower than the sorption rates (Menezes-Blackburn et al., 2016), it is possible that the orthophosphate solubilised and/or mobilized from P_{mic} by inoculants becomes rapidly adsorbed back to soil particles instead of being taken up by plants. Gómez-Muñoz et al. (2018) reported that the effect of *Penicillium bilaiae* is mediated by the level of available P in soil. Such phenomenon may be caused whether by plants demand for P depending on its nutritional status but also by the fact that P solubilised by *Penicillium* cannot be taken up by plant because of parallel P fixation. The effect of P sorption capacity of soil on the outcome of phosphate-solubilising inoculants is partly confirmed by recent study by Mpanga et al. (2019) who found positive effect of inoculants on P nutrition of maize on calcareous, high P sorption capacity soil only after induced rhizosphere acidification by using stabilized ammonium as a source of N. Authors reported no effect of the inoculants when rhizosphere acidification was not induced. The linear regression analysis of our data revealed a significant effect ($r = -0.733$; $p < 0.001$) of P sorption capacity of soil on P uptake (Fig. 3). Wood ash-treated variants had clearly lower P sorption capacity and it is worthy to note that WA treatment (where no change in P uptake or P_{AEM} content was found) also showed lower P sorption capacity than other plain soil variants. The decrease in P sorption capacity of soil as induced by wood ash could be caused by many chemical factors. Surely pH and level of P played a role (Troeh and Thompson, 2005) but more importantly, the content of sulfates (Violante et al., 2002), carbonates (Rahnemaie et al., 2007) and other anions (Guppy et al., 2005) competing with orthophosphate for sorption sites probably caused the decrease in sorption capacity. Although other mechanisms (e.g. effect of arbuscular mycorrhizal fungi) cannot be excluded, we hypothesize that improved P uptake in WA + OMG and WA + BFOD treatments was caused by three mutual effects: acidification with subsequent release of acid-soluble P, and mineralisation of P_{mic}, both accompanied by lowered P sorption capacity of soil. This property of soil is often ignored but its management requires more attention as it may at least partly explain often reported soil-type dependent effects of applied phosphate-solubilising microorganisms.

4. Conclusions

Wood ash is a valuable soil amendment, however, it has extremely low or no direct P-fertilisation effect when applied in agronomically relevant amounts. A combination of wood ash with *Penicillium* sp. PK112 and *Trichoderma harzianum* OMG08 significantly improved P nutrition, and subsequently, the production of maize shoot biomass on acidic soil compared to application of wood ash alone. The tested inoculants therefore represent a promising and cost-effective way to improve the P-fertilisation efficiency of wood ash. Their modes of action were mainly soil acidification and reduction of microbial P content in soil. Our results further suggest that the P sorption capacity of soil may be an integral factor influencing the performance of P-solubilising inoculants.

Declaration of Competing Interest

None.

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2) Basic soil chemical properties after 15 years in long-term tillage and crop rotation experiment

Základní chemické vlastnosti půdy v dlouhodobém (15 let) pokusu s různým zpracováním půdy a osevními postupy

Publikace je zaměřena na vyhodnocení základních půdních vlastností v dlouhodobém pokusu zaměřeném zpracování půdy a rotaci plodin. Tento pokus probíhal na černozemi v Rakousku. Byly srovnávány 4 varianty zpracování půdy: 1) běžná orba, 2) bezorebný systém, 3) hluboké zpracování půdy a 4) mělké zpracování půdy. Sledovanými parametry byly: aktivní a výměnné pH, elektrická vodivost, kationtová výměnná kapacita, celkový obsah dusíku a uhlíku a obsah organického C, vše v 8 různých vrstvách půdy až do 45 cm. Tento článek je základem pro další výzkum v rámci tohoto pokusu. V návaznosti je připravována studie vyhodnocující obsah makroprvků (včetně fosforu a síry). Z výsledků vyplývá, že zatímco obě hodnoty pH a celkový obsah uhlíku stoupaly s hloubkou půdy, vodivost, kationtová výměnná kapacita, celkový N a obsah organického uhlíku s hloubkou půdy klesaly. Zvýšení pH lze přičítat vyššímu množství $CaCO_3$ působícího jako pufr v hlubších vrstvách půdy (Neugschwandtner et al. 2014, Blume et al. 2019). Rozdíly mezi zpracováním půdy se projevily zejména v nejsvrchnější vrstvě půdy, tj. 0-5 cm. Zde byly u běžné orby zaznamenány nižší hodnoty vodivosti ve srovnání s ostatními variantami zpracování půdy. Bezorebný systém vykazoval vyšší hodnoty celkového dusíku a kationtové výměnné kapacity ve srovnání s běžnou orbou. Celkové obsahy uhlíku ve vrstvě 0-5 cm byly vyšší u bezorebného systému a hlubokého zpracování půdy ve srovnání s běžnou orbou. Půdy obhospodařované orbou vykazovaly průkazně nižší hodnoty elektrické vodivosti ve vrstvě 5-10 cm, a to ve srovnání s bezorebným systémem. Průkazný vliv zpracování půdy na sledované parametry nebyl naopak zjištěn u hlubších vrstev půdy. Zpracování půdy dále nemělo signifikantní vliv na obě hodnoty pH, což je v souladu s výzkumem (Aase et Pikul 1995, Limousin et Tessier 2007). Rovněž rotace plodin ve sledovaném období pravděpodobně nehrála významnou roli ve změnách sledovaných hodnot.

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Basic soil chemical properties after 15 years in a long-term tillage and crop rotation experiment**

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Abstract. Basic soil chemical properties were assessed in a long-term tillage and crop rotation experiment 15 years after its establishment on a Chernozem in Raasdorf (Austria) with four tillage treatments – mouldboard ploughing, no-till, deep conservation tillage and shallow conservation tillage – and two crop rotations. The following parameters were assessed: pH_{CaCl_2}, pH_{H_2O}, electrical conductivity, cation exchange capacity, total nitrogen, total organic carbon and total carbon. Among which, pH_{CaCl_2}, pH_{H_2O}, and total carbon increased with soil depth while electrical conductivity, cation exchange capacity, total nitrogen, and total organic carbon decreased with soil depth. The differences between tillage treatments occurred after 15 years in the upper soil layer from 0-5 cm with higher values of electrical conductivity under no-till, deep conservation tillage and shallow conservation tillage than with mouldboard ploughing, higher values of cation exchange capacity and total nitrogen for no-till than for mouldboard ploughing (with deep conservation tillage and shallow conservation tillage showing intermediate values) and more total organic carbon for no-till and deep conservation tillage than for mouldboard ploughing. At a 5-10 cm depth, electrical conductivity was higher for no-till than for mouldboard ploughing. Values of pH_{CaCl_2} and pH_{H_2O} did not differ between tillage treatments in any soil layer. In deeper soil layers, tillage did not affect the analysed parameters. Crop rotation did not affect any of the analysed soil chemical properties.

Keywords: tillage, soil depth, long-term experiment, soil properties, no-till

INTRODUCTION

Tillage systems are generally categorized in terms of conventional tillage using a mouldboard plough to turn over the soil, conservation tillage using a chisel plow, disk plow, harrow disk or cultivators, and no-till where seeds are sown directly into the untilled soil. In conventional tillage, all crop residues are incorporated while in conservation tillage or no-till these residues remain partly or completely on the soil surface.

From a global perspective, the conventional tillage system is increasingly being superseded by the reduced tillage approaches. Whereas the plough is still dominant in Europe, conservation tillage and no-till are broadly applied in North and South America and in Australia (Derpsch *et al.*, 2010). In a review of crop yields from several European countries, yields from no-till were within the 5% range of those obtained by mouldboard ploughing (Soane *et al.*, 2012). Under Pannonian climate conditions on a Chernozem in Eastern Austria, winter wheat yields were generally at similar levels for conventional, conservation and no-till with no-till resulting in higher yields in very dry years and conventional and conservation tillage resulting in higher yields with higher amounts of rainfall during the vegetation period (Neugschwandtner *et al.*, 2015a). Moitzi *et al.* (2019) reported for this experiment, that the direct energy input in no-till and shallow conservation tillage during crop cultivation was considerably lower than in mouldboard ploughing.

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This may result in an increase in the energy efficiency of these tillage systems, especially during dry years, when ploughless tillage systems tended to respond with competitive yields (Neugschwandtner *et al.*, 2015a).

The economic benefits of no-till include a reduction in fuel consumption and working time; both could be reduced by establishing wheat with no-till on a silty loam Chernozem as opposed to conventional establishment (*i.e.* using a heavy cultivator for stubble cultivation and a mouldboard plough and subsequent seeding, using a power harrow and a seeding machine) by about 85% (Moitzi *et al.*, 2013; Szalay *et al.*, 2015). The ecological benefits of no-till include an increase in biotic activity, especially earthworms, and of soil organic carbon, less soil erosion and lower carbon emissions (due to less fuel consumption) (Derpsch *et al.*, 2010).

Tillage operations alter nutrient dynamics on a short-term scale through changes in the physical properties of the soil and the incorporation of crop residues and mineral or organic fertilizers. These effects accumulate in the long-term and an additional system effect builds up (Pekrun *et al.* 2003). Soil physical characteristics like soil aggregate stability, bulk density, pore volume and pore size distribution, infiltration and penetration resistance (Liebhard, 1993b; Liebhard, 1994; Liebhard *et al.*, 1994; Liebhard *et al.*, 1995) and soil chemical characteristics such as soil pH, soil organic matter and soil nutrients (Liebhard, 1993a; Neugschwandtner *et al.*, 2014) are influenced by these long-term changes.

The aim of this study was to assess the influence of four different soil tillage systems and two crop rotations on basic soil chemical parameters in soil layers fifteen years after establishing a static field trial on a Chernozem in eastern Austria. Several soil characteristics which were assessed in 2003, seven years after the start of the experiment, have been reported earlier by Neugschwandtner *et al.* (2014).

MATERIALS AND METHODS

The long-term experiment was carried out in Raasdorf (48° 14' N, 16° 33' E; altitude: 153 m a.s.l) in eastern Austria on the experimental farm of BOKU University. Raasdorf is located close to the east of Vienna, Austria, on the edge of the Marchfeld plain, an important crop production region in the north-western part of the Pannonian Basin. The silty loam soil is classified as a Chernozem of alluvial origin and is rich in calcareous sediments. The mean annual temperature is 10.7°C and the mean annual precipitation is 543 mm (1983-2012).

The long-term experiment, which was established in 1996, is set up with a split-plot design using four replication blocks and involves two factors: the tillage system is assigned to the main plots (24 × 40 m) and crop rotation to the subplots (12 × 40 m). Fertilization is performed on a crop specific basis according to good agricultural practice, *e.g.* winter wheat was fertilized with calcium ammonium nitrate (27% N) at a rate of 130 kg N ha^{-1}.

The tillage variants include: (1) Mouldboard ploughing (MP) after harvest to a soil depth of 25-30 cm. The loosened soil is turned over and therefore residues are fully incorporated into the soil. (2) No-till (NT): Direct drilling in un-tilled soil with a disc drill without the previous removal of residues. A total herbicide (glyphosate) is applied before sowing for weed control. (3) Deep conservation tillage (CTd) to a soil depth of 20-25 cm is performed using a wing share cultivator and every four years a subsoiler is used to a depth of 35 cm. A part of the plant residues remain on the soil surface. (4) Shallow conservation tillage (CTs) to a soil depth of 8-10 cm using a wing share cultivator. A high share of the plant residues remain on the soil surface.

Two flexible crop rotations are performed on subplots with sugar beet (rotation A; grown in four years) or maize (rotation B; grown in four years) as central crops. Both rotations frequently included winter wheat (in a total of seven years), as rotation effects on this crop shall be tested within this long-term experiment. Rotation A had a further two years of maize and in one year each, sunflower and spring durum wheat. Rotation B had a further two years of oilseed rape and one year each of sugar beet and soybean. Both rotations had in the four years (2008-2011) before sampling, the same crops but in a different time course: Rotation A – maize, winter wheat, sugar beet and winter wheat; Rotation B – winter wheat, maize, winter wheat and sugar beet. Crop residues were left on the field.

Soil sampling was performed with soil probes (Purckhauer type, core diameter: 30 mm) in 5 cm steps at depths ranging from 0-30 and in 10 cm steps at depths ranging from 30-50 cm from the 7th to 9th of November, 2011. A mixed sample was composed per plot for each sampled layer consisting of 30 equally sized subsamples randomly collected from the individual plots. The samples were air-dried, homogenized and sieved (2 mm). The crops grown before sampling were harvested in July (rotation A: winter wheat) and in October (rotation B: sugar beet).

The soil pH was determined in a distilled water extract (pH$_{H_2O}$; 50 ml, w/v = 1:10) after 1 h of extraction and in a 0.01 mol l^{-1} calcium chloride solution (pH$_{CaCl_2}$; CaCl$_2$; w/v = 1:2.5) after 2 h of extraction (ÖNORM L 1083, 1999) using a Multi 3420 Multiparameter Meter (WTW GmbH, Weilheim, Germany). Electrical conductivity (EC) was measured simultaneously with pH$_{H_2O}$. The cation exchange capacity (CEC) was determined from the sum of Ca, Mg, K, Na and Al after extraction with 0.1 mol l^{-1} BaCl$_2$ (w/v = 1:10) for 2 h (ISO, 1994). Inductively coupled plasma-atomic emission spectrometry (ICP-OES) using an Agilent 720 (Agilent Technologies Inc., USA) was applied for element determination in the extracts. The total organic carbon (TOC) content was determined spectrophotometrically after the oxidation of organic matter by H_2SO_4 and $K_2Cr_2O_7$ (Sims and Haby, 1971). The amount of total C and total N was determined from about 50 mg of soil by the modified Dumas combustion method at 960°C with a CNS elemental analyser (vario MACRO cube CNS; Elementar Analysensysteme GmbH, Langenselbold, Germany).

Statistical analyses were performed using software SAS version 9.2. An analysis of variance (PROC MIXED) was performed and the means were separated by the least significant differences (LSD), while the F-test indicated the factorial effects at the significance level of $p<0.05$. Based on analysis of variance results, the data are presented as the main effects of depth or as tillage × depth interactions. As no significant differences were observed for crop rotation, means are indicated for each rotation (overall depths and tillage treatments). The Pearson correlation coefficient for analysed parameters was calculated for the depth of 0-5, 5-30, and 30-50 cm.

RESULTS AND DISCUSSION

The experimental site is characterized by a calcareous soil with an alkaline reaction. Values of pH_{CaCl_2} were lower than those of pH_{H_2O} as soil extraction with $CaCl_2$ causes a higher release of hydrogen ions into the solution compared to a soil extraction with H_2O (Gavriloaiei, 2012).

Both pH_{CaCl_2} and pH_{H_2O} decreased with soil depth with pH_{CaCl_2} (Fig. 1A) showing a more homogenous distribution up to a 25 cm depth while pH_{H_2O} gradually decreased with every sampled layer (except for similar values between a 10-15 and 15-20 cm depth). The vertical gradient of pH_{CaCl_2} has already been reported for this experiment after seven years (Neugschwandtner *et al.*, 2014). Similarly to pH, an increase in calcium carbonate ($CaCO_3$) was found in this experiment seven years after its initiation (Neugschwandtner *et al.*, 2014), as $CaCO_3$ buffers the soil pH (Blume *et al.*, 2019).

There were no differences in pH between tillage treatments. This finding is consistent with those of other long-term tillage experiments; *e.g.* on a sandy loam soil in the Northern Great Plains after 12 years (Aase and Pikul, 1995), under temperate conditions on a Luvisol in France after 32 years (Limousin and Tessier, 2007), and in a semi-arid, subtropical environment on a Luvisol in Australia after 9 years (Thomas *et al.*, 2007). A higher acidity with no-till in the upper soil layers was reported from other studies which attributed this effect to acidification processes

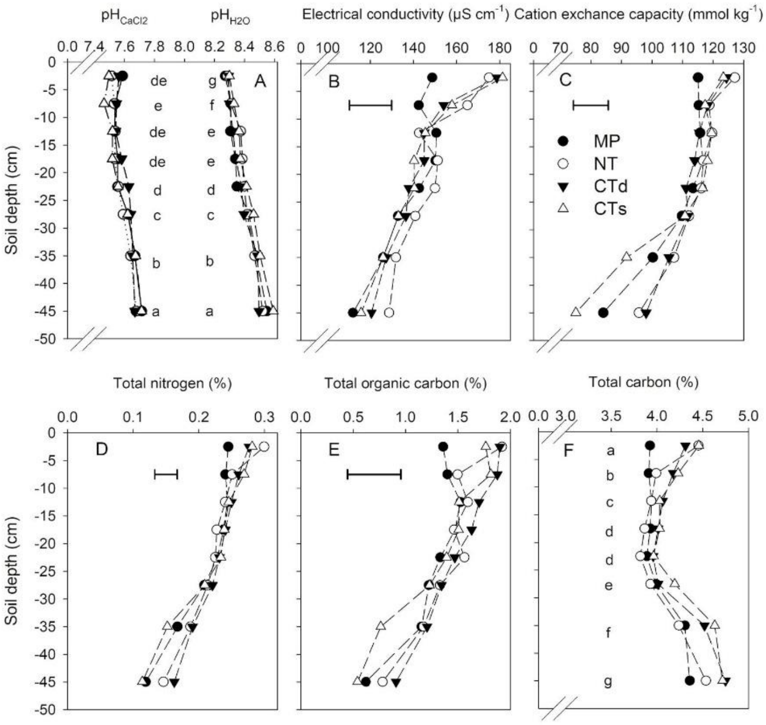

Fig. 1. Basic soil characteristics from 0-50 cm after 15 years of different tillage treatments in Raasdorf (Austria): mouldboard ploughing (MP), no-till, deep conservation tillage (CTd) and shallow conservation tillage (CTs). Different letters indicate significant differences between the soil layers ($p<0.05$). Horizontal bars indicate a significant tillage × depth interaction (LSD, $p<0.05$).

such as the mineralization of organic matter and the nitrification of surface-applied N fertilizer (Franzluebbers and Hons, 1996; Limousin and Tessier, 2007; López-Fando and Pardo, 2009). However, the effects of no-till on pH were reported to occur in a more pronounced way at an early stage; whereas no significant differences between no-till fields run for more than 6 years and conventionally tilled fields were observed (Crozier *et al.*, 1999).

Crop rotation did not affect pH$_{H_2O}$ (A: 8.38, B: 8.40) and pH$_{CaCl_2}$ (A: 7.58, B: 7.59). After 7 years, however, a lower pH$_{CaCl_2}$ had been observed after oilseed rape compared to after maize (Neugschwandtner *et al.*, 2014). The effects of crop rotation can occur, as soil pH is a soil property with short-term changes, *e.g.* during the decomposition of crop residues (Hulugalle and Weaver, 2005), due to rhizosphere processes (Kotková *et al.*, 2008) and with different fertilizer systems (Vašák *et al.*, 2015). There were probably no differences, as the same crops but in a different time course have been grown in both rotations in the last four years.

There was a significant tillage × depth interaction for the electrical conductivity (EC) (Fig. 1B). The EC was lower with MP than with NT, CTd and CT_S at a 0-5 cm depth and with MP compared to NT (with CTd and CTs showing intermediate values) at a 5-10 cm depth. At a 0-5 cm depth, the EC of NT was with 175 µS cm^{-1} 1.18-fold higher compared to MP with 148 µS cm^{-1}. Below a 10 cm soil depth, the EC did not differ among tillage treatments. A higher EC with NT compared to MP was also found by Martínez *et al.* (2013) to a depth of 2 cm with no differences below 2 cm, by Rahman *et al.* (2008) at a 0-10 cm depth and by Perez-Brandán *et al.* (2012) for NT compared to MP and CT at a 0-20 cm soil depth. Higher organic matter contents in NT caused an increase in electrolyte concentrations (Rahman *et al.*, 2008). On the other hand, Chatterjee and Lal (2009) reported diverse results for EC with no differences between NT and MP on three sites and higher values of EC on one side for either NT or MP. A lower EC with NT up to a 1.2 m depth has been described by Dalal (1989), this was attributed to an increased water movement and a higher soil aggregation with NT. Crop rotation did not affect EC (A: 145 µS cm^{-1}, B: 140 µS cm^{-1}). The EC in all treatments was well below the values causing negative yield responses (Steppuhn *et al.*, 2005).

There was a significant tillage × depth interaction for the cation exchange capacity (CEC) (Fig. 1C). In the uppermost soil layer from 0-5 cm, the CEC was ranked as follows: NT ≥ CTd, CTs ≥ MP. At a 0-5 cm depth, the CEC of NT was with 127 mmol kg^{-1} 1.10-fold higher compared to MP with 115 mmol kg^{-1}. The CEC decreased from the first to the second layer (5-10 cm) in the treatments NT, CTd and CTs, thereby reaching similar values as MP. Further down, there was a slight decline of CEC to a depth of 30 cm nd a more pronounced decline up to 50 cm. CEC provides a rough index of the shrink-swell potential and is a measure of soil structural resilience to tillage (Triantafilis *et al.*, 2009). An increase in CEC with no-till was already reported by Lal *et al.* (1990) for coarsely textured soils. The CEC is strongly influenced by soil organic matter due to its high degree of negative charges and therefore its ability to absorb cations (Scheffer *et al.*, 2010). CEC decreases when soil organic matter depletes (Curtin *et al.*, 2015). Crop rotation did not affect CEC (A: 110 mmol kg^{-1}, B: 112 mmol kg^{-1}).

Total N (N$_t$) showed an interaction between tillage × depth. In the uppermost soil layer from 0-5 cm, the highest N$_t$ contents were observed with NT and the lowest in MP (with CTs and CTd showing intermediate values). At a 0-5 cm depth, the N$_t$ of NT was with 0.2449% 1.22-fold higher compared to MP with 0.2995%. Downward up to a 30 cm depth, there are no differences in the N$_t$ content between tillage treatments (Fig. 1D). After seven years, the N$_t$ had been higher in this experiment at 0-10 cm for NT, CTd and CTs than MP with no differences between tillage treatments below a 10 cm depth (Neugschwandtner *et al.*, 2014). A higher N$_t$ with NT in the uppermost soil layer is attributed to the surface accumulation of residues whereas intensive soil mixing dilutes the concentration and accelerates the mineralization of organic matter (Salinas-García *et al.*, 2002; Spiegel *et al.*, 2007; Hou *et al.*, 2012). Furthermore, the soil nitrogen cycle is influenced through tillage operations with a slower oxidation rate of ammonium into nitrate in no-till soil (Laine *et al.*, 2018).

Crop rotation did not affect N$_t$ (A: 0.22%, B: 0.22%) as already reported for this experiment after seven years (Neugschwandtner *et al.*, 2014). However, differences in N$_t$ between crop rotations could have been expected, as the amount of N remaining on the field with crop residues and as soil mineral nitrogen after harvest differ significantly between crops (Neugschwandtner and Kaul, 2015; Neugschwandtner *et al.*, 2015c, d).

A higher total organic carbon content (TOC) was observed with NT and CTd than with MP in the upmost 5 cm (with CTs showing intermediate values) (Fig. 1E). Between 5-10 cm, CTd and CTs had higher TOC contents than NT and MP (not significant). The TOC of NT was compared to MP 1.41-fold higher at a 0-5 cm depth (1.92% versus 1.36%) and 1.07-fold higher at a 5-10 cm depth (1.50% versus 1.40%). A sharp decline of TOC was observed for NT (by 22%) from the first to the second soil layer and for CTs (by 16%) from the second to the third soil layer. In contrast, CTd and especially MP showed a more homogenous TOC distribution in the upper soil layers to a depth of 25 cm. From 25-50 cm, a pronounced decline of the TOC content was observed for all tillage treatments. Limousin and Tessier (2007) and Thomas *et al.* (2007) have also reported an accumulation of TOC with NT in the top soil layer and then a rapid vertical decline. Spiegel *et al.* (2007) reported a 31% higher concentration of organic carbon between 0-10 cm with minimum tillage compared to conventional tillage after 19 years on a similar Chernozem in Austria. MP showed a lower but more homogenous distribution in TOC content, which is attributed to intensive soil mixing and accelerated organic matter mineralization (Salinas-García *et al.*, 2002; Spiegel *et al.*, 2007).

Crop rotation did not affect TOC (A: 1.38%, B: 1.37%). A similar observation was made in this experiment after seven years (Neugschwandtner *et al.*, 2014). However, different crops in rotation show differences in the amount and composition of residues (Neugschwandtner and Kaul, 2016a, 2016b; Neugschwandtner *et al.*, 2015b), which might provoke differences between rotations in TOC. As stated previously, the same crops, but in a different time course have been grown in both rotations over the last four years.

Total carbon (TC) in the soil decreased with soil depth (Fig. 1F). Tillage treatments did not affect the TC. Although in the first 0-5 cm depth, the TC of NT, CTd and CTs tended to be higher compared to MP (tillage × depth – p=0.10, not significant). TC in the soil includes TOC and total inorganic carbon (TIC), which is primarily calcium (and magnesium) carbonate (Guo *et al.*, 2016). Calcium carbonate ($CaCO_3$) was assessed in this experiment seven years after its initiation with no differences in $CaCO_3$ between tillage treatments (Neugschwandtner *et al.*, 2014). The differences in TC among tillage treatments after 15 years reflect those of TOC, so obviously no substantial changes in TIC have occurred. No tillage effect on $CaCO_3$ was also reported by Fernández-Ugalde *et al.* (2009), but Moreno *et al.* (2006) observed that the natural leaching loss of $CaCO_3$ was notably reduced by conservation tillage due to the greater retention of water in the soil profile. Crop rotation did not affect TC (A: 4.15%, B: 4.17%).

Correlations were calculated for the analysed parameters at a 0-5 cm depth, a 5-30 cm depth (up to the end of the plow layer) and at a 30-50 cm depth (subsoil) (Table 1).

In the 0-5 cm soil layer, the following parameters did not correlate with each other: pH_{CaCl_2} with pH_{H_2O} and EC, pH_{H_2O} with N_t, pH_{CaCl_2} and pH_{H2O} with TOC, and TC with pH_{CaCl_2}, pH_{H_2O} and N_t. However, there was a significant negative correlation of pH_{CaCl_2} with CEC and N_t, and of pH_{H_2O} with EC and CEC, as well as a positive correlation among EC, CEC, N_t and TOC, and of TC with EC, N_t and TOC. Our findings at a 0-5 cm depth are partly in agreement with results from the 0-10 cm soil depth of tillage experiments performed on a Aquultic Hapludalfs (Shukla *et al.*, 2006), a Luvisol (Thomas *et al.*, 2007) and an Andisol (Rahman *et al.*, 2008), in which there was also a negative correlation of pH_{H_2O} with EC, a positive correlation among N, EC, and TOC but a negative correlation of pH_{H_2O} with N_t and with TOC.

At a 5-30 cm and 30-50 cm depth, pH_{CaCl_2} and pH_{H_2O} correlated significantly, but showed a negative correlation with EC, CEC, N_t and TOC. Furthermore, EC, CEC, N_t and TOC were positively correlated with each other. At a 5-30 cm depth, TC was positively correlated with pH_{CaCl_2} and pH_{H_2O} and negatively with CEC. No correlation was found for TC at a 5-30 cm depth with EC, N_t and TOC and also with all

Table 1. Pearson correlation coefficient for analyzed parameters

	pH_{H_2O}	EC	CEC	N_t	TOC	TC
			0-5 cm			
pH_{CaCl_2}	0.30	-0.19	-0.56***	-0.43*	-0.23	0.13
pH_{H_2O}		-0.52**	-0.35*	-0.32	-0.19	0.15
EC			0.39*	0.53**	0.46**	0.63***
CEC				0.73***	0.41*	0.17
N_t					0.54**	0.50**
TOC						0.51**
			5-30 cm			
pH_{CaCl_2}	0.46***	-0.31***	-0.55***	-0.50***	-0.42***	0.26***
pH_{H_2O}		-0.69***	-0.41***	-0.60***	-0.26***	0.23**
EC			0.37***	0.49***	0.16*	0.01
CEC				0.65***	0.30***	-0.24**
N_t					0.54***	0.03
TOC						0.05
			30-50 cm			
pH_{CaCl_2}	0.73***	-0.68***	-0.64***	-0.47***	-0.43***	-0.05
pH_{H_2O}		-0.77***	-0.76***	-0.66***	-0.53***	-0.01
EC			0.73***	0.65***	0.51***	0.18
CEC				0.92***	0.70***	-0.05
N_t					0.78***	-0.18
TOC						-0.18

Significant values at: *$p<0.05$, **$p<0.01$ and ***$p<0.001$, (n=32 for 0-5 cm, n=160 for 5-30 cm, n=64 for 30-50 cm).

of the other parameters in the subsoil. Obviously, soil depth had a strong influence over the correlation between the analysed parameters.

CONCLUSIONS

1. Compared to mouldboard ploughing, no-till increased the electrical conductivity, cation exchange capacity, total nitrogen and total organic carbon in the uppermost soil layer (0-5 cm); whereas, pH_{CaCl_2} and pH_{H_2O} were not affected by tillage. In the uppermost soil layer, a positive correlation between electrical conductivity, cation exchange capacity, total nitrogen and total organic carbon was observed.

2. In the 5-10 cm soil depth, electrical conductivity was the only parameter significantly affected by tillage (with higher values for no-till than for mouldboard ploughing).

3. In deeper soil layers, tillage did not affect basic soil characteristics. Different crop rotations had no effect on the analysed basic soil chemical properties after 15 years.

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3) Changes of soil bioavailable phosphorus content in long-term field fertilizing experiment

Změny obsahu přístupného fosforu v půdě v dlouhodobém polním pokusu

Přiložená publikace je studií zaměřenou na vyhodnocení změn přístupného fosforu v dlouhodobých přesných polních pokusech s různými půdně-klimatickými podmínkami. Je zde zahrnuta kontrolní (nehnojená varianta), 2 varianty hnojené čistírenskými kaly, varianta hnojená hnojem, dále pak minerální dusík se slámou, samotný minerální dusík a minerální NPK. Všechny varianty jsou hodnoceny po 18 letech trvání pokusu. Z výsledků vyplývá, že aplikace všech organických hnojiv vedla ke zvýšení obsahu přístupného fosforu v půdě. Příčinou je jednak akumulace organické hmoty v těchto variantách, jednak pozitivní bilance fosforu. Potvrdily se tak závěry mnoha autorů, že zejména aplikace čistírenských kalů má pozitivní vliv na obsah přístupného P (např. Arvas et al. 2011, Wu et al. 2012, Nogueirol et al. 2015). U varianty hnojené hnojem došlo rovněž ke zvýšení obsahu mobilního P v půdě. To lze vysvětlit nejen vyšší aplikační dávkou P ve srovnání s variantou NPK, ale i vlivem huminových látek. Huminové látky a jejich prekurzory dodané v hnoji blokují sorpční místa pro fosfor a ten pak zůstává v mobilní formě (Mikulasova et al. 1997). To je doloženo i praktickým výzkumem od inkubačních po dlouhodobé polní pokusy (Du et al. 2013, Kundratowicz-Maciejewska et Kobierski (2011). Naproti tomu varianty hnojené pouze minerálním dusíkem vykazovaly dle očekávání snížení obsahu mobilního P, a to někdy výraznější než u nehnojené kontroly. V případě hnojení minerálním NPK, kde bylo ročně aplikováno 30 kg P/ha došlo rovněž k záporné bilanci a uvedená dávka P se tak jeví jako nedostačující. Klíčovou informací této publikace je, že k pochopení změn obsahů mobilních fosforečnanů v půdě v závislosti na hnojení nestačí jen informace o fosforu v půdě samotném, ale je nutno zahrnout i další faktory, zejména výnosy a odběr P sklizenými produkty.

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Changes of soil bioavailable phosphorus content in the long-term field fertilizing experiment

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Abstract: The aim of this study is to describe the changes of bioavailable phosphorus content in soil in long-term 18 years field experiments with different fertilizing systems. The field experiments are located at three sites with different soil and climatic conditions in the Czech Republic (Červený Újezd, Humpolec and Prague-Suchdol). Same fertilizing systems and crop rotation (potatoes (maize) – winter wheat – spring barley) are realized at each site with following fertilizing treatments: (1) unfertilized treatment (control), (2) farmyard manure (FYM), (3) and (4) sewage sludge (SS 1 and SS 3), (5) mineral nitrogen (N), (6) mineral nitrogen with straw (N + straw) and (7) mineral nitrogen with phosphorus and potassium (NPK). The long-term fertilizing effect on available P content changes in soil was observed. Bioavailable phosphorus content in soil increased in treatments with organic fertilization after 18 year experiment at all sites. The treatments SS 3 had the highest increase at all sites. The highest bioavailable P content increase compared to control (258 mg/kg) was determined at site Červený Újezd. On the contrary, available phosphorus content decreased at treatments with mineral fertilization and control treatment among all sites. Bioavailable P content decrease in the treatment NPK was observed, although phosphorus was applied. The lowest differences in available P content among all fertilizing treatments were observed at the location Prague-Suchdol.

Keywords: farmyard manure; mineral fertilizing; sewage sludge; soil

Phosphorus (P) belongs to the most important building nutrients and it take part in many life processes (Troeh & Thompson 2005). P concentrations in soils depending on soil horizon (subsoil < topsoil), texture (sandy < loamy), pedogenesis (older < younger), land use (forest < pasture < arable land) and its intensity (extensive < intensive) (Kruse *et al.* 2015). The P adsorption capacity of soil is controlled by several attributes, such as the amount and the type of clay minerals, organic matter, iron and aluminium oxides, and soil pH (Souza *et al.* 2010). Alkine and strongly acid soil reaction depress the bioavailability of phosphorus (Kondratowicz-Maciejewska & Kobierski 2011). Pierzynski *et al.* (2005) reported that total P in most agricultural soils ranges from 50 to 1500 mg/kg, whereas Lindsay (1979) indicated that total P in soils ranges from 200 to 5000 mg/kg. However, only very small ratio of P in soils is available to plants. Organic sources of P are known to long-term increase P availability more than inorganic P fertilizers and enhance efficient use of applied P fertilizer (Mohanty *et al.* 2006). Bioavailable phosphorus content in soil is one of the important indicators of the cultivated land

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fertility, which could supply phosphorus nutrient for crop growth. Monitoring soil available phosphorus content in the cultivated land is significant for fertility adjustment, yield improvement and sustainable development of agriculture (Gu *et al.* 2016).

The Mehlich-3 (M3) method is widely used for extraction of plant-available phosphorus from soil over a wide range of pH values (Kobza & Gáborík 2008; Schroder *et al.* 2009; Zhang *et al.* 2009; Kobza *et al.* 2011; Káš *et al.* 2016). Based on the results of interlaboratory determination of available P reported that the method Mehlich-3 is accurate and precise for the determination of available P in soils (Zhang *et al.* 2009). Statistically significant linear relationships between Mehlich-3 and Mehlich-2, Olsen, CAL, and 0.01 M $CaCl_2$ were found and these methods to accurately determine all criteria are able determination of P supply (Zbíral & Němec 2002; Kulhánek *et al.* 2009). Zamuner *et al.* (2006) reported strong positive correlation between Bray 1 and Mehlich-3 extractanting procedures in soils with moderate to slightly acidic pH values.

The aim of this research work is to evaluate the influence of long-term application of different fertilizers (sewage sludge, farmyard manure, mineral N with straw, mineral N and mineral NPK) on the change of bioavailable phosphorus content in soil.

MATERIAL AND METHODS

The bioavailable phosphorus content changes in long-term field experiments were observed at three sites – Červený Újezd, Lukavec and Praha-Suchdol (Suchdol) with different soil and climatic characteristics (Table 1). Potatoes, winter wheat and spring barely were grown in the crop rotation. At the site in Červený Újezd silage maize is included to the crop rotation instead of potatoes because of agronomical conditions of the experimental site. The size of each experimental plot is 60.0 m^2 at the site Lukavec, 60.5 m^2 at Suchdol site and 80.0 m^2 at Červený Újezd site. The same fertilizing systems are used at all sites. Following treatments were studied: control, Sewage sludge 1 (SS 1), Sewage sludge 3 (SS 3), Farmyard manure (FYM), mineral N, mineral N + straw, mineral NPK. Organic fertilizers are applied once every three years in autumn before the potatoes (silage maize). Mineral fertilizers with phosphorus (in the form of triple superphosphate 21% P) and potassium (in the form of potassium-chloride 50% K) were applied yearly in autumn. Nitrogen is applied yearly in the form of calcium ammonium nitrate (27% N) in spring. Fertilizing system and nutrients doses during crop rotation are shown in Table 2.

The content of bioavailable phosphorus were determined from archive composite soil samples taken at experiments establishment before the first fertilizer application in year 1996 and from soil samples taken after of end the crop rotation i.e. spring barley in the year 2013. The soil samples in the year 2013 were collected by Stratified systematic unaligned sampling when from each parcel were taken four soil samples collected from six subsamples taken into circle of radius 30 cm. The soils samples from arable layer (0–30 cm) were collected using soil probe. The soil samples were air dried at the constant temperature of 30°C. Dry soil samples were sieved throrough a 2-mm mesh. The soil samples were extracted ac-

Table 1. Soil and climatic conditions

Sites	Červený Újezd	Lukavec	Praha-Suchdol
Location	50°4'22"N, 14°10'19"E	49°33'23"N, 14°58'39"E	50°7'40"N, 14°22'33"E
Elevation (m)	398	610	286
Average annual temperature (°C)	7.7	7.7	9.1
Average annual rainfall (mm)	493	666	495
Soil type (WRB 2015)	haplic Luvisol	stagnic Cambisol	haplic Chernozem
NRSC USDA	silty loam	sandy loam	silty loam
CEC (mmol(+)/kg)	118	45	262
C_{ox} (%)	1.17	1.27	1.55
Clay (%) (< 0.002 mm)	5.4	3.2	2.2
Silt (%) (0.002–0.05 mm)	68.1	37.1	71.8
Sand (%) (0.05–2 mm)	26.5	59.7	26.0

NRSC USDA – Natural Resources Conservation Service, USDA; CEC – cation exchange capacity; C_{ox} – total organic carbon

Table 2. Nutrients rate during one crop rotation (kg/ha)

Treatment	Potatoes/maize			Wheat			Barley		
	N	P	K	N	P	K	N	P	K
Control	–	–	–	–	–	–	–	–	–
SS	330[1]	303[2]	59[2]	0	0	0	0	0	0
SS 3	990[1]	909[2]	177[2]	0	0	0	0	0	0
FYM	330[1]	113[2]	326[2]	0	0	0	0	0	0
N	120	0	0	140	0	0	70	0	0
N + straw	138	6[2]	47[2]	140	0	0	70	0	0
NPK	120	30	100	140	30	100	70	30	100

[1]total nitrogen in organic fertilizers; [2]average dose based on the nutrients content of fertilizers; SS, SS 3 – sewage sludge; FYM – farmyard manure

cording to the Mehlich-3 method (Mehlich 1984) to obtain the content of bioavailable phosphorus. Each sample was analysed in two replications. The content of phosphorus was measured using inductively coupled plasma – optical emission spectroscopy (ICP-OES, Agilent Technologies 720, USA). The results bioavailable phosphorus content from 2013 were statistically analysed using the Statistica 12.0 software (StatSoft, USA). The one-way ANOVA and Tukey's tests were used to determine significant differences among the treatments.

RESULTS AND DISCUSSION

The available phosphorus content values at the start experiments in the year 1996 from all sites and all treatments are presented in Table 3. The content of bioavailable phosphorus at the start of the experiment in 1996 ranged between 117 and 174 mg/kg at Červený Újezd site, between 187 and 204 mg/kg at site Lukavec and between 63 and 93 mg/kg at site Praha-Suchdol.

At the all organic fertilizing treatments (SS 1, SS 3 and FYM), available phosphorus content increased after 18 year of observation (Table 4). The increased content of available P is related to organic matter which was applied in organic fertilizers and positive balances of phosphorus in organic treatments when the amount of applied P exceed P uptake by plants. The increase of bioavailable P content in SS 1 and SS 3 treatment was caused due to the high dose of phosphorus applied in sewage sludge. The sewage sludge had average content of total phosphorus 3.0% in dry mass. This is more than 2.5% which reported Sommers (1977). Increase bioavailable phosphorus after application sewage sludge was published by many authors. For example Arvas *et al.* (2011) already observed after one year significant increase bioavailable phosphorus by the sewage sludge application (dose of P in sewage sludge 5.1 kg/ha and 7.7 kg/ha) compared with the control. Wu *et al.* (2012) described increase of available P content about 128 mg/kg compared with control after 12 year sewage sludge application with dose of 90 t/ha/year (content of total P 3.47%). The biggest available P content increase was ascertained at treatment SS 3 at all sites which was caused due to the higher dose of sewage sludge. Nogueirol *et al.* (2015) also published increasing content 56.7, 68.8, 125.2 and 169.0 mg/kg of bioavailable P with increasing doses 0, 65, 130, and 207.5 t/ha of sewage sludge (content of total P 1.1%) in 13-year field experiment.

The FYM treatments had higher available P content than mineral treatments including NPK treatment.

Table 3 Available phosphorus content (mg/kg) at the start of experiments in the year 1996

	Control	SS	SS 3	FYM	N	N + straw	NPK
Č. Újezd	119	118	111	117	142	174	166
Lukavec	198	193	196	187	202	197	204
Suchdol	96	72	89	69	69	63	68

SS, SS 3 – sewage sludge; FYM – farmyard manure

Higher content of bioavailable P in FYM in comparison with NPK was due the higher positive balance of P in FYM treatment at each site. Nest *et al.* (2014) also published on base 8-year field experiment statistically significant higher content of available P in FYM treatment (dose of 62 kg P per ha/year) in comparison to mineral NPK treatment (dose of P 42 kg/ha/year). The available P content increase was caused due to the fact that P applied with FYM was in slightly soluble organic form. Organic matter applied in farmyard manure participated on the increase of phosphorus bioavailability in FYM treatments. Humic acids (contained in FYM) could limit sorption of phosphorus, because humic acid participate the sorption places on the active surface of the soil and increase the release of phosphorus to soil (Mikulasova *et al.* 1997). Higher content of available phosphorus after application humic acids with monocalcium phosphate in comparison with monocalcium phosphate application only in laboratory incubation test were observed (Du *et al.* 2013). Kondratowicz-Maciejewska and Kobierski (2011) on base 22 year field experiment reported significant increase of available phosphorus content in soil with increase of farmyard manure dose. Eghball (2002) also recommended adding compost or manure to increase bioavailable phosphorus content.

Content of bioavailable phosphorus in mineral and control treatments decreased in all treatments after 18 years (Table 4). Content of available phosphorus decreased due to the P uptake by plants and not sufficient P dose in N, N + straw and control treatments as well. Higher decrease of available P content was observed in N treatment in comparison to N + straw treatment due to the P application in straw and greater microbial activity, which is participating on the releasing of available P. Liu *et al.* (2010) observed increase microbial activity after 30 years of straw application. Negative balance of P in the treatment N + straw in comparison to N only was observed at each site. Decrease of available phosphorus content in NPK treatment was caused due to the higher yields and for this reason higher P uptake by plants than in organic treatments. Higher yields on mineral treatment on this experiment were documented by Černý *et al.* (2010). Skwierawska *et al.* (2008) also reported decrease of available P content in control treatment and treatment with application of mineral fertilizers NPK (average yearly dose of P 64.2 kg/ha) after three year experiment.

The smallest differences among bioavailable P content after 18 years (except treatment SS 1 at site Lukavec) were found at site Suchdol. It was caused due to the high P soil sorption capacity. The value of P soil sorption capacity is affected with the soil reaction (Table 1) and 4% carbonates content at Suchdol site (Kodešová *et al.* 2016). The amount of bioavailable P depends on soil P sorption. The soil with low P sorption capacity tends to have higher content of bioavailable P and conversely (Djodic & Mattson 2013).

Figure 1 shows the available P content (after 18 years) and its statistical evaluation at Červený Újezd, Lukavec and Suchdol site, respectively. Significant differences between available phosphorus contents were found at the treatment SS 3, all mineral fertilizing treatments (N, N + straw, NPK) and control treatment, respectively. Antoniadis *et al.* (2015) published significantly higher content of bioavailable P between treatment with high dose 60 t/ha of sewage sludge (dose of P 147 kg/ha) and mineral NPK treatment with dose of P 24.5 kg/ha (in form triple superphosphate) after two years, but significant difference between 20 t/ha (dose of P 49 kg/ha) and 40 t/ha (dose of P 98 kg/ha) of sewage sludge was not found.

SS 1 treatment had significantly higher available P content in comparison to mineral treatments and control treatment at Červený Újezd and Suchdol sites. FYM treatment showed significantly higher available P contents in comparison to N and control treatment at Červený Újezd site. For other cases, statistically significant differences were not found.

Table 4 Available phosphorus content changes (Δmg/kg) after 18 years of experiments

	Control	SS	SS 3	FYM	N	N + straw	NPK
Č. Újezd	–45	107	258	37	–57	–54	–36
Lukavec	–44	40	135	56	–44	–42	–56
Suchdol	–3	83	123	18	–22	–11	–8

SS, SS 3 – sewage sludge; FYM – farmyard manure

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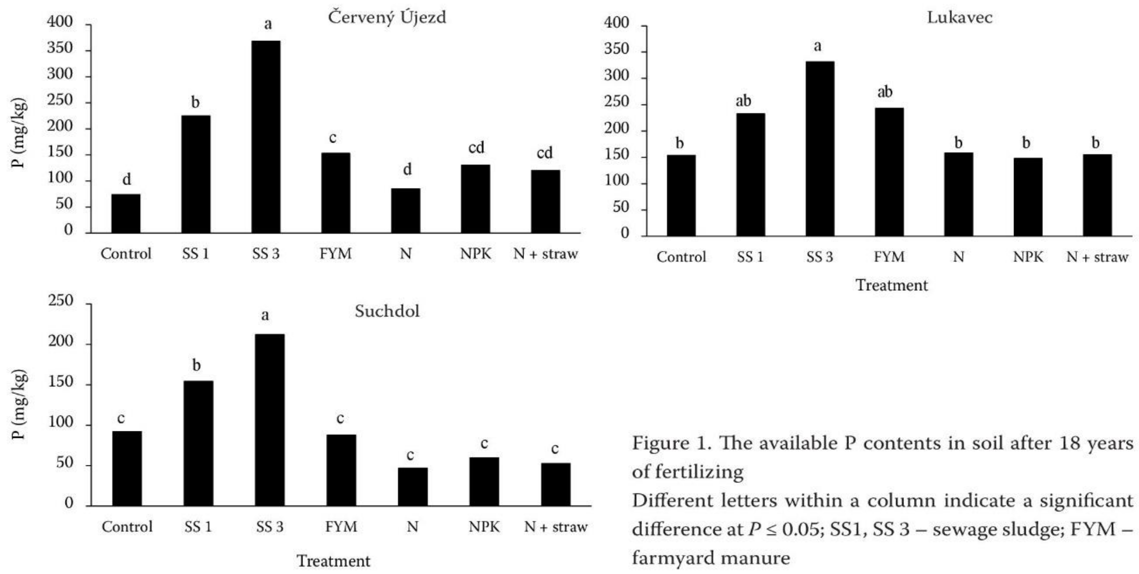

Figure 1. The available P contents in soil after 18 years of fertilizing

Different letters within a column indicate a significant difference at $P \leq 0.05$; SS1, SS 3 – sewage sludge; FYM – farmyard manure

CONCLUSION

Reffering to stated results it is evident that long-term fertilization influences the content of bioavailable phosphorus in soil. Application of farmyard manure and sewage sludge significantly increased available P content in soil. On the contrary, mineral fertilization led to the decrease of available P content in soil including yearly application of phosphorus (30 kg P/ha/year). For the precise evaluation of available phosphorus changes, its relations to soil conditions and grown plants must be taken into account.

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4) Impact of organic and mineral fertilising on aluminium mobility and extractability in two temperate cambisols

Vliv organického a minerálního hnojení na extrahovatelnost a mobilitu hliníku ve dvou kambizemích

Výživu rostlin fosforem je nutno chápat v širších souvislostech. Důvodem je řada půdních, rostlinných i klimatických parametrů působících na přeměny P. Nejen z tohoto důvodu byl vypracován i přiložený článek, kde je mimo jiné řešena sorpce fosforu na půdní hliník. Tato problematika byla sledována v dlouhodobém přesném polním pokusu zahrnujícím kontrolu a 6 dalších variant hnojení včetně organických a minerálních hnojiv. Pokus byl založen v roce 1996 a hliník je zde hodnocen po 21 letech. Pro dosažení relevantních výsledků bylo zvoleno několik extrakčních postupů: vodný výluh, $CaCl_2$, KCl, $CaCl_2$/DTPA, Mehlich 3. Potvrdilo se, že stanovené obsahy hliníku úzce souvisí s hodnotou pH, kdy vyšší obsahy Al znamenají i vyšší aciditu. Ke zvýšení acidity vedla zejména aplikace minerálních hnojiv. Naproti tomu při aplikaci chlévského hnoje došlo ke stabilizaci pH a tím i snížení obsahu hliníku. V rámci pokusu byl sledován i obsah mobilního P v půdě. Zde se jednoznačně potvrdily výsledky Sims et Vadas (2005), tj. negativní korelace mezi obsahem mobilního P a Al v půdě. To dokládá silnou sorpci mobilních fosforečnanů na hliník. Při výrazném zvýšení obsahu hliníku v půdách může dokonce docházet k redukci výnosu plodin. Valle et al. (2009) uvádějí, že při obsahu 46 mg výměnného Al/kg půdy došlo k průkaznému snížení výnosu pšenice. V našich pokusech nebyla překročena hranice 20 mg výměnného Al/kg půdy, a tak je zřejmé, že hliník zde není limitujícím prvkem výnosu. Hlavní význam přiloženého článku představují tři zjištění: 1) obsah mobilního a výměnného hliníku v půdě poskytuje cenné doplňující informace k hodnotě pH, 2) méně používaná extrakce $CaCl_2$/DTPA je užitečným nástrojem ke stanovení mobilního hliníku a 3) stanovení výměnného hliníku pomocí KCl se jeví jako výhodnější ve srovnání s extrakcí $CaCl_2$.

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Impact of organic and mineral fertilising on aluminium mobility and extractability in two temperate Cambisols

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Abstract: Different fertilisation systems cause changes in the content of mobile aluminium (Al) forms in the soil as a result of soil pH changes. Long-term stationary fertilisation experiments established in 1996 at 2 sites were evaluated. Experiments consisted of unfertilised control treatment and 6 other treatments, combining mineral fertilisation with the application of farmyard manure, sewage sludge and straw. To record the changes in mobile Al forms, we used 5 extraction procedures with agents: water, $CaCl_2$, KCl, $CaCl_2$/DTPA (CAT) and Mehlich 3 solutions. At treatment nitrogen (N) + straw, topsoil acidification was observed, resulting in the increased content of mobile Al. At treatments with mineral fertilisation (N, NPK), increased content of mobile forms of Al was recorded. Application of cattle manure and sewage sludge helped to stabilise the soil pH and reduce mobile Al forms. The close correlation between the methods determining the exchangeable Al ($CaCl_2$ and KCl solutions) was observed; however, KCl extraction was more favourable in soils of low Al extractability, as the amount of extracted Al was 3–4 times higher. Positive correlations were determined between Al extracted by Mehlich 3 solution and the content of exchangeable Al. The less frequently used CAT method also turned out to be perspective for mobile Al determination. Furthermore, aluminium content determined in Mehlich 3 extract was compared with mobile phosphorus amounts in H_2O, CAT and Mehlich 3, respectively. All three investigated phosphorus forms showed significantly negative correlations with Al.

Keywords: soil pH; mobile forms of Al; exchangeable aluminium; $CaCl_2$/DTPA (CAT) method; Mehlich 3

Increased soil acidity accelerates solubility of aluminium (Al) compounds, which is a primary source of toxicity to plants at pH < 5.5 (Parker et al. 1989). Special attention thus must be paid to exchangeable aluminium (Drábek et al. 2005) as the dynamic balance is set up between exchangeable and soil solution aluminium. Al^{3+} in soil solution is hydrolysed, which results in acidification of the soil environment as well as worsening of plant growth conditions. The content of exchangeable aluminium usually shows a negative correlation with pH values (Vieira et al. 2008).

Increased content of mobile aluminium in soil often results in yield reduction (Kamprath 1984, Johnson et al. 1997). Valle et al. (2009) determined a significant decrease of wheat grain yields as a consequence of the increase of exchangeable aluminium content from 0.2 $mmol_+$/kg to 5.1 $mmol_+$/kg.

Moore and Edwards (2005), using the gradually increasing nitrogen (N) doses (from 65 up to 260 kg N/ha/year) reported an exponential growth of exchangeable aluminium from 1.0 mg Al/kg (2nd year) up to 100 mg Al/kg (7th year of trials). Similarly, Bouman et al. (1995) determined a significant increase of exchangeable Al and hydrogen (H) at gradually increasing N fertilisation using urea and ammonium nitrate (45–180 kg N/ha/year). Schroder et al. (2011) recorded the effective cation exchange capacity saturation with aluminium (Al_{SAT}) of 33.9% after intensive ammonium nitrate fertilisation (274 kg N/ha/year), whereas at the dose of 34 kg N/ha/year it was only 16.4%. Compared to that, the unfertilised control showed the ratio of only 1.09%. Johnson et al. (1997) observed that the Al_{SAT} content nearing to 16% might induce phytotoxicity in plants. The mathematical

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model presented by Schroder et al. (2011) suggests that each percent of Al_{SAT} decreased the wheat yield on average by 2.8%. Al toxicity manifested in some years by the purple colouring of wheat leaves and a poor root system. Similarly, Wise (2002) recorded a decrease of winter wheat yields at increased aluminium content in the cation exchange complex. Wen et al. (2014) reported increased pH values and soil organic matter content at treatments with long-term (22 years) manure fertilisation, accompanied by the occurrence of amorphous nanominerals such as allophane and imogolite. Both these phenomena led to the transformation of exchangeable aluminium into stable forms. Soil nanominerals allophane and imogolite can sorb much more organic compounds than bulk soil minerals, mainly due to their high level of hydration, large surface and stable surface charge (Mikutta et al. 2006); these processes may contribute to organic matter protection and accumulation.

The results of recent complex observations of agrochemical properties of soils in the Czech Republic show that almost 25.2% of arable soils have pH_{CaCl_2} lower than 5.5 (Smatanová and Sušil 2017). Arable soils acidification can be attributed to intensive mineral nitrogen fertilisation, insufficient organic fertilisation and significantly reduced liming.

The aims of this paper are (1) to determine the impacts of different fertilisation systems on changes in the content of mobile aluminium forms in soils; (2) to compare different methods for soil Al determination, and (3) to investigate the influence of increased soil acidity and Al mobility on soil phosphorus pools.

MATERIAL AND METHODS

Five field trials were established in 1996 at the experimental bases of the Czech University of Life Sciences Prague in different soil-climatic conditions (Vašák 2016). Potential for changes in soil aluminium behaviour due to low soil pH showed only two of five locations – B and C. Because of that, only these locations are evaluated in this manuscript (Table 1).

Within these trials, three crops were rotated in the following order: potatoes, winter wheat and spring barley. Each year, all of the crops were grown. The size of the experimental plots was 60 m^2; each treatment was 4 times replicated. The trial included seven treatments: (1) no fertilisation (control); (2) sewage sludge (SS1); (3) farmyard manure (FYM1); (4) half dose of farmyard manure + N in mineral nitrogen fertiliser (FYM1/2); (5) mineral nitrogen fertiliser (N); (6) NPK in mineral fertiliser (NPK); (7) spring barley straw + N in mineral nitrogen fertiliser (N + St) (Table 2). Organic fertilisers, farmyard manure, sewage sludge and straw, were always applied in autumn (October) to potatoes. Mineral phosphorus and potassium fertilisers were applied to each crop in autumn; mineral nitrogen fertilisers in the form of calcium ammonium nitrate were applied to potatoes and spring barley in spring, before crop establishment. In the case of winter wheat, the dose of nitrogen was divided in half, with the first half applied at BBCH 21, the second at BBCH 31–32. The content of nitrogen was 140 kg N/ha for wheat and 70 kg N/ha for spring barley; FYM1/2 was applied at the rate of 115 kg N/ha for wheat and 50 kg N/ha for barley. The NPK treatment of winter wheat and spring barley included phosphorus at a level of 30 kg P/ha (triple superphosphate) and potassium at a level of 100 kg K/ha (60% potassium salt).

Analytical procedures and statistical evaluation

pH_{CaCl_2}. ISO 10390 (1994) was used to determine pH_{CaCl_2} values.

Water extraction (Al_{H_2O}). Water extraction (Al_{H_2O}) was performed, according to Luscombe et al. (1979).

Table 1. Basic description of investigated locations

Location	B	C
GPS coordinates	49°33'16''N 15°21'2''E	49°33'23''N 14°58'39''E
Altitude (m a.s.l.)	525	610
Mean annual temperature (°C)	7.0	7.7
Mean annual precipitation (mm)	665	666
Soil type	Stagnic Cambisol	
Soil texture	sandy loam	
Clay (%) (< 0.002 mm)	5.8	3.2
Silt (%) (0.002–0.05 mm)	43.6	37.1
Sand (%) (0.05–2 mm)	50.6	59.7
Bulk density (g/cm^3)	1.4	1.3
C_{org} (%)	1.4	1.3
pH_{CaCl_2}	5.1	5.3
CEC (mmol$_+$/kg)	90	45

C_{org} – organic carbon; CEC – cation exchange capacity

Table 2. Experimental design (all nutrients doses are in kg/ha per 3 years)

Treatment	Nitrogen		
	potatoes/maize	wheat	barley
Control	–	–	–
SS1	330[1]	0	0
FYM1	330[1]	0	0
FYM1/2	165	115	50
N	120	140	70
NPK[2]	120	140	70
N + St	120 + St	140	70

[1] Total dose of nitrogen applied in organic fertiliser; Average yearly dose based on the analysis of farmyard manure and of sewage sludge. [2] Applied as follows: N – calcium ammonium nitrate (27% N), P – triple superphosphate (21% P), K – potassium chloride (50% K); FYM – farmyard manure – average doses of fresh FYM were as follows: B – 59.45 t/ha per 3 years (13.42 t of dry mass/ha per 3 years; 2.459% N in dry mass); C – 68.97 t/ha per 3 years (19.32 t of dry mass/ha per 3 years; 1.708% N in dry mass) SS and FYM were applied seven times; straw was applied six times. SS1 – sewage sludge – average doses of fresh sewage sludge were following: 30.18 t/ha over 3 years (9.383 t/ha over 3 years of dry mass). Straw was applied in doses of 5 t/ha over 3 years: 28 kg N – B; 35.5 kg N – C

Calcium chloride soil extraction (Al_{CaCl_2}). The content of Al_{CaCl_2} was determined, according to Novozamsky et al. (1993).

$CaCl_2$/DTPA (CAT) extraction (CSN EN 13651, 2002). For this procedure, 3.00 g of soil sample was weighed into 50 mL tubes. To all samples was applied 30 mL solution of 0.01 mol/L CaCl2 and 0.002 mol/L DTPA). Solution was shaken for 1 h on horizontal shaker.

Potassium chloride extraction (Al_{KCl}). Exchangeable aluminium was extracted in 1.0 mol/L KCl (Bertsch and Bloom 1996).

Mehlich 3 extraction (Al_{M3}). Procedures were followed according to those reported previously (Mehlich 1984).

To determine the relationships between Al and P, phosphorus content was measured together with Al content in water extract, CAT and Mehlich 3 to obtain P_{H_2O}, P_{CAT}, and P_{M3}, respectively.

Determining of different Al fractions. The concentrations of Al and P in all of the above mentioned extracts, as well as concentrations of Na, K, Ca, Mg and Mn in NH_4OAc were determined using optical emission spectroscopy with inductively coupled plasma (ICP-OES) with axial plasma configuration, Varian, VistaPro, equipped with an autosampler SPS-5 (Agilent Technologies, Mulgrave, Australia).

Aluminium saturation was calculated according to Johnson et al. (1997) using the following equation:

$$Al_{SAT}(\%) = \left(\frac{Al_{KCl}}{ECEC}\right) \times 100$$

Where: Al_{KCl} – exchangeable Al ($cmol_+$/kg); ECEC – effective cation exchange capacity, which is the sum of exchangeable Na^+, K^+, Ca^{2+}, Mg^{2+} and Mn^{2+} determined in 1 mol/L NH_4OAc at pH 7.0 ($cmol_+$/L) plus exchangeable Al^{3+} ($cmol_+$/kg).

Statistical analysis. The results were assessed using analysis of variance (ANOVA) with the Fisher *LSD* (least significant difference) post-hoc test. To evaluate the obtained results, the Statistica software (StatSoft Inc. 2015) was used.

RESULTS AND DISCUSSION

The changes of pH_{CaCl_2} at individual treatments are given in Table 3. The values obtained in 1996 (before the establishment of the trial) and in 2017 (after the barley harvest) were compared. Application of manure positively affected soil pH_{CaCl_2}. The application of sewage sludge had a slightly positive effect on pH_{CaCl_2}. With the N + straw treatment, there was a clear tendency to topsoil acidification. The presented results further show a clear negative effect of mineral fertilisation alone (N, NPK).

Table 3. Changes of pH_{CaCl_2} during the period of 1996/2017

Site	Control	SS1	FYM1	FYM1/2	N	NPK	N + St
B	-0.25^{b}	-0.10^{bc}	0.14^{d}	0.00^{c}	-0.31^{a}	-0.15^{b}	-0.35^{a}
C	-0.22^{b}	-0.05^{bc}	0.16^{c}	-0.06^{bc}	-0.20^{b}	-0.43^{a}	-0.47^{a}

Different letters in the row mean significant difference ($P \leq 0.05$) among fertilising treatments. Control – no fertilisation; SS1 – sewage sludge; FYM1 – farmyard manure; FYM1/2 – half dose of farmyard manure + N in mineral nitrogen fertiliser; N – mineral nitrogen fertiliser; NPK – NPK in mineral fertiliser; St – spring barley straw

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Table 4. Aluminium (Al) content in different extraction solutions (mg Al/kg)

Site	Control	SS1	FYM1	FYM1/2	N	NPK	N + St
Al_{H_2O}							
B	41^{a}	44^{a}	42^{a}	44^{a}	43^{a}	42^{a}	43^{a}
C	21^{ab}	18^{ab}	19^{ab}	20^{ab}	20^{ab}	22^{b}	19^{a}
Al_{CAT}							
B	247^{ab}	237^{ab}	229^{a}	241^{ab}	232^{ab}	288^{c}	252^{b}
C	209^{ab}	192^{a}	176^{a}	237^{b}	263^{c}	262^{bc}	253^{bc}
Al_{M3}							
B	1051^{ab}	1069^{ab}	1033^{a}	1058^{ab}	1068^{ab}	1097^{b}	1077^{b}
C	950^{a}	938^{a}	921^{a}	935^{a}	994^{a}	1017^{a}	1030^{a}
Al_{CaCl_2}							
B	1.2^{ab}	0.8^{a}	1.2^{ab}	1.0^{ab}	1.4^{b}	1.5^{ab}	1.2^{ab}
C	1.6^{ab}	1.2^{a}	1.6^{a}	1.7^{ab}	3.1^{ab}	3.0^{b}	2.9^{b}

Different letters in the row mean significant difference ($P \leq 0.05$) among fertilising treatments. Control – no fertilisation; SS1 – sewage sludge; FYM1 – farmyard manure; FYM1/2 – half dose of farmyard manure + N in mineral nitrogen fertiliser; N – mineral nitrogen fertiliser; NPK – NPK in mineral fertiliser; St – spring barley straw; Al_{H_2O} – water extraction; Al_{CAT} – CAT extraction; Al_{M3} – Mehlich 3 extraction; Al_{CaCl_2} – calcium chloride soil extraction

Extraction procedures

Water-soluble (Al_{H_2O}) and exchangeable aluminium (Al_{KCl}, Al_{CaCl_2}). In Table 4 are given the Al_{H_2O} contents. The differences among fertilising treatments were not statistically significant. On the other hand, water as an extract released a significant amount of Al, which was 42.7 mg Al/kg at location B and 19.9 mg Al/kg at location C, respectively. It is from 9 to 36 times higher Al content compared to $CaCl_2$ extraction.

The content of exchangeable Al was determined using 1 mol/L KCl, which is a commonly used method nowadays (Drábek et al. 2005), as well as using 0.01 mol/L $CaCl_2$, previously evaluated by Johnson et al. (1997) (Table 4). Compared to 0.01 mol/L $CaCl_2$, Al extracted by 1 mol/L KCl was more effective, as the amount of extracted aluminium was 3–4 times higher. It is a positive feature, especially in soils of $pH_{CaCl_2} < 5.5$, where the amount of extractable Al is usually low. Higher extraction strength of the KCl agent results from a higher concentration of replacing cation (K^+) and consequently from higher acidity caused by H^+ released from exchange sites to the solution. On the other hand, a very tight correlation was determined between both methods (Table 5), which suggests that both of them may be used. Extraction in 0.01 mol/L $CaCl_2$ solution is closer to real conditions at site at a given time (Novozamsky et al. 1993). In our trials, the content of exchangeable aluminium (Al_{KCl}) did not reach over 20 mg Al/kg (site B) and 11 mg Al/kg (site C). These concentrations are low and should not be toxic for plants. Valle et al. (2009) reported a significant decrease of wheat grain yields, at the content of exchangeable Al above 5.1 $mmol_+$/kg (i.e. 45.9 mg Al/kg). Similarly, low contents (1.04 $mmol_+$/kg) as in our observations were analysed by Norton et al. (2018) at soils of pH 5.5.

To assess aluminium toxicity, aluminium saturation (Al_{SAT}) of the effective cation exchange capacity (ECEC) was calculated (Johnson et al. 1997). In our trials, the content of Al_{SAT} was always < 3% – i.e. very low Al saturation level (Table 6). Kamprath (1984) stated that at Al_{SAT} lower than 10%, the wheat yield was not affected. Johnson et al. (1997) published the limit level of Al_{SAT} lower than 16%. Higher saturation resulted in phytotoxicity of wheat plants grown in Oklahoma (USA) soils. Moreover, using the Al_{SAT} criterion, growth and yield of plants in our trials were not negatively influenced by the content of toxic aluminium forms.

CAT method. Results of $CaCl_2$/DTPA extraction (CAT) are shown in Table 4. It is a method frequently used for determination of available forms of nutrients in substrates (Dubský et al. 2019). The acid character of CAT solution, as well as DTPA chelating effects, result in a strong extraction effect of this agent. Out of all the tested methods used in these localities, CAT showed

Table 5. Pearson correlation coefficient values for different extraction solutions

	Al_{CAT}/Al_{M3}	Al_{H_2O}/Al_{M3}	Al_{CAT}/Al_{H_2O}	Al_{KCl}/Al_{CaCl_2}	Al_{KCl}/Al_{M3}	Al_{KCl}/Al_{CAT}	Al_{KCl}/Al_{H_2O}
B	0.806^{***}	−0.118	0.023	0.759^{***}	0.792^{***}	0.713^{***}	-0.469^{**}
C	−0.018	-0.402^{*}	0.514^{**}	0.901^{***}	0.746^{***}	0.810^{***}	−0.134

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; Al_{CAT} – CAT extraction; Al_{M3} – Mehlich 3 extraction; Al_{H_2O} – water extraction; Al_{KCl} – potassium chloride extraction

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Table 6. Saturation of effective cation exchange capacity (%) by aluminium

Site	Control	SS1	FYM1	FYM1/2	N	NPK	N + St
B	0.50^b	0.21^a	0.24^a	0.60^{abc}	0.79^{bc}	3.04^d	1.09^c
C	0.40^b	0.00^a	0.00^a	0.09^{ab}	1.10^b	1.10^b	2.43^c

Different letters in the row mean significant difference ($P \leq 0.05$) among fertilising treatments. Control – no fertilisation; SS1 – sewage sludge; FYM1 – farmyard manure; FYM1/2 – half dose of farmyard manure + N in mineral nitrogen fertiliser; N – mineral nitrogen fertiliser; NPK – NPK in mineral fertiliser; St – spring barley straw

the tightest correlation with the pH_{CaCl_2} value changes caused by different fertilisation systems (Figure 1). In our trials, the CAT method mostly showed significant negative correlations with mobile phosphorus in soils (Table 7). A negative relationship between the content of soil mobile aluminium forms and phosphorus was also described by Sims and Vadas (2005).

Mehlich 3 method (Al_{M3}). No tight correlations between the changes in pH values and Al_{M3} content were determined. The possible potential of using this method is demonstrated by positive correlations with exchangeable aluminium (Al_{KCl}) (Table 5). Mehlich 3 procedure stands for a "robust" method, having probably a limited power in case of aluminium. Both organically and inorganically sorbed aluminium was determined. Nevertheless, the advantage is its potential to determine a range of nutrients; using the analytical method with the ICP measurement, data on mobile aluminium may be obtained easily, too. This procedure is suitable mainly at soils with pH_{CaCl_2} < 5.5. Compared to the method of non-crystalline Al determination using the ammonium oxalate, the advantage of Mehlich 3 is a significantly shorter exposition time. Our experiments also revealed a negative correlation between the Al_{M3} content and mobile phosphorus (P_{H_2O}, P_{CAT}, P_{M3}) (Table 7), which indirectly confirms the hypothesis that Al_{M3} may be used to predict the P sorption capacity in soils (Fernández Marcos et al. 1998).

According to Fernández Marcos et al. (1998), as to determining mobile non-crystalline aluminium, Mehlich 3 results are equal to those of ammonium oxalate (Al_o). Also, Sen Tran et al. (1990) reported a very good correlation with oxalate (Al_o). At high Al_o concentrations, this relation is no more linear. Decreased Mehlich 3 extract sensitivity to non-crys-

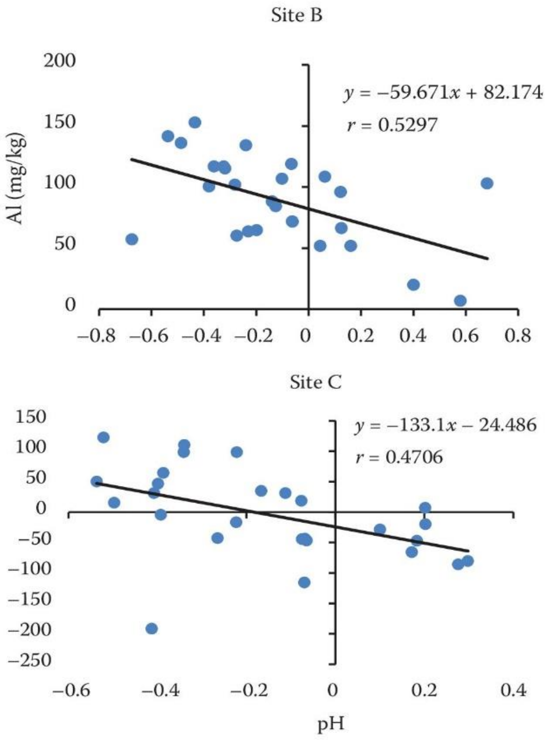

Figure 1. Changes of Al_{CAT} in accordance with changes of pH_{CaCl_2}

Table 7. Pearson correlation coefficients for content of aluminium (Al) and phosphorus (P)

Site	P_{H_2O}	P_{CAT}	P_{M3}
Al_{M3}			
B	−0.370**	−0.353**	−0.389**
C	−0.291*	−0.204	−0.061
Al_{CAT}			
B	−0.529***	−0.522***	−0.562***
C	−0.602***	−0.554***	−0.678***
Al_{KCl}			
B	−0.465**	0.191	−0.468**
C	−0.555***	−0.502***	−0.509***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; Al_{M3}/P_{M3} – Mehlich 3 extraction; Al_{CAT}/P_{CAT}– CAT extraction; Al_{KCl} – potassium chloride extraction; P_{H_2O} – water extraction

talline aluminium at high Al_o concentrations may be caused by low fluorine concentration (0.015 mol/L) and further by the short time of extraction (only 5 min). Fernández Marcos et al. (1998) assumed that the Mehlich 3 method is suitable for determining mobile aluminium in a wide range of soils. The authors further confirmed the hypothesis that Al_{M3} might be successfully used for prediction of phosphorus sorption capacity in soils. Similar results about the use of the Mehlich 3 method for assessment of changes in the content of mobile aluminium were published in the study of Monterroso et al. (1999); the authors also reported a significant Al_{M3} correlation with the content of non-crystalline aluminium.

Effect of the fertilisation systems

The evaluation of individual extraction agents does not allow drawing unequivocal and conclusive decrease of mobile aluminium forms at treatments using cattle manure and sewage sludges (Table 4). Significantly conclusive results, however, arise from the values of aluminium saturation of the sorption complex (Al_{SAT}). Our previous research (Balík et al. 2018) prove the increase of the total organic carbon in topsoil (C_{org}), microbial biomass carbon (C_{mic}) as well as easily-extractable carbon (C_{DOC}) at these two treatments. We suppose that these factors contributed to a decrease in the Al_{SAT} values, which means reduced aluminium mobility. Similarly, Wen et al. (2014) in long-term experiments with manure amendment determined a decrease of the Al_{SAT} values and an increased ratio of the amorphous aluminium. The content of amorphous Al significantly correlated with the organic carbon content in topsoil. As described by Rutkowska et al. (2015), long-term manure application decreased the exchangeable aluminium content at the NPK treatment by 15% and the NP treatment by 30%. Application of organic substances has a positive impact on the immobilisation of toxic Al ions, thanks to the production of stable organo-mineral complexes (Qin et al. 2010). Also, increased production of soil nanominerals (allophane, imoglite) after the manure application may be a novel mechanism to reduce soil acidification (Mikutta et al. 2006). Similarly, Tao et al. (2019) reported a significant decrease of exchangeable aluminium in long-term experiments with manure application.

Mineral fertilisation alone resulted in a tendency of topsoil acidification and higher aluminium mobility. Especially the Al_{SAT} ratio increased. This weaker acidification process than expected may be explained by two factors: (a) average N fertilisation intensity (120 kg N/ha/year); (b) form of N fertiliser used (calcium ammonium nitrate). Still, the process of acidification (pH_{CaCl_2}) and increase of aluminium mobility (Al_{H_2O}, Al_{CAT}, Al_{M3}, Al_{KCl}) is evident in all analyses carried out, which is in good agreement with many literature sources (Bouman et al. 1995, Moore and Edwards 2005, Schroder et al. 2011, Rutkowska et al. 2015).

Interestingly, N + straw treatment resulted in acidification of soil and increased aluminium mobility; it was evident mainly from the Al_{SAT} ratio. It is well known that aluminium has a high affinity to organic substances and may form permanent bonds with various organic fractions. Their stability depends on the type of organic substance and the number of hydroxyl and phenolic groups directly bound to aluminium (Parker 2005). It is probable that straw, mainly at site C, does not contribute to increased stability of the organo-mineral complex and it is mineralised quickly after application. In contrast to our results, Qina et al. (2010) reported an increase in soil pH and a decrease of water-soluble and exchangeable aluminium after rice straw application for 12 years.

A close correlation between the methods determining the exchangeable aluminium (Al_{CaCl_2} and Al_{KCl}) was observed; however, KCl extraction appeared more favourable. The less frequently used CAT method turned out to be more perspective for mobile aluminium determination and has several advantages: (*i*) good reaction to changes of mobile Al forms related to changes of soil pH values; (*ii*) positive correlations with exchangeable sorbed Al and *vice versa* (*iii*) negative correlation with mobile P forms; (*iv*) determined Al contents in common soils did not fall below the ICP detection level. A very good tool to express the ongoing acidification processes and the content of mobile aluminium forms is effective cation exchange capacity saturation with aluminium. The content of mobile and exchangeable aluminium forms, as supposed, showed a negative correlation with mobile P. Application of cattle manure and sewage sludges helped stabilise the soil pH and reduce mobile forms of aluminium. At treatment N + straw, topsoil acidification and increased aluminium mobility were observed, resulting mainly in the increased content of mobile Al_{SAT}. At treatments with mineral fertilisation (N, NPK), a tendency to increased content of mobile forms of aluminium was observed.

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5) Sulfur nutrition index in relation to nitrogen uptake and quality of winter wheat grain

Vztah výživového indexu sírou k odběru dusíku a kvalitě zrna ozimé pšenice

V publikaci je řešena úzká spojitost hnojení sírou s hnojením dusíkem. Z výsledků Hřivna et al. (2015) a Klikocka et al. (2015) jednoznačně vyplývá, že hnojení sírou má pozitivní vliv nejen na výnos zrna pšenice, ale i na jeho specifickou hmotnost a další kvalitativní parametry, jako jsou obsah bílkovin, lepku a výsledky Zelenyho sedimentačního testu. V praxi je již dlouhodobě využíván index hnojení dusíkem (např. Justes et al. 1994, Yue et al 2012). Cílem tohoto výzkumu bylo vyhodnotit podobný index pro síru (SNI), tj. poměr obsahu síry v nadzemní biomase ke kritickému obsahu síry - Sc, a to ve vztahu k odběru dusíku a pekařské kvalitě ozimé pšenice. Tato problematika byla hodnocena ve čtyřletých maloparcelkových přesných polních pokusech na 3 různých stanovištích v ČR. Podmínkou byl fakt, že dusík nebyl limitující živinou. Pro vyhodnocení SNI byl využit model zřeďovací křivky pro síru vyvinutý Reussi et al. (2012). Na základě tohoto modelu byla vypočtena optimální hranice obsahu síry v nadzemní hmotě pšenice, a to pouze na základě hmotnosti nadzemní hmoty. Při výnosu biomasy nepřesahující hodnotu 1.0 t/ha byla vypočtena konstantní hladina pro kritický obsah síry - Sc=0,55. Dále byl zjištěn velmi silný vztah mezi SNI a poměrem N:S v nadzemní hmotě rostlin. Optimální poměr N:S a současně i kvalitativní parametry zrna (Zelenyho test, obsah bílkovin a mokrého lepku) vykazovaly rostliny s indexem SNI přesahujícím hodnotu 0,80 ve fázi počátku odnožování, 0,70 v pozdní fázi odnožování a 0,60 na počátku metání. Studovaný model dle Reussi et al. (2012) naproti tomu vykazoval spolehlivé výsledky jen do dosažení fáze konce dlouživého růstu stonků (BBCH 51). Ve studii byly dále sledovány vztahy SNI s výnosy zrna pšenice. Zde však bylo dosaženo pouze velmi slabých vzájemných korelací.

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RESEARCH

Sulphur nutrition index in relation to nitrogen uptake and quality of winter wheat grain

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ABSTRACT

A sulphur nutrition index (SNI) is an analogue of the N nutrition index, which is a widely used simple indicator of plant N status. The aim of this study was to relate the SNI (ratio of S concentration in shoot biomass to critical S concentration - Sc) to N uptake, grain yield and breadmaking quality of winter wheat (*Triticum aestivum* L.) grain during 4-yr small-plot field experiments realized under non-limiting N conditions in three different locations in the Czech Republic. The model of S dilution curve (Sc) developed by Reussi et al. (2012) for spring wheat was used for expression of the SNI. According to the model, optimal S concentration in shoot biomass was calculated using nothing but shoot biomass weight. The constant value of Sc = 0.55 was determined for shoot biomass weight lower than 1.0 t ha^{-1}. A very strong correlation was recorded between the SNI and an N to S weight ratio (N:S) in shoot biomass. Both, optimal N:S weight ratio in shoot biomass and qualitative parameters of grain (particularly Zeleny sedimentation volume, grain protein content and wet gluten content) were recorded if the SNI exceeded values of 0.80 at the beginning of stem elongation, 0.70 at the late boot stage, and even 0.60 at the beginning of heading despite the fact that the Reussi et al.'s model of S dilution curve was originally applicable only until the end of stem elongation. Correlation between the SNI and relative grain yield was weak.

Key words: Critical concentration, dilution curve, gluten, protein content, sulphur status, *Triticum aestivum*, yield.

INTRODUCTION

Low content of bioavailable S forms in soils was recorded e.g. in Poland (Siebielec et al., 2017). Concurrently, negative S balance was mentioned by Lozek et al. (2011) in soils in Slovakia. Concurrently, S uptake positively influences both uptake and utilization of N (Salvagiotti et al., 2009). Precise application of S particularly in intensive wheat production systems is then necessary (Flaete et al., 2005). A concurrent management of N and S is important to reduce potential pollution of residual soil nitrate by increasing N recovery from the soil while sustaining high N use efficiency (Salvagiotti et al., 2009).

Critical values for leaf concentrations of total S, sulphate and glutathione change over time and are not suitable for diagnosing S deficiency early in the growth season. In contrast, an N:S ratio in shoot biomass is a more reliable indicator of plant S status (Blake-Kalff et al., 2000). Optimal values of the N:S ratio in shoot biomass were expressed for various growth stages of wheat by many authors: after 6 wk of growth (Qian and Schoenau, 2007), during tillering (Reussi et al., 2011), during stem elongation (Calvo et al., 2008), during ear emergence (Steinfurth et al., 2012), and particularly in grain (Zhao et al., 1999; Tea et al., 2007).

However, one of the problems using the N:S ratio is that a surplus of one of these elements may be interpreted as a deficiency of the other one (Scherer, 2001). Furthermore, determination of the N:S ratio requires two precise analytical measurements (Blake-Kalff et al., 2000). Hence, Reussi et al. (2012) expressed S dilution critical curve from the beginning of tillering to the end of stem elongation of spring wheat. This model takes account of decreasing plant S concentration during wheat growth because the optimal S concentration in shoot biomass is calculated using nothing but shoot biomass weight (Reussi et al., 2012). The calculation of S dilution critical curve enables to express the S nutrition index (SNI) analogous to the N nutrition index (NNI), which is widely used for quantifying the N status (Sadras and Lemaire, 2014). Sulphur nutrition index is calculated as a ratio of measured S content in shoot biomass and the critical S concentration calculated from the model of S dilution critical curve. For SNI > 1 the crop S status can be considered as non-limiting, and for SNI < 1 the crop S status can be considered as limited by S supply. On the other hand, the model according to Reussi et al. (2012) of S dilution critical curve is relevant to weight of shoot biomass higher than 1.0 t DM ha^{-1}, which is a problem for S status determination at earlier growth stages.

The aim of this study was to answer the following questions: Can SNI be considered as an appropriate indicator of winter wheat S status under Central European field conditions? Does the value of SNI = 1 lead to the highest grain yield and the best quality of winter wheat grain? Is it possible to use the S dilution critical curve for earlier or later growth stages of winter wheat than the model according to Reussi et al. (2012) states?

MATERIAL AND METHODS

Small-plot field experiments with winter wheat (*Triticum aestivum* L.) 'Sulamit' were realized during a 4-yr period (2007-2010) at three locations with different soil-climatic conditions in the Czech Republic (Central Europe): Hněvčeves (L1; 50°18' N, 15°42' E, 265 m a.s.l., Haplic Luvisol (FAO classification), average precipitations and temperature - 560 mm, 8.4 °C, pH($CaCl_2$) 6.3, available nutrient contents (Mehlich 3): 89 mg P kg^{-1}, 292 mg K kg^{-1}, 158 mg Mg kg^{-1}, 2350 mg Ca kg^{-1}), Humpolec (L2; 49°32' N, 15°21' E, 525 m a.s.l., Gleic Cambisol, average precipitations and temperature - 678 mm, 6.7 °C, pH($CaCl_2$) 6.1, available nutrient contents (Mehlich 3): 90 mg P kg^{-1}, 193 mg K kg^{-1}, 183 mg Mg kg^{-1}, 2250 mg Ca kg^{-1}) and Ivanovice na Hané (L3; 49°18' N, 17°5' E, 225 m a.s.l., Chernozem, average precipitations and temperature – 554 mm, 9.3 °C, pH($CaCl_2$) 7.3, available nutrient contents (Mehlich 3): 137 mg P kg^{-1}, 390 mg K kg^{-1}, 245 mg Mg kg^{-1}, 4458 mg Ca kg^{-1}). Water soluble S varied among years, average contents were: 12.3 mg kg^{-1} for L1, 15.1 mg kg^{-1} for L2, and 8.2 mg kg^{-1} for L3.

The sowing density was 450 seeds m^{-2}, the size of each plot was 39 m^2, of which 15 m^2 were harvested by a small-plot combine harvester; grain yield was determined by weighing grains from individual plots and the result was converted to 0% moisture. Samples of shoot biomass at the BBCH 30 growth stage (beginning of stem elongation), BBCH 45 growth stage (late boot stage) and BBCH 51 (beginning of heading) were taken from a 0.25 m^2 area. The N:S ratio was determined using the weight concentrations expressed in g kg^{-1} of N and S in shoot biomass according to Blake-Kalff et al. (2000).

Levels of 150 kg N ha^{-1}, 200 kg N ha^{-1} and 150 kg N ha^{-1} + 40 kg S ha^{-1}, respectively, were applied in mineral fertilizers using two techniques of fertilizers application: topdressing in three split N doses on the soil surface during spring vegetation and N fertilizers injection into soil at the beginning of spring vegetation, respectively. Each treatment had four replicates. Detailed characteristics of the experimental scheme are given by Sedlar et al. (2015).

Plant N status was assessed by critical N concentration (N_c) expressed by Justes et al. (1994). Nitrogen nutrition index (NNI) was calculated as a ratio of measured shoot total N concentration (%) and N_c (Sadras and Lemaire, 2014). To reduce the influence of N status on evaluation of S uptake, only plant samples with optimal range of N concentration in shoot biomass were included in our results, i.e. samples with NNI = 0.90-1.10 at the BBCH 30 and BBCH 45 growth stages and NNI = 0.85-1.15 at the BBCH 51 growth stage.

Total N concentration in shoot biomass was determined by the Kjeldahl method using the Vapodest 50s (C. Gerhardt, Königswinter, Germany). Grain protein content was expressed as N × 5.7. Sulphur content in shoot biomass was determined using with optical emission spectroscopy with inductively coupled plasma (ICP-OES Agilent 720; Agilent Technologies, Santa Clara, California, USA). Zeleny sedimentation volume and a wet gluten content in DM of grain were determined using the NIR OmegAnalyzer G (Bruins Instrument, Salem, Massachusetts, USA). Due to a significant effect of location on the previously mentioned variables, all variables were expressed as relative values related to the averages of individual locations (Table 1).

Critical S concentration (S_c) in shoot biomass was calculated according to Reussi et al. (2012):

$$S_c = 0.37\ DM^{-0.169} \quad [1]$$

where DM is shoot DM (t ha^{-1}). Based on our results, the constant value S_c = 0.55 was used for DM < 1.0 t ha^{-1} (see Results and Discussion).

Table 1. Average values of variables at individual experimental locations (L1-L3) across all experimental years.

Location	TGW	Zeleny sedimentation volume	GPC	WGC	Grain yield
	g	mL	—— %	——	t ha^{-1}
L1	47.8c	45.7b	13.0a	26.5a	11.10a
L2	40.2a	52.2a	12.9a	24.9b	7.20b
L3	44.5b	52.8a	13.8b	28.8c	8.93c

Values within the column marked with the same letter are nonsignificantly different according Tukey test ($p \leq 0.05$).
TGW: Thousand grain weight; GPC: grain protein content; WGC: wet gluten content.

The SNI was calculated analogously to NNI expressed by Sadras and Lemaire (2014):

$$SNI = S_m/S_c \qquad [2]$$

where S_m is total S content measured in shoot biomass (%) and S_c is a critical S concentration calculated from the model of S dilution critical curve (Reussi et al., 2012).

Linear regression and logarithmic regression were used for calculation of correlations among studied variables. A statistical analysis of data was carried out using Statistica 13 (TIBCO Software Inc., Palo Alto, California, USA). A standard ANOVA with the Tukey test was used to evaluate differences of variables among individual experimental locations.

RESULTS AND DISCUSSION

Critical S concentration for low shoot biomass

The calculation of critical S concentration for shoot DM weight lower than 1.0 t ha^{-1} has not been defined by the model of S dilution critical curve (S_c) expressed by Reussi et al. (2012). However, in our experiments, 58% of all plant samples achieved lower weight of shoot DM than 1.0 t ha^{-1} at the BBCH 30 growth stage indicating a need for definition of the critical S concentration for low shoot biomass. For the calculation of the NNI, Justes et al. (1994) and Yue et al. (2012) expressed a N_c for low shoot biomass by a constant value calculated as an average N concentration in shoot biomass of non-limiting N treatments.

Based on our results, the constant value of the critical S concentration for low shoot biomass was taken to be $S_c = 0.55$. The value of 0.55 is an average S concentration in shoot biomass when shoot DM did not exceed 1.0 t ha^{-1} under non-limiting S conditions (data not shown). In that case, wheat plants had a N:S ratio in shoot biomass lower than 14.9:1 (median 7.32:1) at the BBCH 30 growth stage which was optimal for the highest grain yields of wheat according to the results of Blake-Kalff et al. (2000). To reduce the influence of plant N status on evaluation of S uptake, only plant samples with optimal N concentration in shoot biomass were included in our results, i.e. samples with values of NNI = 0.95-1.05 (0.99 median). On top of that, only plants with sufficient S content in grain i.e. grain N:S = 11-15:1 (median 12.6:1) (Tea et al., 2007) and simultaneously with sufficient S content in straw, i.e. straw N:S = 3-7 (4.79 median) (Steinfurth et al., 2012) were evaluated. The average S concentration was calculated out of 20 cases in total.

SNI at the BBCH 30 and 45 growth stages

Figure 1 describes a very strong correlation between the SNI at the BBCH 30 growth stage and the N:S ratio in shoot biomass at the BBCH 30 growth stage. After substituting the x-value in regression equation (Figure 1) for the upper limit of the optimal N:S ratio stated by Blake-Kalff et al. (2000), i.e. N:S = 14.9, SNI was equivalent to the value of SNI = 0.73 at the BBCH 30 growth stage.

The correlation between the SNI at the BBCH 45 growth stage and the N:S ratio in shoot biomass at the BBCH 45 growth stage was also very strong. After substituting the x-value in regression equation (Figure 2) for the N:S ratio determined by Calvo et al. (2008) at the BBCH 37 growth stage after the highest S supply, i.e. N:S = 11-13, SNI was equivalent to values of 0.84-0.72 at the BBCH 45 growth stage.

According to the findings of Hrivna et al. (2015) and Klikocka et al. (2016), S fertilization improves wheat grain yield, grain specific weight, protein content, gluten content and Zeleny sedimentation volume. Therefore, linear regressions

Figure 1. Correlation between the sulphur nutrition index at the BBCH 30 growth stage and the N:S ratio in shoot biomass at the BBCH 30 growth stage using the constant value of critical S concentration [S_c] = 0.55 for shoot DM < 1 t ha^{-1} under non-limiting N conditions (N nutrition index = 0.90-1.10). The number of cases was 138.

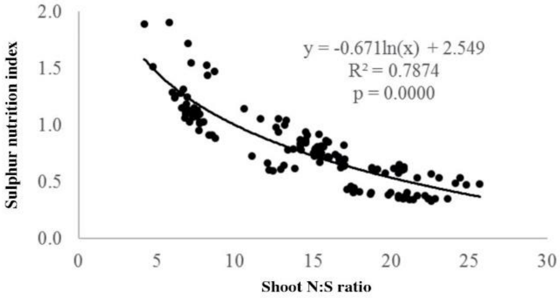
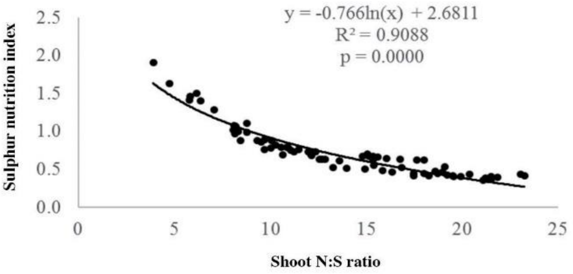


Figure 2. Correlation between the sulphur nutrition index at the BBCH 45 growth stage and the N:S ratio in shoot biomass at the BBCH 45 growth stage under non-limiting N conditions (N nutrition index = 0.90-1.10). The number of cases was 74.



describing correlations between the SNIs (y-values) at the BBCH 30 and BBCH 45 growth stages and both quantity and quality of winter wheat grain (x-values) are given in Table 2. The strongest correlation was recorded between the SNI at the BBCH 30 growth stage and relative Zeleny sedimentation volume in which case the correlation was moderate. The other variables correlated with the SNI at the BBCH 30 growth stage only weakly. To express the optimal values of the SNI, the x-value in regression equation was substituted for a value of 1 which is an average of studied relative variables. In that case, SNI achieved a value of 0.85 for average Zeleny sedimentation index. In case of SNI > 0.88, all studied relative parameters reached above-average values. With respect to grain quality of winter wheat, the SNI should be higher than the results suggest from correlation between the SNI at the BBCH 30 growth stage and the N:S ratio in shoot biomass at the BBCH 30 growth stage. On the other hand, only very weak correlations were recorded between the SNI at the BBCH 45 growth stage and studied relative variables (Table 2).

Table 2. Relations between relative both quantitative and qualitative parameters of winter wheat grain (x-values) and the sulphur nutrition index (SNI) (y-value) at the BBCH 30 (using the constant value of critical S concentration [S_c] = 0.55 for shoot DM < 1 t ha^{-1}) and BBCH 45 growth stage, respectively.

	SNI 30 (y)			SNI 45 (y)		
Relative parameter (x)	Linear regression equation	R^2	SNI-value if x = 1	Linear regression equation	R^2	SNI-value if x = 1
TGW	$y = 1.1338x - 0.2489$	0.111***	0.88	$y = 0.0228x + 0.6622$	0.000	0.69
Zeleny SV	$y = 0.6919x + 0.1563$	0.273***	0.85	$y = -0.0912x + 0.7599$	0.003	0.67
GPC	$y = 0.5941x - 0.0252$	0.051*	0.87	$y = -0.8242x + 1.5042$	0.032	0.68
WGC	$y = 0.766x + 0.1033$	0.095***	0.87	$y = -0.0644x + 0.7355$	0.001	0.67
Grain yield	$y = 0.5018x + 0.3726$	0.015	0.87	$y = -0.06255x + 1.2887$	0.032	0.66

*, **, ***Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

TGW: Thousand grain weight; Zeleny SV: Zeleny sedimentation volume; GPC: grain protein content; WGC: wet gluten content.

SNI at the beginning of heading

According to the results of Reussi et al. (2012), the model of S dilution curve was originally applicable only to the end of stem elongation of wheat. However, a very strong correlation was found between the SNI at the BBCH 51 growth stage and an N:S ratio in shoot biomass at the BBCH 51 growth stage (Figure 3). After substituting the x-value in regression equation (Figure 3) for the N:S ratio in shoot biomass stated by Steinfurth et al. (2012) as the optimum for the ear emergence growth stage, i.e. N:S = 13-14, SNI was equivalent to values of 0.64-0.59 at the BBCH 51 growth stage.

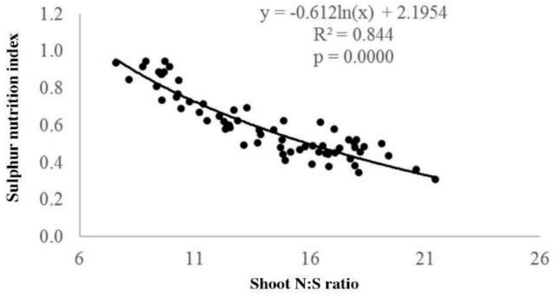
Moderate correlation was recorded between the SNI at the BBCH 51 growth stage and a relative Zeleny sedimentation volume and relative wet gluten content (Table 3). After substituting the x-value in regression equation (Table 3) for an average value of variable, i.e. x = 1, the SNI corresponded to values of SNI = 0.61 and SNI = 0.60, respectively. These values comply with the optimal SNI values, i.e. SNI = 0.64-0.59, derived from the relation between the SNI and the N:S ratio in shoot biomass for the BBCH 51 growth stage (Figure 3).

Table 3. Relations between relative both quantitative and qualitative parameters of winter wheat grain (x-values) and the sulphur nutrition index (SNI) (y-value) determined at the BBCH 51 growth stage.

Relative parameter (x)	Linear regression equation	R^2	SNI-value if x = 1
TGW	$y = 1.0357x - 0.4337$	0.029	0.60
Zeleny SV	$y = 0.6511x - 0.0380$	0.196***	0.61
GPC	$y = 0.9152x - 0.3171$	0.062*	0.60
WGC	$y = 1.0097x - 0.4094$	0.158***	0.60
Grain yield	$y = 0.6295x - 0.0401$	0.070*	0.59

*, **, ***Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
TGW: Thousand grain weight; Zeleny SV: Zeleny sedimentation volume; GPC: grain protein content; WGC: wet gluten content.

Figure 3. Correlation between the sulphur nutrition index at the BBCH 51 growth stage and the N:S ratio in shoot biomass at the BBCH 51 growth stage under non-limiting N conditions (N nutrition index = 0.85-1.15). The number of cases was 66.



The SNI at the BBCH 51 can be utilizable with respect to late S fertilization because Steinfurth et al. (2012) state that S fertilization at ear emergence growth stage prevents S deficiency in late stages of wheat growth. Zorb et al. (2009) state that the late S fertilization was found to improve the composition of gluten proteins and baking quality of winter wheat grain.

The fact that a nutrition index equal to 1 may not be always optimal for yield and quality of harvested products was also shown in case of NNI of various crops (Debaeke et al., 2012; Hu et al., 2014; Sedlar et al., 2017).

The SNI only weakly correlated with relative grain yield of winter wheat. Klikocka et al. (2016) also recorded higher effect of S fertilization on grain quality of wheat rather than on grain yield. According to the results of Kulhanek et al. (2014), nonsignificant effect of S fertilization on grain yield of winter wheat was also found under field conditions of the Czech Republic.

CONCLUSIONS

Calculation of the S nutrition index (SNI) using the model of Reussi et al. (2012) of S dilution curve was proved to be a reliable indicator of winter wheat S nutrition status even at the beginning of heading (BBCH 51). Calculation of the SNI was feasible also at the beginning of spring vegetation when shoot dry biomass weight was lower than 1.0 t ha^{-1}. In that case, the constant value of the critical S concentration in shoot biomass 0.55 was determined. Both optimal N:S weight ratio in shoot biomass and qualitative parameters of grain (particularly Zeleny sedimentation volume, grain protein content and wet gluten content) were recorded if the SNI exceeded values of 0.80 at the beginning of stem elongation (BBCH 31), 0.70 at the late boot stage (BBCH 45) and 0.60 at the beginning of heading (BBCH 51). Correlation between the SNI and relative grain yield was weak.

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6) Potential of Mehlich 3 Method for extracting plant available sulfur in Czech agricultural soils

Potenciál metody Mehlich 3 ke stanovení rostlinám přístupné síry zemědělsky využívaných půdách ČR

Metoda Mehlich 3 je již řadu let využívána ke stanovení obsahu mobilního P, K, Ca a Mg v půdě. Nespornou výhodou je možnost měření více prvků v jednom extraktu a dále celosvětové využití pro karbonátové i nekarbonártové půdy, např. ve Spojených státech, některých evropských a jihoamerických zemích, v Austrálii i některých asijských regionech (Gartley et al. 2002, Ring et al. 2004, Kulhánek et al. 2009 a 2014, Bortolon et al. 2011, Rayment et lyons 2012). Při pokroku současné měřící techniky (využití optické emisní spektrometrie) se nabízí možnost využití této metody pro stanovení síry, mikroelementů, popřípadě i některých rizikových prvků. Cílem této práce bylo určit využitelnost metody Mehlich 3 pro stanovení rostlinám přístupné síry. Za tímto účelem bylo analyzováno 98 vzorků půd z různých půdně klimatických podmínek. V těch byla stanovena síra extrakcí Mehlich 3 (Mehlich 1984) a dalšími extrakčními postupy, zahrnujícími přístupné i pevně vázané formy S. Dosažené výsledky ukazují na velmi dobrý potenciál metody Mehlich 3 pro stanovení přístupné síry v půdě. To bylo potvrzeno těsnými korelacemi s obsahem přístupné síry v půdě stanovené vodným výluhem a rovněž adsorbované síry stanovené 0,032 mol/l NaH_2PO_4, kde korelační koeficienty přesakovaly dokonce hodnotu 0,85. Naopak korelace výsledků Mehlich 3 s nepřístupnými frakcemi síry (zejména organickými) byly zpravidla neprůkazné. Je tedy možné tvrdit, že extrakcí Mehlich 3 je stanoven pouze signifikantní podíl přístupné minerální síry, což dokládají i podobné absolutní hodnoty (S v Mehlich 3 a přístupné S). Předběžné výsledky srovnání obsahu síry stavnovené v Mehlich 3 s obsahy síry v rostlinách dále ukazují průkazný vzájemný vztah u řepky ozimé a pšenice ozimé. Proto je připravována další podrobná studie (cca ze 350 stanovišť) zaměřená na srovnání obsahu síry v půdě, v rostlině a odběru S rostlinu, včetně sledování stability výsledků dosažených Metodou Mehlich 3 v rámci vegetační sezóny.

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Potential of Mehlich 3 method for extracting plant available sulfur in the Czech agricultural soils

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ABSTRACT

Kulhánek M., Černý J., Balík J., Sedlář O., Suran P. (2018): Potential of Mehlich 3 method for extracting plant available sulfur in the Czech agricultural soils. Plant Soil Environ., 64: 455–462.

Mehlich 3 is an extractant used worldwide for extracting bioavailable nutrients in soils; however, its extraction abilities for sulfur (S) are still not well described. The aim of this preliminary study was to compare the results of Mehlich 3 determined soil S fraction (S_{M3}) with the results of sulfur fractionation, mainly focusing on bioavailable S (S_{av} – sum of water-extractable (S_w) and adsorbed (S_{ads}) sulfur). Air dried soil samples from commonly used agricultural soils were chosen for the analyses. The following S fractions were determined: (i) S_w; (ii) S_{ads}; (iii) S_{av}; (iv) 1 mol/L HCl extractable (S_{HCl}); (v) estersulfate (S_{es}); (vi) organic (S_{org}) and (vii) total (S_{tot}). The median value of S_{M3} (18.3 mg/kg) was similar to S_{av} (17.9 mg/kg). From the correlation and regression analysis it is clear that S_{M3} results are in close relationship with S_{av} form. On the other hand, the relationships between S_{M3} and organic S (including S_{HCl}) were very weak. Based on the obtained results it can be concluded that Mehlich 3 method has a good potential to determine bioavailable sulfur in commonly used agricultural soils. However, especially the plant response should be further studied to confirm this theory.

Keywords: sulfur bioavailability; macronutrient; soil extractant; soil testing; soil tests comparison

Soil testing plays a vital part in providing field-based nutrient-management feedback to growers. To improve responses from nutrient inputs and to minimize environmental degradation, cost-effective, rapid soil tests are required to enable site-specific recommendations (Ostatek-Boczynski and Lee-Steere 2012). Universal soil extractants are being used routinely in soil test laboratories engaged in soil fertility evaluation, mainly to improve efficiency and applicability to a wide range of soils. Mehlich 3 method (M3) is widely used since it is capable of easily and rapidly determining the elements, phosphorus, potassium, calcium, magnesium, sodium, boron, copper, iron, manganese and zinc, and it is an extractant suitable for all acid soils and soilless mixtures (Jones 1990, Rao and Sharma 1997, Monterroso et al. 1999). Mehlich (1984) proposed a solution, M3, to expand utility to a wide range of soil types. It is widely used, particularly in the United States as well as in some European, South American, Australian and Asian countries for both, calcareous and non-calcareous soils (Gartley et al. 2002, Ring et al. 2004, Kulhánek et al. 2009, Bortolon et al. 2011, Rayment and Lyons 2012, Kulhánek et al. 2014).

However, there is only a few studies focused on M3 as an extractant for sulfur (Rao and Sharma 1997, Matula 1999, Ketterings et al. 2011, Kowalenko et al. 2014, Zbíral et al. 2018). It is probably due to the fact that sulfur was an underestimated macroelement because of a good S supply from atmospheric deposition and wide use of S-containing

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fertilizers almost until the end of the 20th century (Scherer 2001, 2009). However, the content of mineral sulfur forms in soil rapidly decreased in the last three decades (Balík et al. 2009, Scherer 2009). Therefore, mineral sulfur deficiency starts to be an actual problem in many locations (Eriksen 2005, Lehmann et al. 2008, Balík et al. 2009, Scherer 2009, Kulhánek et al. 2016). Because of these facts, relatively new methods have been developed and further improved for extracting of bioavailable and other soil sulfur forms (e.g., Shan et al. 1992, Blair et al. 1993, Morche 2008, Förster et al. 2012). Yet, most of these methods are focused only for sulfur determining and because of that, they are usually more expensive in comparison to multi-elemental methods. Therefore, it is obvious to compare the results of these methods with M3, which, in case of good correlations, can be further used for determining of bioavailable S forms with the advantage of simultaneous measurement with other macro and micronutrients.

It has been reported that anions, such as acetate and nitrate, are capable to extract S from the soils. The ability for replacing SO_4^{2-} tends to be low as compared to the phosphate (PO_4^{3-}) anion (Chao and Thomas 1963). The M3 extractant contains acetate and nitrate anions, hence, the possibility of using this extractant for S cannot be ruled out (Rao and Sharma 1997). On the other hand, some of the extractants focused on mineral or directly bioavailable sulfur can extract significant amounts of organic S (Alewell 1993). Because of this, it is important to know the relationships between M3-determined S and organic sulfur compounds.

The aim of this research is to compare the results of M3-determined sulfur with different sulfur fractions in the set of soil samples taken up from the sites with a wide range of pH values as well as the sulfur contents. Due to the use of correlation analysis, it will be possible to estimate the sulfur form, which is in the closest relationship to the M3 method. Based on the results it will be possible to predict the potential of M3 to extract plant available S.

MATERIAL AND METHODS

Experimental setup. Archive, air dried (< 2 mm) soil samples from the long-term field experiments of the Czech University of Life Sciences in Prague and the Central Institute of Supervising and Testing in Agriculture in Brno were chosen for the analyses. These samples represent a common range of agronomy-used soil types, fertilizing treatments, as well as the high range of water-extractable S contents and pH values (Table 1, Figure 1). Total number of samples analysed was 98. All of them were taken up after the harvest of currently grown crops (summer/autumn) as it is usually done by collecting the samples for determining other nutrients using M3.

Laboratory analysis. Soil texture was determined using the areometric method according to Casagrande and Loos (1934). The following analyses were realized: C_{org} (total organic carbon content in soil) – oxidation with potassium dichromate and sulfuric acid (ISO 14235, 1998). N_{tot} (total nitrogen content in soil) – Kjeldahl method (ISO 11261, 1995). The pH values – 0.01 mol/L $CaCl_2$ extracts (ISO 10390, 2005).

For the contents of S_w (water-extractable (readily available) S); S_{ads} (adsorbed S) and S_{HCl} (0.1 mol/L HCl extractable S) – the fractionation after Morche (2008) was used: a soil sample was firstly extracted with demineralized water (1:10 v/w), followed with 0.032 mol/L NaH_2PO_4 (1:10 w/v) and 1 mol/L HCl (1:20 w/v) extraction. For the S_{HI} (hydroiodic acid reducible S), the method after Shan and Chen (1995 modified by Morche 2008) was used: 0.5 g of soil was weighed into a boiling flask. This was connected to the Jonshon-Nishita digestion system followed with addition of 15 mL of reducing solution (mixture of hydroiodic, formic and hypophosphoric acid in the ratio 4:1:2); this suspension was heated (in 140°C oil bath) for 50 min under nitrogen atmosphere. The developed H_2S gas was trapped in 10 mL of 0.05 mol/L NaOH solution and here converted to sulfate.

Total S (S_{tot}) content was determined in the digests obtained by the following decomposition procedure: Aliquots (0.5 g) of soil samples were decomposed with a mixture of 8 mL concentrated nitric acid, 5 mL of hydrochloric acid, and 2 mL of concentrated hydrofluoric acid. The mixture was heated in an Ethos 1 (MLS GmbH, Leutkirch im Allgäu, Germany) microwave-assisted wet digestion system for 33 min at 210°C. After cooling, the digest was quantitatively transferred in Teflon® vessel and evaporated to dryness at 160°C. The digest was then dissolved in a 3 mL nitric and hydrochloric acid mixture (1:3), transferred into a glass tube, filled up by deionized water and measured. The

Table 1. Basic characteristics of the used soils

Site	*n*	Soil type	Altitude (m a.s.l.)	Average yearly temperature (°C)	Average yearly rainfall (mm/m^2)	Soil texture < 0.001 (mm)	Soil texture 0.001–0.01 (mm)	Soil texture 0.01–0.05 (mm)	Soil texture 0.05–0.25 (mm)	Soil texture 0.25–2 (mm)	N_{tot} (%)	C_{org} (%)	pH
1	6	Arenic Luvisol	345	7.1	798	10.5	17.8	56.1	13.8	1.80	0.08	0.99	5.7
2	12	Haplic Luvisol	265	8.2	574	4.36	21.3	60.0	5.20	13.5	0.11	0.98	5.9
3	6	Slightly Gleyic Cambisol	472	7.4	573	6.70	13.7	16.6	18.8	44.0	0.12	0.80	5.8
4	14	Haplic Cambisol	525	7.0	665	5.84	24.0	25.4	9.40	41.2	0.09	1.24	5.1
5	6	Gleyic Fluvisol	425	7.5	535	18.1	20.7	38.5	14.1	8.70	0.12	1.03	6.1
6	6	Dystric Cambisol	645	6.1	605	5.90	16.6	20.5	25.6	21.4	0.14	1.09	5.7
7	6	Haplic Luvisol	460	7.6	606	10.8	16.9	16.1	19.3	37.0	0.14	0.88	7.1
8	6	Mesobasic Cambisol	505	7.7	632	7.90	17.8	24.5	20.9	28.9	0.13	0.99	5.5
9	6	Haplic Luvisol	196	9.2	551	17.3	14.8	26.4	30.4	11.5	0.12	1.06	6.6
10	6	Haplic Albeluvisol	290	8.0	650	10.1	18.0	61.8	8.70	1.50	0.12	0.97	5.9
11	6	Haplic Chernozem	300	8.4	581	16.2	16.0	40.9	23.9	3.10	0.12	1.34	7.2
12	12	Haplic Chernozem	286	9.1	495	3.18	17.4	56.6	6.14	19.9	0.12	1.11	7.4
13	6	Haplic Chernozem	247	8.3	454	21.7	16.5	26.6	24.0	11.2	0.17	1.53	6.4

n – number of soil samples per locality (archive samples from different fertilizing treatments and years); N_{tot} – total nitrogen content in soil; C_{org} – total organic carbon content in soil

certified reference material RM 7003 Loam was applied for the quality assurance of the analytical data. For the comparison with other fractions, M3 extraction (Mehlich 1984) was used to obtain S_{M3} (Mehlich 3 extractable S), where the soil samples were extracted using 0.2 mol/L CH_3COOH, 0.25 mol/L NH_4NO_3, 0.015 mol/L NH_4F, 0.013 mol/L HNO_3, 0.001 mol/L $(HOOCCH_2)_2NCH_2CH_2N$ (EDTA) in the ratio 1:10 (w/v) with 5 min of shaking.

The concentrations of S in soil digests and extracts were determined using the optical emission spectroscopy with inductively coupled plasma

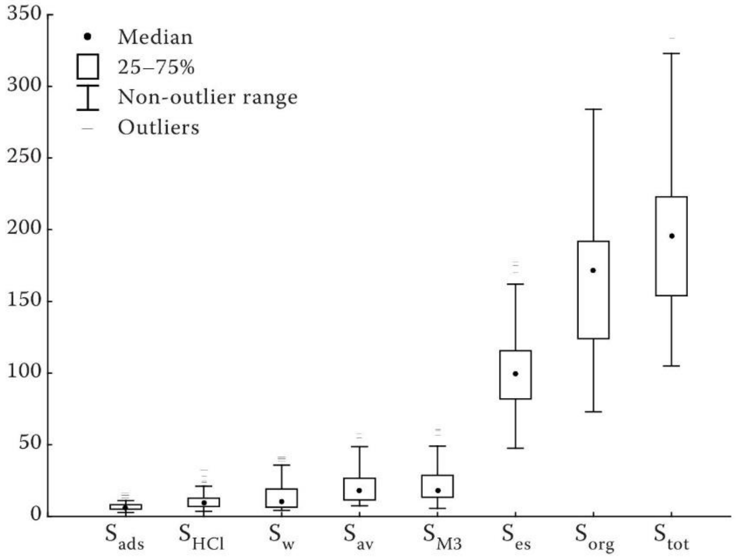

Figure 1. Basic statistical characteristics of the results (values in mg/kg) (*n* = 98). S_{ads} – adsorbed S; S_{HCl} – 0.1 mol/L HCl extractable S; S_w – water-extractable (readily available) S; S_{av} – bioavailable S; S_{M3} – Mehlich 3 extractable S; S_{es} – estersulfate S; S_{org} – organic S; S_{tot} – total S

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(ICP-OES) with axial plasma configuration, Varian, VistaPro, equipped with autosampler SPS-5 (Mulgrave, Australia). Operating measurement wavelength for ICP-OES was 180.7 nm for S. The measurement conditions were: power 1.2 kW, plasma flow 15.0 L/min, auxiliary flow 0.75 L/min, nebulizer flow 0.9 L/min.

Measurement using ICP-OES was chosen intentionally in this research because it is commonly used for analysis of M3 extracts allowing simultaneous extraction of bioavailable macro and micronutrients in soil. This is the main advantage of M3 against other extractants specialized on S only. On the other hand, ICP-OES measures also some part of organic S in extracts focused on inorganic S only. The ratio of organic S in S_w and S_{ads} extracts is usually very low, but in S_{HCl} extracts on non-calcareous soils it is usually significant. Because of that, ion chromatography (IC) (Kowalenko and Grimmett 2007) or capillary electrophoresis (Zbíral 1998, 1999) are commonly used for the precise measurement of the inorganic S.

Derived measures and data analysis. The content of S_{av} (bioavailable S) was calculated as the sum of S_w and S_{ads}, because water soluble and adsorbed S are generally believed to be immediate S source for plants (Förster et al. 2012), the content of S_{es} (estersulfate S) sulfur as $S_{es} = S_{HI} - S_{av}$ and the approximate content of S_{org} as $S_{org} = S_{tot} - (S_{av} + S_{HCl})$, respectively.

For the basic statistical evaluation (medians, averages, linear regression), Excel (2016) was used and for further analyses (box-plots, normality of distribution and Pearson's correlation analysis) Statistica ver. 13.2 (Dell software 2016) was used.

RESULTS AND DISCUSSION

Descriptive statistics. Figure 1 represents the basic statistical evaluation of the obtained results. The median values of extracted S increased in the order $S_{ads} < S_{HCl} < S_w < S_{av} < S_{M3} < S_{es} < S_{org} < S_{tot}$, and the contents reached 6.15, 9.41, 10.3, 18.0, 18.2, 99.7, 171 and 196 mg S/kg, respectively. The interesting fact is that the value determined using the M3 extractant corresponds closely with the content of bioavailable sulfur, where the value obtained with M3 was only by 0.2 mg S/kg higher in comparison to S_{av}. The content of S_{av} represented 9.19% of S_{tot}. The sum of all determined S forms (except S_{tot}) reached the higher values than S_{tot}. It was caused by the extraction methods overlapping; e.g. S_{es} represents the significant part of S_{org}.

Correlation analysis. The most important part of this research was to evaluate the relationships between M3 and other determined S forms. For this purpose, the correlation and regression analysis was carried out. The results of the Pearson's correlation analysis are mentioned in Table 2. From the obtained results, it is clear that the S_{M3} values corresponded well with the contents of S_{ads} and S_w, where the correlation coefficients reached 0.73 (with S_{ads}) and 0.886 (with S_w), respectively. Therefore the correlations between S_{M3} and S_{av} were also very high (0.882). On the other hand, the relationships between the S_{M3} and organic S compounds were weak. The correlation coefficient between S_{M3} and S_{org} was 0.349 (with lower significance $P \leq 0.01$) and with S_{es} reached the nonsignificant value 0.112, respectively. The significance increased by comparison of S_{M3} with

Table 2. Pearson correlation coefficients between the sulfur (S) fractions (n = 98)

	S_w	S_{ads}	S_{av}	S_{HCl}	S_{M3}	S_{org}	S_{es}
S_{ads}	0.809***						
S_{av}	0.992***	0.877***					
S_{HCl}	0.274**	0.297**	0.288**				
S_{M3}	0.886***	0.731***	0.882***	0.391***			
S_{org}	0.248*	0.066	0.217*	0.406***	0.349**		
S_{es}	0.076	–0.099	0.040	0.413***	0.112	0.761***	
S_{tot}	0.436***	0.255*	0.411***	0.515***	0.517***	0.975***	0.728***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; S_{ads} – adsorbed S; S_{av} – bioavailable S; S_{HCl} – 0.1 mol/L HCl extractable S; S_{M3} – Mehlich 3 extractable S; S_{org} – organic S; S_{es} – estersulfate S; S_{tot} – total S; S_w – water-extractable (readily available) S

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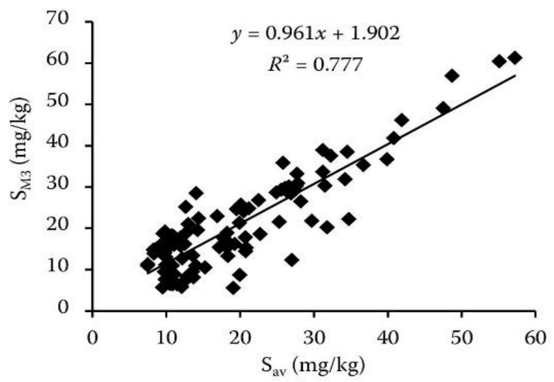

Figure 2. Regression analysis between S_{M3} (Mehlich 3 extractable S) and S_{av} (bioavailable S) (n = 98)

S_{tot} (r = 0.517). It was probably caused due to the fact that S_{tot} contains also inorganic S forms, which showed a close relationship with S_{M3}. The correlations between S_{M3} and S_{HCl} were significant at $P < 0.001$, but the r value was only 0.391. It has been described in many studies that a significant part of organic S is extracted by hydrochloric acid (discussed further). Further analyses confirmed highly significant relationships of S_{tot} with S_{org}, as well as with S_{es}. The correlations between S_{org} and S_{es} were also very high.

Regression analysis. It was carried out to confirm the results of correlation analysis (Figure 2, Table 3). Based on the main aim of this study, the relationships between S_{M3} and other soil S fractions are shown here. From the comparison of exponential, logarithmic, polynomic and linear regression, the last one showed the best results in most of the cases. Figure 2 shows the relationship between S_{M3} and S_{av}. This confirmed the results from the previously mentioned correlation analysis. The regression coefficient $R^2 = 0.777$ confirms very close relationships between M3-determined S and bioavailable S. With 77.7% of reliability it can be also calculated that $S_{M3} = 0.961 \times S_{av} + 1.902$.

The following results of regression analysis between S_{M3} and other S fractions are presented in Table 3. The results correspond with the data obtained by correlation analysis. It confirms the ability of M3 extractant to determine the bioavailable S forms due to the close relationships with both, S_w and S_{ads}, respectively. On the other hand, regression relationships with organic S forms and S_{HCl} are very weak.

DISCUSSION

According to the previous studies, readily available sulfur is in form of the SO_4^{2-} ions dissolved in the soil solution. The main ratio of these ions can be extracted with distilled water and other weak solutions, e.g., $CaCl_2$ or $LiCl_2$ (Walker and Doomenbal 1972, Tabatabai 1982). However, this form is not the only one that can be taken up with plants. There are also sulfates bound with the weak binds on the soil particles – adsorbed S. These can be usually easily released into the soil solution and subsequently taken up with plant roots. Therefore, these ions are also believed to be plant available (Förster et al. 2012). Because of that, the plant available sulfur content represents the sum of S_w and S_{ads}, which also corresponds with many literature sources (Bohn et al. 1986, Tisdale et al. 1993). The majority of the sulfur taken up by plants is derived from the soil organic S pool (Boye et al. 2010), from which S is transformed in adsorbed and water-extractable forms as well. Because of that, organic S pool is also very important (especially from the long-term point of view); however, it could not be directly taken as a bioavailable pool.

From the obtained data it is clear that S_{av} represents only a small part of the total sulfur. As

Table 3. The results of regression analysis between S_{M3} (y value) and other sulfur (S) fractions

	a	*b*	R^2		*a*	*b*	R^2
S_{M3}:S_W	1.184	5.274	0.784	S_{M3}:S_{es}	0.043	16.94	0.013
S_{M3}:S_{ads}	3.659	–3.021	0.534	S_{M3}:S_{org}	0.081	7.782	0.122
S_{M3}:S_{HCl}	0.827	12.43	0.153	S_{M3}:S_{tot}	0.106	0.083	0.267

Linear regression parameters $y = ax + b$; a – slope; b – intercept; R^2 – regression coefficient; S_{M3} – Mehlich 3 extractable S; S_w – water-extractable (readily available) S; S_{ads} – adsorbed S; S_{HCl} – 0.1 mol/L HCl extractable S

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confirmed e.g. by Tisdale et al. (1993), Wang et al. (2006), Balík et al. (2009) or Förster et al. (2012) bioavailable (mineral sulfur) forms represents less than 10% of S_{tot}. In our case, the average value of 9.19% was obtained. The relatively higher value is probably caused due to the fact that using the ICP-OES some part of dissolved organic S is measured (Shan et al. 1992, Kowalenko and Grimmett 2007).

The interesting fact is that the average value determined by the M3 extractant (18.2 mg/kg) corresponds closely with the average content of S_{av} (18.0 mg/kg). The higher S_{M3} values were expected because the M3 is one of the strongest extractants to determine bioavailable forms of phosphorus (Kulhánek et al. 2009, Wuenscher et al. 2015) and other macroelements (Zbíral and Němec 2005, Bortolon et al. 2011, Ostatek-Boczynski and Lee-Steere 2012).

Similar relationships between S_{M3} and S_w were found in the study of Kowalenko et al. (2014). These authors evaluated 109 contrasting soils, where the average values of S_{M3} and S_w were almost the same. Based on their results it is possible to assume that water extracts similar soil S pool as M3 and multiple-element soil extraction methods show a considerable promise for soil S testing.

The order of $S_{es} < S_{org} < S_{tot}$ is logical, because S_{org} represents the main part of S_{tot} and S_{es} a large part of S_{org}. Similar distribution of soil sulfur fractions was confirmed e.g. by Morche (2008), Scherer (2009), Kulhánek et al. (2011), Förster et al. (2012).

The correlation analysis shows close relationships ($r = 0.883$) between both bioavailable S forms (S_w and S_{ads}) and S_{M3}. Therefore it is clear, that M3 extractant determines a significant amount of bioavailable S. The relationship between S_{HCl} and S_{M3} is much lower ($r = 0.391$). It is probably due to the extraction abilities of 1 mol/L HCl. This extractant was developed to determine occluded S form that is bound in Ca and Mg co-precipitated forms. This form is only possible to measure using the ion chromatography (Förster et al. 2012). Yet, in the case of ICP measurement, a significant amount of organic S-compounds is determined especially in low-carbonates soils (Shan et al. 1992). The results of Morche (2008) show that in soils with less than 1% $CaCO_3$, most of the S extracted with 1 mol/L HCl is organically bonded S. In our case, 79.6% of the tested soils had less than 1% of $CaCO_3$. Therefore, it is possible to assume that a significant ratio of the ICP-measured S_{HCl} was organically bonded S. The lower correlation coefficient between S_{HCl} and S_{M3} also shows that M3 extractant probably does not extract the significant part of organic S. It is confirmed also by lower correlations between S_{M3} and S_{org} and no significant correlation between S_{M3} and estersulfate S.

Additional observations confirmed close relationships between S_{ads} and S_w, S_{ads} and S_{av} and S_w and S_{av}. The close correlations between S_{org} and S_{tot} as well as between S_{es} and S_{tot} confirm that significant ratio of soil sulfur is organically bonded (Morche 2008, Scherer 2009, Kulhánek et al. 2011, Förster et al. 2012 and others).

As mentioned before, M3 is a widely used extractant allowing to determine simultaneously many important macro and micronutrients in different soils (Jones 1990, Rao and Sharma 1997 and others). Therefore, determination of S using M3 could represent a cost-saving way to evaluate bioavailable sulfur together with other nutrients. In this preliminary study, close relationships between S_{M3} and soil sulfur forms were found, which are described as bioavailable in the literature. In the other set of plant and soil samples taken up from fields with a wide range of different soils ($n = 152$) during vegetation, significant correlations ($r > 0.350$; $P < 0.001$) between S_{M3} content and total S content in winter wheat and winter rape were found. However, the correlations were not confirmed for maize. These data are not published here, because the further research of plant response is needed.

The study of the set of 98 samples from different commonly used agricultural soils showed that S content determined using the M3 method closely corresponds with the amounts of bioavailable S fractions. That was confirmed with both, correlation and regression analyses as well by the fact that relationships between S_{M3} and organic S fractions were usually very weak. M3 can be also evaluated as a suitable extractant for bioavailable sulfur for the commonly used arable soils. However, this manuscript is only a preliminary study and brings a lot of new questions. To confirm this preliminary study, further research of S_{M3} and S content and uptake by different plant species is needed. It is also important to further study the relationships between M3-determined soil sulfur and soil pH, organic matter content and enzymatic activity.

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7) Using of active microorganisms of *Pseudomonas* genus during cultivation of maize in field conditions

Využití aktivních mikroorganismů rodu *Pseudomonas* pro pěstování kukuřice v provozních podmínkách.

V současné době je intenzivně řešena možnost použití různých biostimulantů určených k mobilizaci fosforu z hůře přístupných forem jak v půdě, tak v dodaných hnojivech. Existuje již řada pozitivních výsledků, které však zpravidla pocházejí z inkubačních testů ve sterilním prostředí nebo z nádobových pokusů. V případě bakterií *Pseudomonas sp.* se jedná např. o studie Vallabhaneni (2016), Israr et al. (2016) Yusran et al. (2009). Je však obecně známo, že od nádobových pokusů k běžnému využití v polních podmínkách vede ještě dlouhá cesta. Proto byly založeny maloparcelkové přesné polní pokusy zaměřené na aplikaci bakterie *Pseudomonas sp.*, kmen DSMZ 113134 do půdy, které probíhaly 3 roky na stanovišti Humpolec. Zároveň byly zkoušeny různé možnosti aplikace, a to jak v kombinaci s hnojivy (mletý fosfát, trojitý superfosfát, dusíkatá hnojiva s amonným a nitrátovým N), tak různými způsoby aplikace (plošná, lokální, aplikace *Pseudumonas sp.* v kapalné i granulované formě). Cílem bylo zjistit vliv uvedených strategií na výnos kukuřice a obsah živin v nadzemní hmotě se zaměřením na fosfor. Statistické vyhodnocení ukázalo, že vliv biostimulantů na zvýšení obsahu fosforu v nadzemní hmotě kukuřice byl neprůkazný, a to ve všech sledovaných ročnících. Naopak se ukázal průkazný vliv ročníku na obsah P v rostlinách. Shrnutí všech pokusů naznačuje, že aplikace *Pseudomonas sp.* má pozitivní vliv na obsah K, Mg a S v nadzemní hmotě kukuřice a zároveň na celkový odběr Ca a S. Celkovou malou efektivitu biostimulantů v našich pokusech je možno přičítat zejména půdním vlastnostem stanoviště, které pravděpodobně nebyly vhodné pro přežití aplikovaného kmene bakterie. V mnohých studiích je publikován pozitivní vliv biostimulantů (včetně *Pseudomonas*) na regulaci různých patogenů (Mikicinski et al. 2016, Vallabhaneni 2016, Wu et al. 2017). Je tedy možné usuzovat, že případný pozitivní účinek biostimulantů na obsah a odběr živin může být pouze „vedlejším efektem“ lepšího zdravotního stavu rostlin. Pro potvrzení této domněnky je třeba další podrobný výzkum.

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Use of active microorganisms of the *Pseudomonas* genus during cultivation of maize in field conditions

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ABSTRACT

Holečková Z., Kulhánek M., Hakl J., Balík J. (2018): Use of active microorganisms of the *Pseudomonas* genus during cultivation of maize in field conditions. Plant Soil Environ., 64: 26–31.

The aim of this research is to estimate the influence of a bioeffector (BE) application on dry matter yield and nutrient content (P, K, Ca, Mg, S) in maize (*Zea mays* L.). Between 2014 and 2016, a field experiment with silage maize as a testing plant was realized on sandy loam Cambisol. The application of *Pseudomonas* sp. in combination with phosphorus (rock phosphate (RP) or triple superphosphate (TSP)) and nitrogen fertilizers (ammonium nitrate with urea, ammonium nitrate with limestone, calcium nitrate or ammonium sulfate with a nitrification inhibitor) and with different application strategies was studied. The effects of a bioeffector application on the increase of dry matter yields were not confirmed. An important influence on the BE application and its activity was probably those of soil and site conditions and competition of the researched microorganisms with other present microorganisms. Higher yields of dry matter were shown in treatments where P fertilizers were applied. There was almost no difference between the application of RP and TSP. This could be caused by the fact that the soil had a slightly acidic pH value. In this case, the RP showed similar results to the TSP. The application of bioeffector significantly increased Mg, K and S contents in maize above-ground biomass. An increase of the Ca content was almost significant and a tendency towards a higher average content of phosphorus was also recorded.

Keywords: plant nutrition; bioavailability; biocontrol; bacteria; fungi; organic farming

In past decades, agriculture and crop production were almost completely dependent on mineral fertilizers, and hence on natural sources of nutrients which are scarce and limited. As a result, there is need to develop new alternative ways to improve bioavailability of nutrients from the applied fertilizers. Development of these alternative ways requires understanding of plant-soil relationship, but also good knowledge of agriculture and environment (Whithers et al. 2014). One of many alternative ways is the tested application of products containing live and active microorganisms in plant production. These commercially produced preparations, so-called bioeffectors, contain two main components: live microorganisms (bacteria or fungi) and active natural compounds (plant and herbal extracts, dried seaweeds and soil and compost extracts).

Pseudomonas well suited as biocontrol and growth-promoting agents (Vallabhaneni 2016). These microorganisms can enhance availability of deficient or immobile nutrients in soils after solubilizing their mineral forms. The use of *P. putida* improved the growth and yield of various crops such as rice, tomato or wheat. The application of *P. putida* either alone or in combination with phosphorus improved plant growth, plant uptake (N, P, K) and antioxidative activity (Israr et al. 2016). Yusran et al. (2009) reported that the application

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of *Pseudomonas* sp. and *Bacillus amyloliquefaciens* (individually or in combination) into soil in a pot experiment led to improved state of tomato roots. They were healthy and showed significantly higher colonization by arbuscular mycorrhizal fungi. Liu et al. (2015) reported a positive effect of seed inoculation with diazotrophic bacteria on shoot dry weight and yield of maize. Products with this composition can be used in both conventional and organic farming and are developed for a wide range of agricultural and ornamental plants (Hogenhout et al. 2009, Neumann 2012). Most nutrients (mainly phosphorus) in soil are in a form that is barely available for plants. The main effect of bioeffectors should be to improve the bioavailability of nutrients for plants (Lošák et al. 2016). These products also contribute to a better plant health because they are also used against various diseases and pests. By promoting plant growth, they contribute to higher yields and better production quality (Janarthanam 2013, Vallabhaneni 2016, El-Gremi et al. 2017, Holečková et al. 2017).

This work has three main aims: (1) to assess the effect of the *Pseudomonas* sp. application in combination with P fertilizers on silage maize yield, and on the content of selected nutrients (P, K, Ca, Mg, S) in field conditions; (2) to confirm a potential of bioeffector Proradix (PDX) to promote maize growth and acquisition of mineral nutrients in soil with satisfactory available P content in the Czech Republic; (3) to test new, costs-saving, ways of bioeffector (BE) application together with different ways of nitrogen application.

MATERIAL AND METHODS

Small-plot field experiment with different ways of application of Proradix (*Pseudomonas* sp., strain DSMZ 113134, 5 × 10^{10} colony forming units/g, Sourcon Padena, Tübingen, Germany (further only PDX) to silage maize was realized in the years 2014–2016. The small-plot experiment was established at the Humpolec site (49°33'16''N; 15°21'18''E). Characteristics of the experimental field are following: Cambisol, sandy loam, 525 m a.s.l., average yearly temperature and rainfall 8.2°C and 573 mm, pH_{CaCl2}: 5.7. The content of bioavailable nutrients in soil estimated using the Mehlich 3 method is: 71 mg P/kg, 180 mg K/kg, 1200 mg Ca/kg and 120 mg Mg/kg. The plot size was 4.20 × 7.50 m. The distance between rows was 70 cm (6 rows per plot) and the number of seeds was 95 000 per ha. Maize seeds were untreated cv. Colisée in 2014 and 2015 and cv. Kartagos in 2016 (both from KWS, Einbeck, Germany). Twenty randomly selected plants from two centre rows were harvested from each plot.

Experimental design A. Different application strategies and combinations with P fertilizers were tested in the experiment A. For broad application, a dose of 22.7 kg/ha of PDX was used (dose per plot was diluted in 9 L of water) and for local application ten times lower amount (2 L of water solution per plot). Local application was conducted using a spike wheels applicator GFI 3A (Maschinen und Antriebstechnik GmbH, Güstrow, Germany). Broad application was conducted with sowing, where PDX was applied into soil to a depth of 10 cm immediately after spraying at soil surface. Local application was done at the 5th developed leaf. All fertilizers were used before sowing and applied in 10 cm soil profile. Two phosphorus fertilizers were applied with sowing – (i) fine milled rock phosphate – RP (7.9% P) and (ii) triple superphosphate – TSP (21% P). The whole experiment was fertilized with nitrogen (120 kg N/ha in calcium ammonium nitrate 27% N) and potassium (50 kg K/ha in Patentkali 24.9% K, 6% Mg) at sowing. The experimental design is shown in Table 1.

Experimental design B. The main aim of the experiment B (Table 2) was to test the cost-saving application strategies of PDX. PDX was always applied in bands (10 cm depth) into the rows next

Table 1. Experimental design A

Treatment	PDX Application	PDX	N	P	K
		(kg/ha)			
Zero control	0	0	120	0	50
Water control	broad	0	120	0	50
RP	broad	0	120	26	50
TSP	broad	0	120	26	50
PDX + RP	broad	22.7	120	26	50
PDX + TSP	broad	22.7	120	26	50
PDX + RP	local	2.27	120	26	50
PDX + TSP	local	2.27	120	26	50

PDX – bioeffector Proradix; RP – rock phosphate; TSP – triple superphosphate

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Table 2. Shortened experimental design B with terms of bioeffectors and fertilizers application

Treatment	PDX application	PDX	N	N application
		(kg/ha)		
Zero	–	0	0	–
N1	–	0	80 + 60 + 0	broad
N2	–	0	0 + 80 + 60	broad
Local N	–	0	0 + 140 + 0	local
N1 + PDX li	local	2.27	80 + 60 + 0	broad
N1 + PDX gr	local	2.27	80 + 60 + 0	broad
Local N + PDX li	local	2.27	0 + 140 + 0	local
Local N + PDX gr	local	2.27	0 + 140 + 0	local
N2 PDX liquid	local	2.27	0 + 80 + 60	broad

N1 – 80 kg N/ha 3 days before sowing + 60 kg N/ha at 2–3 developed leaf; N2 – 80 kg N/ha at 2–3 developed leaf + 60 kg N/ha; PDX – bioeffector Proradix (li – liquid; gr – granulated)

to the seeds (Figure 1). Two forms of PDX were used: (i) liquid (PDX li), where the application rate was 9 L of solution per plot or (ii) granulated (PDX gr) form where the granules were made by spraying the PDX stock solution on pumice stone (size 0–3 mm, Palkowitschia s.r.o., Prague, Czech Republic). The final dose of PDX was always 2.27 kg/ha. PDX was always applied 4 days after sowing. The PDX application was combined with three ways of nitrogen fertilizing: (i) 80 kg N/ha 3 days before sowing + 60 kg N/ha at 2–3 developed leaves (N1); (ii) 140 kg N/ha via the CULTAN strategy at 2–3 developed leaves (Figure 1; Local N) and (iii) 80 kg N/ha at 2–3 developed leaves + 60 kg N/ha (N2). For N1 and N2, calcium ammonium nitrate was used.

The aim of the CULTAN method is building of ammonium nitrogen depot in soil using high pressure injectioning. Roots grow around this reserve decreasing the ammonium toxicity through N uptake from the non-toxic reserve surface. As a result, N uptake is continuous and plants have the N supply available during the entire cropping season, which saves the costs for commonly used repeated N application (Sommer 2005).

Experimental design C. Ammonium releases the H^+ proton in microorganisms and plants can therefore improve solubilization of Ca-phosphates as a side effect (Neumann and Römheld 2002).

The aim was hence to test the potential of PDX to release phosphorus from rock phosphate improved with ammonium nitrogen fertilizing. The experimental design is shown in Table 3. The source of nitrogen was calcium nitrate – CN (15% N) or ammonium sulfate + dimethylphenylpiperazinium (DMPP) nitrification inhibitor – AS (21% N). CN was applied in two doses (short before sowing) and AS all at once short before sowing. The nitrate form in CN is very mobile in soil. Because of that, the dose was divided in two parts and the first (bigger) part was applied very shortly (one day) before sowing to be the source of available nitrogen for germinating plants. The second part was applied during vegetation to provide the nitrogen supply during vegetation. RP and TSP were applied also in one dose short before sowing. All fertilizers as well as PDX were applied broad.

Maize from all three experiments was always harvested in dough vegetation stage. For experiment A it was on 3rd September, for experiment

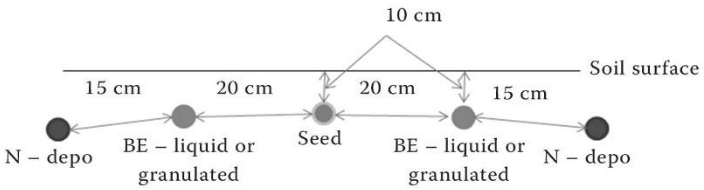

Figure 1. Scheme of the bioeffectors (BE) application together with Local N

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Table 3. Experimental design C

Treatment	PDX application	PDX	N	P
		(kg/ha)		
Zero	–	0	0	0
CN	–	0	107 + 54	0
AS	–	0	161	0
CN + RP	–	0	107 + 54	130
AS + RP	–	0	161	130
CN + RP + PDX	broad	22.7	107 + 54	130
AS + RP + PDX	broad	22.7	161	130
CN + TSP	–	0	107 + 54	130
AS + TSP	–	0	161	130

CN – calcium nitrate; AS – ammonium sulfate + dimethylphenylpiperazinium (DMPP) nitrification inhibitor; RP – rock phosphate; TSP – triple superphosphate; PDX – bioeffector Proradix

B on 11th September, and for experiment C on 14th September.

Analyses. Twenty average plants from two centre rows of each plot were always harvested to estimate the silage maize yield. A representative sample of three selected plants from each plot was air dried and finely milled for further analyses. Total content of macronutrients in the above-ground biomass was estimated using dry decomposition (Mader et al. 1998). The extracts were measured by an inductively coupled plasma optical emission spectrometry (ICP-OES, Varian VistaPro, Victoria, Australia). Nutrient uptake was calculated based on the content of elements in the above-ground biomass and the yield of maize from the small-plot.

Data evaluation. Due to different designs of the experiments each year, the data were analysed by ANOVA, where the year, bioeffector application and P fertilization were included. All analyses were carried out by Statistica ver. 12. software (California, USA) at a significance level of 0.05 (Tukey's *HSD* (honest significant difference)).

RESULTS AND DISCUSSION

Three-way analysis of variance (ANOVA) with interactions between external factors (year, bioeffector and P-fertilizer) was used to investigate differences in yield of dry matter and P, K, Ca, Mg and S content from 2014 to 2016 in the above-ground biomass of maize. Differences in dry matter yields over years depending on the application of bioeffector and P-fertilizers are shown in Table 4.

The application of bioeffector or P-fertilizers did not significantly affected dry matter yields. On the other hand, only the effect of year was particularly significant for dry matter yield where the highest content was observed in 2016 and the lowest in 2014.

Differences in the element content over years, application of bioeffector and P-fertilizers are shown in Table 5.

The results show that the application of bioeffector significantly increased the potassium, magnesium and sulfur content in maize above-ground biomass. The increase of Ca content was almost significant and a trend towards a higher average content of P was also observed. Also Lošák et al. (2010) described the effect of year in 2-year field experiments with graded fertilizer doses applied to maize. Application of P-fertilizers did not affect the content of any included element. On the other hand, the effect of year was significant for all elements, as the highest content was observed in 2015 and the lowest in 2016. The highest contents of K, Ca and S were probably caused by higher rainfall in 2015 or by site conditions.

Differences in the element uptake over years, application of bioeffector and P-fertilizers yield of dry matter are shown in Table 6.

The data obtained validated the results of nutrient contents mentioned in Table 5. The application of

Table 4. Effect of Proradix (PDX), phosphorus fertilization and year on the yield of biomass

		Dry matter yield (t/ha)
BE	0	18.6
	PDX	16.1
	P	0.478
P-fertilizer	0	15.1
	RP	19.5
	TSP	20.8
	P	0.035
Year	2014	10.8^a
	2015	15.1^a
	2016	26.7^b
	P	< 0.001

RP – rock phosphate; TSP – triple superphosphate. Different letters are significantly different at the 0.05 level

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Table 5. Effect of bioeffector Proradix (PDX), phosphorus fertilization and year on the concentration of elements (mg/kg) in maize above-ground biomass

		P	K	Ca	Mg	S
BE	0	2438	13 728^{a}	2688	1453^{a}	789^{a}
	PDX	2539	15 486^{b}	2891	1538^{b}	881^{b}
	P	0.210	< 0.001	0.095	0.049	0.012
P-fertilizer	0	2329	14 883	2878	1481	857
	RP	2416	14 601	2770	1505	820
	TSP	2521	14 338	2721	1500	829
	P	0.197	0.708	0.629	0.905	0.729
Year	2014	2674^{b}	11 072^{a}	1757^{a}	1545^{b}	568^{b}
	2015	2851^{c}	22 031^{b}	5089^{b}	1950^{c}	1464^{c}
	2016	1942^{a}	10 718^{a}	1523^{a}	991^{a}	473^{a}
	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

BE – bioeffector; RP – rock phosphate; TSP – triple superphosphate. Different letters are significantly different at the 0.05 level

bioeffector significantly increased Ca and S uptake in maize above-ground biomass. The application of P-fertilizers led to a slightly increased P uptake by plants. However, a decreasing tendency in Ca and S uptake was observed. Lower calcium and sulfur intake after application of P-fertilizers, compared to the control treatment, could have been caused by chemical sorption of HPO_4^{2-} and calcium. In case of sulfur, there could have been an antagonistic relation between HPO_4^{2-} and SO_4^{2-}. No effects depending on P fertilizing were significant. On the other hand, an effect of year was significant for all elements, as the highest content was observed in 2015 and the lowest in 2014. Also in this case, the highest nutrient uptake for all nutrients was probably caused by higher local rainfall in 2015 or by site conditions. Many authors carried out studies and experiments where they applied *Pseudomonas* and the results showed that the application of bacteria increased resistance of plants to many diseases (Mikicinski et al. 2016, Vallabhaneni 2016, Wu et al. 2017). Therefore, a higher uptake of all nutrients in 2015 could have been caused by the fact that plants began to defend themselves more against pathogens and thus they took up more nutrients.

Israr et al. (2016) reported that this bacteria genus is used as a biological fertilizer which, together with mineral fertilizers, can serve as an effective approach to enhance plant nutrition requirements. And that these microorganisms can increase avail-

Table 6. Effect of bioeffector Proradix (PDX), phosphorus fertilization and year on element uptake (kg/ha) in maize above-ground biomass

		P	K	Ca	Mg	S
BE	0	40.7	219.7	39.2^{a}	22.4	11.8^{a}
	PDX	40.2	251.1	48.2^{b}	24.7	14.5^{b}
	P	0.042	0.020	0.035	0.111	0.041
P-fertilizer	0	38.7	256.8	55.1^{b}	24.0	15.9^{b}
	RP	40.1	214.0	30.8^{a}	22.5	9.9^{a}
	TSP	45.2	202.0	29.5^{a}	23.1	9.9^{a}
	P	0.352	0.238	0.522	0.063	0.310
Year	2014	28.6^{b}	119.8^{a}	18.9^{a}	16.7^{b}	6.16^{a}
	2015	43.6^{a}	334.6^{c}	79.1^{c}	29.4^{a}	22.5^{c}
	2016	50.6^{a}	270.8^{b}	38.7^{b}	25.6^{a}	12.3^{b}
	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

BE – bioeffector; RP – rock phosphate, TSP – triple superphosphate. Different letters are significantly different at the 0.05 level

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ability of deficient or immobile nutrients in soil after dissolution of their mineral forms. This fact has been confirmed for K, Mg and S content, as well as Ca and S uptake. On the other hand, Kifle and Laing (2016) carried out the study, which included experiments with corn where they applied the *Pseudomonas* bacteria. The results showed that the application had a positive effect only on seed germination, not on increased grain yield, dry matter or plant height. This study confirms this fact in the yield of dry matter.

In this research, the influence of bioeffectors on the K, Mg and S content in maize above-ground biomass and also on Ca and S uptake was statistically validated. After P-fertilizers application, Ca and S uptake decreased in contrast to the control treatment without P-fertilizer. A statistically significant effect of experimental year on the content and uptake of all elements and on the yield of dry matter was observed.

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8) Microorganisms in plant protection

Mikroorganismy v ochraně rostlin

Mnoho studií dokazuje, že mikrobiální biostimulanty působí pozitivně z hlediska ochrany rostlin. Principem je zpravidla eliminace patogenních činitelů, jako jsou zejména choroby, ale i škůdci. Otázkou tedy je, do jaké míry je zlepšené hospodaření s fosforem a jinými živinami způsobeno jejich mobilizací z hůře dostupných forem nebo lepším celkovým zdravotním stavem rostlin. Proto byly zpracovány dva literární přehledy – jeden zaměřený na působení biostimulantů v ochraně rostlin a druhý na biostimulanty ve výživě rostlin. Na základě poznatků z prvního uvedeného přehledu je zřejmé, že biostimulanty představují perspektivní cestu k ekologické likvidaci některých patogenů. Toto zjištění je podstatné zejména díky narůstajícímu tlaku na omezení používání pesticidů. Mnoho srovnávaných studií však vychází zejména z laboratorních a nádobových pokusů (např. Takenaka et al. 2003, Wu et al. 2017, Vallabhaneni 2016). Zde bude ještě nutné ověření výsledků v provozních podmínkách. Velkým příslibem je použití uvedených biostimulantů ve skleníkových provozech, kde je možné do značné míry připravit vhodné prostředí pro jejich působení. Za zmínku stojí např. výsledky z provozních pokusů, kde Kröber et al. (2014) potvrdili působení *Bacillus amyloliquefaciens* proti *Rhizoctonia solani* u salátů. Murphy et al. (2000) prokázali účinnost stejné bakterie proti virové skvrnitosti rajčat. Velký potenciál představují i biostimulanty houbového původu, např. *Trichoderma*, *Penicillium* nebo *Pythium*. V laboratorních podmínkách již bylo potvrzeno konkurenční působení proti celé řadě rostlinných chorob (Samuels et al. 2014, Vitti et al. 2016, Altinok et Erdogan 2015, Yang et al. 2009, Hase et al. 2008 a další). V současné době dále probíhá intenzivní výzkum působení těchto biostimulantů v podmínkách blízkým praktickému využití.

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Microrganisms in Plant Protection

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Abstract: Biocontrol agents (BA) are products which contain live microorganisms or their spores as the active substances. Their application could be one possible way that should: i) improve resistance to diseases and pathogens, ii) growth of roots and aboveground biomass and iii) nutrient uptake by plants. The agent's function is based on many different mechanisms. Experiments with BA were carried out under different conditions (fields, pots, greenhouses), with different varieties of tested plants as well as using different application strategies (seed incrustation, application on the leaves and others). Therefore, many different results were published in scientific journals. The aim of this study is to review published results focused on the usage of BA within a plant protection. It might be useful mainly for the ecological farming and healthy food production. This review summarizes the most recent knowledge in this scientific field.

Keywords: biocontrol agents, microoragnisms, plant protection, organic farming.

1. Introduction

The enormous growth of Earth's population requires to provide adequate food resources and to find out alternative strategies for a sufficient crop production. One of the crop production's crucial factors is the achievement of effective plant protection. However the plant protection agents can often expose to danger the environment and human health by food chain's pollution with different chemical compounds. The nowadays problem is limited areas of productive agricultural land and an increasing occurrence of plant diseases and pests within the crop production also. It is also necessary to look for other approaches and strategies (Neumann 2012; Hogenhout *et al.* 2009). The use of pesticides is a traditional method but it causes negative side impacts on the environment by progressive resistance of the pathogens to active substance. This proccess incites a futher research to find out more alternative strategies that would eliminate pathogens. Recently, there has been an effort made towards development of harmless products that are based on microorganisms and their influences (bacteria, fungi) and active natural substances (extracts from soil, compost or seaweeds, microbial residues, plant extracts). The proposal and design of new strategies requires better public discourse about the consequences of the farming impacts on the environment resulting in a better understanding of the soil-plant relationship. It is expected that the development of new strategies will have a significant economical and environmental impact, particularly for future generations (Roy 2017; Withers *et al*. 2014).

2. Microorganisms in plant protection

This chapter lists a selection of microorganisms that are used in the plant protection and crop production. In current time the methods of biological control with the use of microorganisms attract attention of research as a promising alternative to chemical control. Biological protection with the use of antagonistic microorganisms has proved to be a viable alternative. Development of the Biocontrol agents (BA) increases due to the potential use of these substances in organic farming (El-Gremi *et al*. 2017). The current focus on a plant disease management has been shifted from chemical pesticides to more ecofriendly biopesticides in order to reduce an environmental pollution and to minimize the risk of development of pesticide-resistant strains of plant pathogens. Many bacteria have the potential to reduce crop losses through biocontrol mechanism (Vallabhaneni 2016). BAs are divided into two main groups, according to which type of microorganism contain: fungal strains (*Trichoderma*, *Penicillium* and *Sebacinales)* and bacterial strains (*Bacillus* and *Pseudomonas)* (Neumann 2012). BA are highlighted here with more evidences through field or pot experiments and greenhouse studies. The experiments and studies include a broad spectrum of crops such as corn, rice, soybeans, tomatoes, cotton, energy cane, oil palms, millets, oilseeds, banana, coconut, lime, coffee, tea, rubber, flower, spices, herbs, lawns, ornaments, trees, biofuel and forage grass (Janarthanam 2013), sugar beet, tobacco, cucumber, watermelon, muskmelon, cucumber, tropical crops (Choudhary & Johri 2009).

2.1. Fungal BioControl

As mentioned before BA can be divided into two main groups - fungal and bacterial. Several fungal representatives have been selected and described further in this section. At the end of this section (2.1.) in Table 1. There are selected bacteria and their impact on plant protection.

2.1.1. *Trichoderma* ssp.

Strains of the genus *Trichoderma* spp. are wild filamentous fungi occurring in the most of soils and different habitats. *Trichoderma* is a fungal genus that includes species that are currently being used as BA or as biofertilizer (Dominguez *et al*. 2016; Hermosa *et al*. 2012). *Trichoderma* is known for producing several enzymes and antibiotics. The varietes of physiological, antifungal and insecticidal effects are attributed to this species. It operates against a broad spectrum of plant pathogens. These fungi increase the growth of plants' above ground biomass as well as the development of the root system (El-Gremi *et al*. 2017; Galletti *et al*. 2015; Ferrigo *et al*. 2014; Do Vale *et al*. 2012; Raja 2007). It has also been observed that selected *Trichoderma* strains can improve plant nutrient uptake (Yedidia *et al*. 2001) which has indirect influence on the plant health as well. The above mentioned increase of growth occurs due to its strong anti-pathogenic activity, biosynthesis of hormones, improving nutrient uptake from the soil, root development, or increasing the rate of carbohydrates metabolism and photosynthesis as well (El-Gremi *et al*. 2017). The main hydrolytic enzymes secreted by the *Trichoderma* strains are proteases, chitinases and endochitinases. The glycoside hydrolase family, including chitinases, and other enzymes are representing 51% of the total secretome (totality of secreted organic molecules and inorganic elements by biological cells, tissues, organs, and organisms). Few representatives are classified in the protease family (8.9%), others (17.6%) are mostly intracellular proteins. The endochitinases are proteins involved in chitin degradation. The mechanism of chitinases action can be divided into two major groups: endochitinases and exochitinases. In general, endochitinases belongs into chitinases that cleave chitin randomly inside the chain. Exochitinases are subclassified into chitobiosidases and chitobiases. All of these enzymes act in a mutual, synergistic on chitin and on cell wall degradation (Do Vale *et al*. 2012; Duo-Chuan 2006). Chitinases are produced e.g. by bacteria, algae, fungi, plants, insects, nematodes, molluscides, vertebrates, including human and also certain viruses (Gooday 1999). *Trichoderma* is also the main component in several commercially produced biofungicides. The biofungicide is intended to apply in a foliar application, seed protection and into a soil. The soil application is used for the treatment and suppression of various diseases caused by pathogens such as *Botrytis*, *Fusarium*, and *Penicillium* spp. This group of fungicides is used against pests also. It improves a plants' health and environmental monitoring (Gomes *et al*. 2015; Samuels *et al*. 2014). This filamentous fungus increases the resistance of plants against biotic and abiotic stresses and therefore indirectly increases e.g. nitrogen use efficiency. The plants' deep and developed roots allow to withstand drought that was confirmed at e.g. for maize and ornamentals. The above mentioned characteristics are applied as a seed treatment against various pathogens and mycotoxins (Galletti *et al*. 2015; Ferrigo *et al*. 2014; Raja 2007).

2.1.2. *Trichoderma harzianum*

Trichoderma harzianum is wild filamentous fungus; it occurs in soil. *Trichoderma* belongs to the fungi that includes species which are currently used as biological control agents (Dominguez *et al*. 2016; Hermosa *et al*. 2012). Mycoparasitic fungi, such as *T. harzianum*, produce an arsenal of chitin-degrading enzymes to hydrolyze the host cell wall and can also generate high contents of cellulases under appropriate culture conditions (Do Vale *et al*. 2012). Strain T22 was also reported as one enabling to improve the efficiency of photosynthesis and growth of tomatoes (El-Gremi *et al*. 2017). As a notable BA, *Trichoderma harzianum* can antagonize a diverse array of phytopathogenic fungi, including *Botrytis cinerea*, *Rhizoctonia solani* and *Fusarium oxysporum*. Elucidating the biocontrol mechanism of *T. harzianum* in response to the pathogens enables to be exploited in the control of plant diseases (Yang *et al*. 2009). Vitti *et al*. (2016) researched the influence of *T. harzianum* (strain T-22) application under laboratory conditions on the occurrence of *Cucumber mosaic virus* in tomato. And the results prove that early inoculation of this strain is able to induce a defense response. The reduction of mosaic occurrence affects enzyme (dismutase and catalase) and phytohormones (ethylene, abscisic acid, salicylic acid, and jasmonic acid) production. As well Kerroum *et al*. (2015) carried out a study with tomatoes. This study involved pot experiments and confirmed the antagonistic effect of *T. harzianum* against *F. oxysporum* f. sp. radicis-lycopersici that causes root crown rot of tomatoes. Altinok & Erdogan (2015) conducted laboratory and pot trials with *T. harzianum*, strains T16 and T23. These strains significantly inhibited growth of the pathogenic fungus *Fusarium oxysporum*. Ahmad *et al*. (2015) realized a pot trial with *Brassica juncea* testing the influence of soil salinity on brassica after application of *T. harzianum*. Soil salinity stress caused that the plants were smaller with slower growth, changes of plants' physical and biochemical properties and decrease in the biomass yield was found out. Results showed that the seedling plants treated with *T. harzianum* were significantly more resistant to stress conditions caused by salinity in comparison with untreated plants.

2.1.3. *Pythium oligandrum*

The biocontrol agent *Pythium oligandrum*, a soil-inhabiting oomycete, colonizes the rhizosphere of many crop species and it is responsible for the reduction of diseases caused by a number of soil-borne fungal pathogens (Al-Rawahi & Hancock, 1997). *P. oligandrum* promotes plant growth, as a result of interactions' complex, which includes an indirect effect through control of pathogens in the rhizosphere and/or a direct one mediated by plant-induced resistence. The increased plant growth is caused by the interaction between *P. oligandrum* and roots. It is proved that during this interaction the fungus produces auxin compound - tryptamine (Le Floch *et al*. 2003). This fungus produces an elicitor that activates plant defence reactions (Takenaka *et al*. 2003). Therefore, it is postulated that *P. oligandrum* is able to reduce disease through a plant-mediated resistance mechanism i.e. referred as induced resistance. Hase *et al*. (2008) proved that treatment of tomato roots (*Solanum lycopersicum*) with *P. oligandrum* induces an increased amount of ethylene, reducing the severity of bacterial disease caused by *Ralstonia solanacearum*. Hase *et al*. (2008), Glazebrook (2005) next published that plant growth regulators play important role in the plant defence responses to pathogens i.e. jasmonic acid and salicylic acid. Therefore Hase *et al*. (2008) conducted study and laboratory experiment with the involvement of jasmonic acid and salicylic acid. These acids are dependent on signal transduction pathways in resistance to *R. solanacearum*. The experiments were carried out with tomato roots treated with *P. oligandrum* at two tomato cultivars. The first used tomato cultivar was Micro-Tom, i.e. wild-type and the second one was Moneymaker, the type that does not accumulate a salicylic acid. The occurrence of *R. solanacearum* was suppressed in the both tomatoes cultivars after application of *P. oligandrum*. The enhanced resistance was induced at 5 days after treatment. It seems be proved that *P. oligandrum* generally induces resistance to *R. solanacearum* in tomatoes. Takenaka *et al*. (2003) published conclusions that the application of *P. oligandrum* enhances resistance to root-rot-causing agents *Aphanomyces cochlioides* and *Rhizoctonia solani* in sugar beet. Holmes *et al*. (1998) conducted study and pot experiments, where sugar beet seeds were treated with *P. oligandrum* against damping-off of sugar beet. The results indicated that used of *P. oligandrum* significantly reduced a disease caused by *P. ultimum* but at pH values between 7.0 and 7.5 only.

Table 1. Plant protection promoting fungi as BA against various plant diseases.

Fungi	Experimental conditions	Disease	References
Trichoderma ssp.	Laboratory	Diseases caused by pathogens such as *Botrytis*, *Fusarium* or *Penicillium* spp	Samuels *et al*. (2014)
Trichoderma harzianum	Laboratory	*Cucumber mosaic virus* in tomato	Vitti *et al*. (2016)
	Laboratory and pot experiments	*Fusarium oxysporum*	Altinok & Erdogan (2015)
	Laboratory	*Botrytis cinerea*, *Rhizoctonia solani*, *Fusarium oxysporum*	Yang *et al*. (2009)
Pythium oligandrum	Laboratory	*Ralstonia solanacearum* in tomato	Hase *et al*. (2008)
	Laboratory	Root-rot caused by *Rhizoctonia solani* and *Aphanomyces cochlioides* in sugar beet	Takenaka *et al*. (2003)
	Laboratory	Damping-off caused by *P. ultimum* in sugar beet	Holmes *et al*. (1998)

2.2. Bacterial BioControl

Several promising bacterial representatives have been selected and described further in this section. And also at the end of this section (2.2.) in Table 2. There are selected fungi and their impact on plant protection.

2.2.1. ***Pseudomonas*** **spp.**

Pseudomonas sp. is ubiquitous microorganism in agricultural soils, well adapted to grow in the rhizosphere. *Pseudomonas* is well suited as biocontrol and growth-promoting agents (Vallabhaneni 2016). They are often used as BA because they display a broad range of mechanisms to control diseases (Arseneault *et al.* 2016). The inoculation of seeds or roots with fluorescent *Pseudomonas* has been a widely used in practice to increase plant vigor and productivity in tobacco. The *Pseudomonas* has a beneficial effect against a wide range of root phytopathogens, e.g. *Rhizoctonia solani*, *Pythium aphanidermatum* and *Fusarium oxysporum* belong to them. The mechanisms suggested to achieve such inhibition include: production of antibiotics, iron-chelating compounds, hydrolytic enzymes and biosurfactants, competition for favourable nutritional sites or as mycorrhiza helping bacteria (Vallabhaneni 2016). Proteins produced by certain species of *Pseudomonas* increase resistance to *Xanthomonas oryzae var. oryzae* in rice and to Tobacco *Mosaic Virus*. These proteins cause hypersensitivity reactions, higher expression levels of genes related to defense against pathogens and promoting of growth. Therefore they have a potential for development as protein-type BA. When they are applied to tobacco or rice plants then proteins derived from harpin are able to induce resistance to Tobacco *Mosaic Virus* and to *leaf blight disease* in rice with varying degrees. The functional peptide fragments, which were identified there, may result in the effective control of diseases as well as increase a productivity of crops. The condition is that they are developed into a form of microbial pesticides for agricultural applications. This could be an environmentally friendly alternative to some of the chemical pesticides currently in use (Wu *et al.* 2017). The appearence of fluorescent *Pseudomonas* in the rhizosphere microflora depends on characteristics such as soil texture, rhizosphere pH, soil matrix potential, soil water flow, temperature, plant species (Vallabhaneni 2016). Mikicinski *et al.* (2016) used the isolate of *Pseudomonas graminis* (strain 49M) under laboratory and greenhouse conditions but in an orchard also. The aim was to protect apple blossoms and apple terminal shoots. This study identified

Pseudomonas graminis, strain 49M's ability to suppress the fire blight in an immature pear and apple flower and its fitness on flowers in an orchard. The strain 49M is highly protective against fire blight on different plant tissues (up to 73.3% on flowers and 86.2% on terminal shoots, compared to the controls) during the entire bloom period in an orchard. This is the first report showing that *Pseudomonas graminis* strain 49M is a prospective candidate for a future development as the biopesticide that will be used against the fire blight. Vallabhaneni (2016) conducted a study and his results suggest that the *Pseudomonas fluorescens* utilization to control *Rhizoctonia solani* is the promising strategy of disease management. This statement is supported by the fact that all tested *P. fluorescens* isolates reduced the disease severity in tobacco seed beds. Such reduction was evident due to the decrease of affected seedlings number, decrease in the number of sclerotium formation and symptoms' disappearance of severe disease on seed beds. Nine isolates of *P. fluorescens* were selected and evaluated in terms of their antagonistic activity against *R. solani* under vitro conditions. Knot *et al*. (2013) reported that the *Pseudomonas* increases germination of *Poa pratensis* seeds under laboratory conditions, especially of 2-4 years old seeds. Yusran *et al*. (2009) reported the application of *Pseudomonas* and *Bacillus amyloliquefaciens* (individually or in a combination) into soil caused that the state of tomato roots improved in a pot trial. They were healthier and significantly higher colonized by arbuscular mycorrhizal fungi.

2.2.2. *Bacillus amyloliquefaciens*

Bacillus amyloliquefaciens is gram-positive, aerobic and endospore-forming bacteria. They are often used as is commercial chemicals in industry (Zhang *et al*. 2016; Chowdhury *et al*. 2015; Kröber *et al*. 2014). They are one of the beneficial agents used for the plant growth promotion and the suppression of soil-borne diseases in agriculture as well. *B. amyloliquefaciens* produces many metabolites such as are e.g. enzymes (chitinase, peroxidases and proteases), casein, elastin, gelatin, starch, nitrites, esculin and arbutin, phosphatases, adenine, cellulose, guanine, hypoxanthine, pectin, testosterone, tyrosine, many types of antibiotics (e.g. bacillomycins, fengycin, difficidin) and other substances (El-Gremi *et al*. 2017; Chowdhury *et al*. 2015; Lagerlöf *et al*. 2015; He *et al*. 2013; Priest *et al*. 1987). Production of antibiotic that inhibite a growth of antifungal pathogens El-Gremi *et al*. (2017), as well as antibacterial and antinematocidal effects for plants and also the ability to produce a wide variety of secondary metabolites, which aims to suppress competing bacteria, fungi, viruses or nematodes in the rhizosphere of plants. Lagerlöf *et al*. (2015), Kröber *et al*. (2014), He *et al*. (2013), Chen *et al*. (2009) and Koumoutsi *et al*. (2004) declare that the bacteria reduce the influence of plant abiotic stress conditions such as drought, salinity or lack of nutrients. Proteins secreted by *Bacillus amyloliquefaciens* FZB42 protect plants against disease by eliciting innate immunity (Kierul *et al*. 2015). He *et al*. (2013) reported that *Bacillus amyloliquefaciens* belongs to beneficial soil microorganisms, which colonize the plant roots and stimulate the growth of its host. The use of these bacteria offers great potential to increase the yield and reduce the plant disease caused by numerous microorganisms. Kim *et al*. (2015) reported that these bacteria attract attention by their increasing importance in the last time, particularly by their fungicidal effect. PT14 strain proved its property to be a broad spectrum of antifungal activity against *Fusarium solani* and *Fusarium oxysporum*. Nevertheless this strain was not active against bacterial strains. Furthermore Lagerlöf *et al*. (2015), Talboys *et al*. (2014), Fan *et al*. (2012), Burkett-Cadena *et al*. (2008) reported that *B. amyloliquefaciens* promotes a plant growth that is based primarily on the production of secondary metabolites suppressing competing microbial pathogens and diseases occurring in the rhizosphere of plants. It encourages a root development and improves seed germination as well. Some plants, e.g. maize (Baudoin *et al*. 2003), soybean (Yang *et al*. 2012), lupin (Egle *et al*. 2003), rice (Aulakh *et al*. 2001) produce a lactic acid in root exudates. This acid with the other root exudates becomes a energy source for *B. amyloliquefaciens*. Chowdhury *et al*. (2015) carried out experiments which demonstrated that FZB42 strain is able to reduce the disease severity of bottom root caused by soil-borne pathogen *Rhizoctonia solani* on lettuce. Kröber *et al*. (2014) reported results of their pot and field experiments which demonstrated that the strain FZB42 is able to effectively colonize the rhizosphere of lettuce (*Lactuca sativa*) and promotes a significant suppression of bottom rot disease caused by *Rhizoctonia solani*.

2.2.3. *Bacillus subtilis*

Bacillus subtilis is a ubiquitous gram-positive bacteria commonly found in water, soil, air and decomposition of plant residues. However, the primary presence of these bacteria was found in soil (Tam *et al*. 2006; Kunst *et al*. 1997). The bacteria produce endospores that allow it to endure and overcome some extreme temperatures and dry periods. *B. subtilis* produce a series of proteases and other enzymes. This bacterium is considered a benign organism, as it has not properties that cause disease and is not pathogenic or toxic for humans, animals or plants (Kunst *et al*. 1997). Many years ago Korzybski *et al*. (1978) and Katz and Demain (1977), published that the *B. subtilis* produces a wide spectrum of antibacterial and antifungal compounds and furthermore also antibiotics such as difficidin and oxydifficidin that are effective against the broad range of aerobic and anaerobic bacteria.

These bacteria are widely used in agriculture to promote plant growth. They may be taken into account as a promising approach how to protect plants against diseases (Ma *et al*. 2015). Orio *et al*. (2016) reported that the application of *B. subtilis* had a strong effect against fungal pathogen that causes pink disease of roots (*Setophoma terrestris*) at onions.

Table 2. Plant protection promoting bacteria as BA against various plant diseases.

Bacteria	Experimental sites	Disease	References
Pseudomonas spp.	Laboratory condition	*Xanthomonas oryzae* in rice and *Tobacco Mosaic Virus* in tobacco	Wu et al. (2017)
	Greenhouse condition, pot experiment	Fire blight of pear and apple	Mikicinski *et al*. (2016)
	Laboratory condition	Diseases caused by *Rhizoctonia solani* in tobacco	Vallabhaneni (2016)
	Laboratory condition, pot experiment	Meloidogyne javanica in tomato	Siddiqui & Shaukat (2004)
Bacillus amyloliquefaciens	Laboratory condition	Disease of bottom root caused by *Rhizoctonia solani* on lettuce	Chowdhury *et al*. (2015)
	Greenhouse and field conditions	Rot disease caused by *Rhizoctonia solani* on lettuce	Kröber *et al*. (2014)
	Laboratory condition	*Erwinia carotovora* in *Arabidopsis*	Ryu et al. (2004)
	Field condition	Tomato mottle virus	Murphy et al. (2000)

Bacillus subtilis	Laboratory condition	Pink disease of roots at onions	Orio et al. (2016)
	Laboratory condition	*Erwinia carotovora* in *Arabidopsis*	Ryu et al. (2004)
	Greenhouse and field conditions	Downy mildew in pearl millet	Raj et al. (2003)

3. Conclusions

The current research is focused on the partial replacement of chemicals used in agriculture to protect plants against pests and diseases. Within this context it is examined an usage of BAs, where e.g. the interactions between organisms leads to damage to other plant pathogen organism.

Many studies reported positive influence of fungal as well as bacterial BAs on plant health and growth, respectively. These studies were mostly realized in laboratory conditions, where many negative factors can be excluded. Therefore, before transferring these technologies in agronomic practice, pot and especially field trials are strongly needed to confirm the laboratory results in field conditions. Nowadays, there are only several studies that confirmed the positive influence of BAs in the pots or fields. Generally, BAs presents the promising way in plant protection, which required further testing.

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9) Influence of bioeffectors application on maize growth, yields and nutrient uptake

Vliv aplikace biostimulantů na růst, výnos a odběr živin kukuřicí

Za účelem ověření působení biostimulantů z hlediska mobilizace živin a jejich lepšího využití rostlinami byly založeny nádobové pokusy s kukuřicí. Testovány byly biostimulanty obsahující *Trichoderma harzianum*, kmen T-22, *Pseudomonas sp.*, kmen DSMZ 113134 a *Bacillus amyloliquefaciens*, kmen FZB42. Tyto biostimulanty byly aplikovány do půdy jak samotné, tak v kombinaci buď s mletým fosfátem, nebo trojitým superfosfátem. Hlavním cílem bylo zjištění potenciálního působení biostimulantů na uvolnění pevně vázaného P z mletého fosfátu. To bylo zjišťováno prostřednictvím stanovení obsahu fosforu v nadzemní hmotě rostlin a jeho odběru. Dále byly sledovány i obsahy a odběry dalších rostlinných živin, výnosy a výška rostlin kukuřice. Výsledky ukazují, že pozitivní vliv na výšku rostlin vykazoval pouze přístupný fosfor dodaný ve formě trojitého superfosfátu. To se projevilo zejména v raných vegetačních fázích. Při hodnocení výnosu sušiny nadzemní hmoty byly dokonce patrné negativní tendence u variant s biostimulanty, zejména u *Bacillus amyloliquefaciens* na nehnojené půdě. Dá se tak předpokládat, že v půdě chudé na fosfor dochází více ke konkurenčnímu boji o tuto živinu, než o spolupráci biostimulantu s rostlinou. To do značné míry potvrzují i výsledky rozborů rostlin, kde nebyl zjištěn jednoznačně pozitivní vliv biostimulantů na obsah ani odběr živin kukuřicí. Výsledky tohoto pokusu tedy nepotvrdily stanovenou hypotézu, že aplikace biostimulantů povede ke zvýšení výnosů kukuřice a zlepšení odběru P a ostatních živin. Možnou příčinou jsou nevhodné půdní podmínky, zejména nízká hodnota pH sledované půdy. V literatuře jsou naproti tomu často uváděny pozitivní výsledky. Např. Yursan et al. (2009) potvrdili příznivý vliv *Pseudomonas sp.* a *Bacillus amyloliquefaciens* na zdraví a růst kořenů rostlin rajčat. Kumar et al. (2015) prokázali lepší růst *Cajanus Cajan* po aplikaci *Pseudomonas fluorescens*. Celkově lze konstatovat, že publikované výsledky mohou do značné míry vést k nadhodnocení působení biostimulantů. Důvodem je jednak mnohem snazší publikovatelnost průkazných výsledků, jednak tlak firem produkujících biostimulanty na jejich propagaci. Pro dosažení relevantních výsledků a jejich ověření je tak třeba ještě řada pokusů a dlouhodobý výzkum.

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Influence of Bioeffectors Application on Maize Growth, Yields and Nutrient Uptake

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Abstract: Application of bioeffectors should improve the mobilisation of nutrients (especially phosphorus) from less available forms in soil, improve plant growth and contribute to mycorrhiza development. Bioeffectors should also increase resistance to diseases and pathogens. Consequently, bioeffectors should lead to a higher yields. The aim of this research is to estimate the influence of bioeffector application on plant growth and nutrient uptake of maize (*Zea mays, L*. var. Colisee). Three bioeffectors in combination with two phosphorus fertilisers were tested in a pot experiment with cambisol Humpolec. The bioeffectors used were: Trianum (*Trichoderma harzianum*), Proradix (*Pseudomonas sp.*) and RhizoVital (*Bacillus amyloliquefaciens*) in combination with triple superphosphate and rock phosphate. The use of bioeffectors did not positively influence nutrient uptake, dry matter or plant growth. Results of the pot experiments did not show significant positive effects of bioeffector application on plant growth, dry mass or availability of nutrients from less soluble forms in the soil.

Keywords: bioeffector, maize, nutrients, phosphorus, soil.

Introduction

Phosphorus in soil is an irreplaceable macro-element necessary for plant growth and development. Despite its necessity in plant metabolism is its content in the soil is relatively low (Mengel 1991; Blume et al. 2010). In the majority of soil types a higher phosphorus content is found in the close-to-surface layers due to increased biological activity, which results in the accumulation of organic material. Application of organic and mineral fertilisers can often influence soil phosphorus amount. The content of phosphorus in soil can vary depending on the nature of parent material, texture and other farming factors (the ratio and type of supplied phosphorus and method of soil cultivation) (Ivanič et al. 1984; Sharpley 1995). Phosphorus in soil can be divided in two basic groups: inorganic phosphorus and organic phosphorus (Sharpley et al. 1987). The amount of phosphorus bound in the soil fractions depends mainly on the timing of fertilizers application, including the impact of earlier interventions (McGehan and Lewis 2002). The degree of availability for plants depends on chemical, physical-chemical and physical properties of the particular soil type, seasonal dynamics of water, air and temperature regimes, biological activity of soil, the plant species, etc. (Sharpley 1995; Nash et al. 2014). Today's society relies on inorganic phosphorus compounds (fertilisers, feed or food additives) to exploit the limited natural resources of phosphates. For these reasons, there is an overall need to develop more sustainable mechanisms to maintain phosphorus availability for crops and livestock but using a smaller amount of supplied mineral phosphorus, which will lead to improvement of soil functions. Creation of a new strategy requires better public awareness about the consequences of farming approaches on the environment, a better understanding of phosphorus dynamics in the soil-plant relationship, the creation of new innovative technologies to reduce the dependance of the population on mined phosphate and increase the efficiency of phosphorus fertilisation. The development of new strategies is expected to have a significant economical and environmental impact, particularly for future generations (Withers et al. 2014). Due to a growing world population it is expected that demand for food and feed will increase. Limited availability of productive agricultural land and increasing dependance on mineral fertilisers make it necessary to develop alternative strategies for plant nutrition (Hogenhout et al. 2009; Neumann 2012). In 2012 a project was introduced that includes the use of so-called bioeffectors in crop production. This project should contribute to the reduction of mineral fertilisers used in agriculture and to proper and efficient land use and involves testing under real conditions at different geographic locations (Smalla et al. 2012). It is an integrated project focused on the development of new approaches based on activity of live microorganisms and active natural substances (Hogenhout et al. 2009). Bioeffectors can contribute, depending on soil and climate conditions, to overcome limitations in the availability of nutrients. These compounds contain microorganisms (bacteria, fungi) and active natural substances, such as extracts from soil or compost, microbial residues, plant extracts or products of biological processes. These products are developed for a wide variety of crops (e.g. maize, wheat, tomatoes, rape, spinach, grass, ornamentals). Their effective use should cause the mobilisation of nutrients from less bioavailable forms in soil (Neumann 2012; Smalla et al. 2012) and further support root growth (Ferrigo et al. 2014; Galletti et al. 2015) and mycorrhiza development (Yusran et al. 2009). The aim of this study is to evaluate the effect of bioeffectors on maize plant growth and selected nutrient uptake by the above ground mass of a plant, particularly focusing on phosphorus management.

Materials and methods

Pot experiments were established in a vegetation hall on the 30th of April 2014. Five maize seeds (*Zea mays*, variety Colisée) were sown into the pots (volume 5 L). On the 28th of May 2014, plants were selected on the final count of three per pot.

The tested soil was obtained from experimental stations of the Crop Research Institute (Humpolec site). Further site characteristics are mentioned in Table 1.

Table 1. Characteristics of experimental fields.

Site	Humpolec
Latitude	49°33'15'' N
Longitude	15°21'02'' E
Altitude (m above sea level)	525
Mean yearly temperature (°C)	7.0
Mean yearly rainfall (mm)	665
Soil type	cambisol
Soil sort	sandy loam
pH[1)]	5.1
P (mg/kg)[2)]	77 (± 10) B[3)]

[1)] Estimated in air-dried soil, 0.01 mol/l $CaCl_2$, 1:10 w/v

[2)] Average basic data estimated using Mehlich 3 method

[3)] Category B = low content

The substrate was composed of soil and quartz sand at a 2:1 ratio. In this experiment three bioeffectors in combination with two fertilisers were tested with the same dose of nitrogen and potassium (Table 2).

Table 2. Scheme of pot experiments.

Treatment No.	Treatment	Treatment No.	Treatment
1	BE0 + NK	7	BE2 + RP + NK
2	BE1 + NK	8	BE3 + RP + NK
3	BE2 + NK	9	BE0 + TSP + NK
4	BE3 + NK	10	BE1 + TSP + NK
5	BE0 + RP + NK	11	BE2 + TSP + NK
6	BE1 + RP + NK	12	BE3 + TSP + NK

Nitrogen was supplied in the form of calcium nitrate (0.50 g N pot^{-1}) and potassium in the form of K-fertilizer Patentkali (0.85 g K pot^{-1}). The effectiveness of bioeffectors was tested using rock phosphate (RP) and triple superphosphate (TSP), which were applied at the same dose of phosphorus (0.26 g P pot^{-1}). All treatments were compared with a control to which was applied only an inactive bioeffector (demineralized water). The experimental plants were harvested on the 13th of August 2014.

Bioeffectors used in the pot experiment, together with the active substance (in parentheses) were: (i) BE 0: Control (water only); (ii) BE 1: Trianum (*Trichoderma harzianum*, strain T-22, 10^9 spores g^{-1}, Koppert Biological Systems), 0.1175 g pot^{-1}; (iii) BE 2: Proradix (*Pseudomonas sp.,* strain DSMZ 13134, 6.6x10^{10} colony forming units (cfu g^{-1}, Sourcon Padena GmbH & Co.KG), 0.1375 g pot^{-1}; (iv) BE 3: RhizoVital (*Bacillus amyloliquefaciens*, strain FZB42, 2.5x10^{10} cfu g^{-1}, ABiTEP GmbH), 0.35 ml pot^{-1}. All bioeffectors were applied locally to the seeds in the form stock solution at a dosage of 25 ml per pot (5 ml of stock solution to each seed).

Plant height was measured four times during the experiment (5th of June 2014, 18th of June 2014, 3rd of July 2014, 13th of August 2014). After harvesting the pot experiments, the above ground biomass weight, % of dry mass, the content of macro- and selected micro-nutrients in above ground biomass and their uptake, were measured. For the estimation of nutrients, fine milled above ground dry biomass was analysed via dry decomposition at 500°C. Thereafter, samples were transferred to a solution of 1.5% nitric acid (provided by Mader et al. 1998). The extracts were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian VistaPro, Australia). All results were statistically analysed (tests for the normality of distribution, One way ANOVA, Scheffes test at significance level 0.05) using the statistical software application STATISTICA (StatSoft 2016).

Results

The pot experiment was based on the hypothesis that the application of bioeffectors would increase the amount of available phosphorus and other important nutrients for plants. This would result in better phosphorus and other nutrient uptake, greater plant growth and higher yields (Table 3). Table 3 shows plant height measured during the experiment. It is obvious that in the initial growth stages (recorded on 5th and 18th of June), plant height was influenced mainly by TSP use. On the 18th of June a significant positive effect of rock phosphate application on plant height was recorded as well. In later stages, nonsignificant differences between the studied variants occured. This was probably due to competition among the plants in the pots. In terms of plant height variant BE0 showed the smallest plant heights on the 5th and 18th of June and the 3rd of July; however, the impact of the application of various bioeffectors was not statistically verified.

Table 3. Height of maize plants (cm) on specific dates (three plants).

Treatment	Height 5th June	Height 18th June	Height 3rd July	Height 13th August
1	24.2^a	47.8^a	88.8^a	157^a
2	27.2^a	56.1^a	91.6^a	147^a
3	26.9^a	57.1^a	94.6^a	152^a
4	28.1^a	56.5^a	95.9^a	149^a
5	29.3^a	65.3^b	103^a	136^a
6	31.3^a	66.1^b	99.5^a	140^a
7	32.3^a	70.5^b	103^a	137^a
8	31.7^a	67.4^b	98.0^a	134^a
9	45.7^b	82.1^c	102^a	135^a
10	43.1^b	84.4^c	105^a	126^b
11	51.4^b	82.4^c	102^a	128^a
12	46.4^b	78.3^c	99.5^a	132^a
F-test	42.5	38.3	3.57	4.71
p ≤ *	0.01	0.01	n.s.	0.05

* p = significance level

Figure 1 indicates the average above ground dry biomass yield for each variant (Fig. 1). The highest dry mass weight was recorded for treatment BE1 + TSP. This was probably caused by application of TSP. The lowest weight of dry matter was recorded for treatment 4 (BE3 + NK) and treatment 7 (BE2 + RP + NK). From Figure 1 it is obvious that the application of selected bioeffectors had no statistically significant effect on the dry matter yield.

Figure 1. Dry matter weight of the above ground biomass of maize (g per three plants).

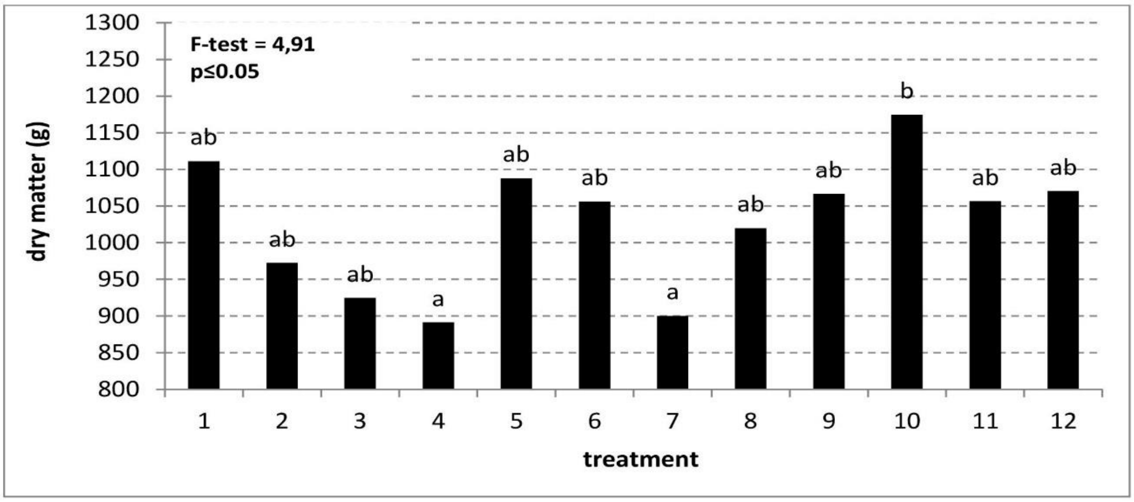

Table 4 lists the nutrients content in the above ground biomass of maize (Table 4). Significant differences between treatments were obtained only for nitrogen and calcium, whereby the nitrogen content under treatment 3 was higher in comparison to treatments 6, 9, 10 and 11. Under treatment 3 the bioeffector BE2 + NK was applied. On the other hand under treatment 6 (BE1 + RP + NK) was applied, under treatment 9 (BE2 + RP + NK) was applied, under treatment 10 (BE1 + TSP + NK) was applied and under treatment 11 (BE2 + TSP + NK) was applied. Calcium content under treatment 1 was significantly higher in comparison to that under treatments 7 and 10. Under treatment 1 (BE0 + NK) was applied, while under treatments 7 and 10, BE2 + RP + NK and BE1 + TSP + NK were applied, respectively. The rest of the results were not statistically significant but the highest contents of analysed elements were found in the following treatments: phosphorus = var. 11; potassium = var. 4; magnesium, sulphur, iron, copper and zinc = var. 1 and manganese = var. 5. The lowest contents of analysed elements were found in the following variants: phosphorus = var. 6; potassium = var. 9; magnesium = 6; sulphur = 6 and 10; iron = var. 2; copper = var. 9 and zinc and manganese = var. 10.

Table 4. Average content of nutrients in plants (mg/kg).

Var.	N	P	K	Ca	Mg	S	Fe	Cu	Zn	Mn
1	5296^{ab}	1031^{a}	12729^{a}	2219^{b}	1259^{a}	529^{a}	57.38^{a}	1.44^{a}	23.49^{a}	29.17^{a}
2	5164^{ab}	1033^{a}	12339^{a}	1446^{ab}	1043^{a}	470^{a}	21.42^{a}	1.22^{a}	11.16^{a}	24.85^{a}
3	6678^{b}	1260^{a}	12127^{a}	1676^{ab}	1215^{a}	497^{a}	32.74^{a}	1.37^{a}	12.49^{a}	32.32^{a}
4	5115^{ab}	1038^{a}	13815^{a}	1438^{ab}	1032^{a}	458^{a}	54.52^{a}	1.24^{a}	11.66^{a}	29.33^{a}
5	4894^{ab}	1033^{a}	11485^{a}	1840^{ab}	1197^{a}	457^{a}	50.76^{a}	1.32^{a}	10.27^{a}	32.78^{a}
6	4010^{a}	1011^{a}	11344^{a}	1406^{ab}	930^{a}	405^{a}	30.56^{a}	0.95^{a}	9.24^{a}	24.66^{a}
7	5404^{ab}	1257^{a}	11862^{a}	1201^{a}	944^{a}	424^{a}	39.83^{a}	0.96^{a}	10.58^{a}	26.46^{a}
8	4836^{ab}	1072^{a}	10781^{a}	1534^{ab}	1052^{a}	469^{a}	54.85^{a}	1.18^{a}	10.05^{a}	26.78^{a}
9	3583^{a}	1277^{a}	10258^{a}	1293^{ab}	913^{a}	444^{a}	39.48^{a}	0.84^{a}	8.74^{a}	28.79^{a}
10	4388^{a}	1270^{a}	10811^{a}	1062^{a}	822^{a}	405^{a}	21.71^{a}	0.92^{a}	7.93^{a}	23.76^{a}
11	4418^{a}	1293^{a}	10478^{a}	1331^{ab}	951^{a}	471^{a}	26.16^{a}	0.97^{a}	8.96^{a}	29.38^{a}
12	4808^{ab}	1247a	12536^{a}	1392^{ab}	988^{a}	413^{a}	32.55^{a}	1.01^{a}	9.23^{a}	32.08^{a}
F-test	6.81	1.73	2.73	4.81	3.28	2.16	1.26	3.68	1.70	1.41
p ≤ *	0.01	n.s.	n.s.	0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

* p = significance level

Table 5 shows the average amount of element uptake by plants for each treatment. This research focused especially on phosphorus because phosphorus sources are limited and bioeffectors are developed specifically to increase phosphorus availability (Table 5). Significant differences between treatments ($p \leq 0.01$) were obtained only for nitrogen, calcium and sulphur, whereby the nitrogen uptake under treatment 3 was higher in comparison to treatment 9. Under treatments 3 and 9, BE2 + NK and BE0 + TSP + NK were applied, respectively. Statistically verified differences in calcium uptake were identified under treatment 1 and treatments 2, 4, 7, 9, 10 and 11. Under treatment 1 the highest uptake of calcium was recorded and BE0 + NK was applied. Under treatments 2, 4, 7, 9, 10 and 11 (BE1 + NK, BE3 + NK, BE2 + RP + NK, BE0 + TSP + NK, BE1 + TSP + NK and BE2 + TSP + NK) were applied, respectively. The final statistically verified difference was of sulphur uptake, whereby the sulfur content under treatment 1 was higher in comparison to treatment 7. Under treatment 1 (BE0 + NK) was applied and under treatment 7 (BE2 + RP + NK) was applied. The rest of results were not statistically significant but the highest uptakes of analysed elements were under the following treatments: phosphorus = var. 10; potassium, magnesium, iron, copper and zinc = var. 1 and manganese = var. 5. The lowest uptakes of analysed elements were under following variants: phosphorus = var. 4; potassium, magnesium, copper and manganese = var. 7; iron - var. 2 and zinc = var. 10.

Table 5. Average uptake of nutrients by plants (mg per three plants).

Var.	N	P	K	Ca	Mg	S	Fe	Cu	Zn	Mn
1	5853^{ab}	1139^{a}	14114^{a}	2458^{a}	1393^{a}	585^{b}	64.79^{a}	1.61^{a}	26.68^{a}	32.59^{a}
2	5023^{ab}	1005^{a}	12013^{a}	1407^{b}	1015^{a}	458^{ab}	20.83^{a}	1.19^{a}	10.87^{a}	24.20^{a}
3	6167^{a}	1146^{a}	11116^{a}	1539^{ab}	1113^{a}	455^{ab}	29.85^{a}	1.25^{a}	11.42^{a}	29.77^{a}
4	4546^{ab}	922^{a}	12310^{a}	1285^{b}	921^{a}	408^{ab}	48.80^{a}	1.11^{a}	10.36^{a}	26.19^{a}
5	5323^{ab}	1124^{a}	12508^{a}	1998^{ab}	1301^{a}	497^{ab}	55.00^{a}	1.44^{a}	11.16^{a}	35.54^{a}
6	4211^{ab}	1062^{a}	12030^{a}	1494^{ab}	982^{a}	428^{ab}	32.58^{a}	1.00^{a}	9.67^{a}	25.98^{a}
7	4837^{ab}	1139^{a}	10657^{a}	1083^{b}	858^{a}	384^{a}	35.93^{a}	0.86^{a}	9.54^{a}	23.22^{a}
8	4942^{ab}	1093^{a}	10977^{a}	1557^{ab}	1070^{a}	477^{ab}	54.95^{a}	1.20^{a}	10.24^{a}	27.20^{a}
9	3829^{b}	1357^{a}	10922^{a}	1380^{b}	973^{a}	472^{ab}	42.25^{a}	0.89^{a}	9.30^{a}	30.88^{a}
10	5115^{ab}	1489^{a}	12739^{a}	1244^{b}	960^{a}	473^{ab}	25.59^{a}	1.07^{a}	9.21^{a}	27.60^{a}
11	4676^{ab}	1375^{a}	11087^{a}	1417^{b}	1011^{a}	500^{ab}	27.88^{a}	1.04^{a}	9.53^{a}	31.25^{a}
12	5130^{ab}	1326^{a}	13345^{a}	1487^{ab}	1054^{a}	440^{ab}	34.88^{a}	1.07^{a}	9.88^{a}	34.70^{a}
F-test	4.16	2.78	2.00	5.89	3.57	3.31	1.31	3.44	1.675	1.87
p ≤ *	0.01	n.s.	n.s.	0.01	n.s.	0.01	n.s.	n.s.	n.s.	n.s.

* p = significance level

Discussion

To date several studies have evaluated the different effects of bioeffectors on plants and included various different parameters. For example, Yusran et al. (2009) reported that after Proradix and RhizoVital application (individually or in combination) to soil in a pot experiment, a significant improvement in the state of tomato plant roots occurred. The roots were healthier and showed significantly higher colonisation by arbuscular mycorrhizal fungi. In our experiments these parameters are not rated but the health of the plants should improve plant growth and yield. However, we did not confirm a significant positive effect of bioeffector application on maize yield. Kumar et al. (2015) conducted pot experiments to support Pigeon Pea (*Cajanus cajan* L.) plant growth after inoculation with bacteria *Pseudomonas fluorescens*. For the study 75 fluorescent Pseudomonas strains from different agro-ecosystems in India were isolated. The isolated strain P17 showed considerable support for growth in terms of root length, dry matter, chlorophyll, carbohydrates, nitrogen, calcium, iron and manganese. *Pseudomonas sp*. strain P17 was identified as a potential rhizobacteria to support plant growth and increase nutrient uptake. In our experiments we tested *Pseudomonas sp*., strain DSMZ 13137 and found that it did not have a positive influence on plant growth or nutrient uptake. Further, Chiarini et al. (1998) conducted a pot experiment in greenhouse conditions with *Sorghum bicolor* and inoculation with microorganisms *Burkholderia cepacia*, *Pseudomonas fluorescens* and *Enterobacter sp*. The results showed that all three microorganisms have the ability to colonise the root system of *Sorghum*, but only the *B. cepacia* and *P. fluorescens* supported plant growth via inoculation with one microorganism only. Dual inoculation had no further effect on plant growth. Our results did not show a positive influence of *Pseudomonas sp.* strain DSMZ 113134 on increasing plant growth or uptake of nutrients. Dual inoculation was not evaluated in our experiments.

In this research was not influence of bioeffectors was confirmed on plant height nor on yield or dry matter weight. Higher values were probably caused by the addition of TSP. Similar plant heights in the later stages could be caused by the correlative stimulating effects of the roots and subsequent growth of the above ground plant parts, or the production of fytohormones (gibberellins, cytokinins, auxins) (Šebánek et al. 1991). Statistically significant differences between treatments on nutrients content in the above ground were obtained only for nitrogen and calcium. And statistically significant differences on uptake of nutrients by plants were obtained only for nitrogen, calcium and sulfur.

Conclusions

Although some positive results in other studies, bioeffectors did not positively influenced maize yields, as well as the macro- and selected microelements content in above ground biomass of plants in our experiments. Results from the pot experiments showed only that the TSP application increased the plant height during the initial growth stages as well as P uptake with above ground biomass of harvested plants. Therefore it is clear that bioeffectors works only in specific conditions and should be further tested.

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10) Co-application of wood ash and *Paenibacillus mucilaginosus* to soil: the effect on maize nutritional status, root exudation and composition of soil solution

Aplikace dřevěného popela spolu s bakterií *Paenibacillus mucilagfinosus* do půdy: vliv na výživný stav kukuřice, kořenovou exsudaci a složení půdního roztoku

Tato publikace je opět zaměřena na kombinaci odpadního materiálu s obtížně dostupným fosforem a biostimulantu. Tentokrát se jedná o dřevěný popel v kombinaci s *Paenibacillus mucilaginosus*. Sledovanými parametry byly: výživný stav, exsudace a složení půdního roztoku u kukuřice. Na základě výsledků bylo zjištěno, že dřevěný popel představuje důležitý zdroj minerálních živin, které se však vyskytují v rostlinám obtížně přístupných formách. Aplikace *Paenibacillus mucilaginosus*, kmen ABi13, vedla ke změnám půdního mikrobiomu, s největším vlivem na *Actinobacteria* a naopak nevedla na nehnojených půdách s nízkým pH a obsahem živin k průkaznému zvýšení výnosu biomasy kukuřice. Důvodem byla především neefektivní mobilizace fosforu. Některé studie naproti tomu ukazují, že inokulace bakteriemi u půd chudých na živiny a mikrobiální aktivitu, nebo půd s celkově nevhodnými podmínkami pro růst, vedla k celkově lepšímu růstu rostlin (Egamberdiyeva 2007, Strigul et Kravchenko 2006, Fliessbach et al. 2009, Mäder et al. 2011). Opačných tendencí bylo dosaženo při aplikaci *Paenibacillus mucilaginosus* spolu s dřevěným popelem. Zde došlo k průkaznému zvýšení obsahu P v půdním roztoku oproti variantám hnojeným samotným popelem bez biostimulantu. Zároveň však došlo také k inhibici růstu kukuřice z důvodu odčerpání nitrátového dusíku aplikovaným biostimulantem. To potvrdily i změny v chování nízkomolekulárních organických kyselin v rhizosféře kukuřice. Jedná se o první studii, kdy byl přímo potvrzen vliv rhizobakterií na zvýšení rozpustnosti fosforu v dodaném dřevěném popelu. Zároveň však vyvstala řada nových otázek, mj. jakým způsobem *Paenibacillus mucilaginosus* imobilizuje nitrátový dusík? Po zodpovězení této otázky se nabízí další možnost využití sledovaného *Paenibacillu* jako prostředku pro postupné uvolňování dusíku do přístupných forem. Studie dále ukazuje, že při hodnocení účinnosti biostimulantů je třeba se zaměřit nejen na status fosforu, ale i ostatních makro- i mikroprvků.

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REGULAR ARTICLE

Co-application of wood ash and *Paenibacillus mucilaginosus* to soil: the effect on maize nutritional status, root exudation and composition of soil solution

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Abstract
Aims Improvement in nutrient efficiency of recycled fertiliser products represents a crucial step for sustainable agriculture. In this context, ash from biomass combustion belongs to the materials of interest.
Methods Novel strain of potential plant growth-promoting bacterium (*Paenibacillus mucilaginosus* ABi13) was tested for its ability to increase the plant availability of nutrients from wood ash (WA) in P-deficient soil-plant systems. Maize plants were grown in soil microcosms in semi-natural conditions, enabling rhizospheric- and bulk-soil solution analysis with special emphasis on low-molecular-mass organic acids (LMMOA).
Results Wood ash, as a sole fertiliser, increased biomass yield and improved nutritional status of maize plants. Concomitantly, application of WA led to lower root exudation rates of malate and isocitrate likely due to improved P status of plants. *P. mucilaginosus* ABi13 was inefficient in mobilising P from plain, acidic soil, but increased P solubility in ash-amended soil. However, *P. mucilaginosus* ABi13 consequently decreased NO_3^- concentrations in soil solution and induced N deficiency in maize, which led to decreased biomass yield and LMMOA exudation rates.
Conclusions This study demonstrated the importance of plant nutritional status on the final outcome of PGPR inoculation and contributes to our understanding of interactions between introduced PGPR, soil microbiome and plants.

Keywords *Paenibacillus mucilaginosus* · Low molecular mass organic acids · Recycled fertiliser products · Soil solution · Wood ash · Maize · Rhizosphere

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Introduction

The majority of biomass ash produced is represented by wood ash, since wood is the single most important biomass fuel worldwide (FAO 2017). Major mineral nutrients contained in wood ash are Ca, K, Mg and P, which are present in various forms of minerals. Wood ash exhibits several other valuable properties, which may significantly improve soil quality. In addition to possible improvement in hydraulic conductivity or water holding capacity, high alkalinity of wood ash reduces soil acidity, which, in turn, reduces Mn and Al toxicity and nutrient leaching into groundwater (Demeyer et al. 2001). Transfer of some risk elements, e.g. Cd, from soils to crop production can be also reduced by ash application (Ochecová et al. 2014; Li et al. 2016). However, the plant-availability of ash-bearing macronutrients is generally very low and is inversely proportional to combustion temperature due to mineral-phase transformation into various forms of insoluble silicates and phosphates (Vassilev et al. 2013). This is especially so with industrial wood ash where combustion temperatures are rarely lower than 800 °C (Vos 2005). Very low plant-availability of ash-bearing nutrients associated with low nutrient use efficiency discourages from wide use of wood ash as a recycled fertiliser product. Nonetheless, effective recycling of biomass ash as fertilisers is an important step in agricultural sustainability as wood ash recycling on agricultural land achieves a closing of the loop for phosphorus and several other nutrients. Consumption of P and K fertilisers in particular, which are associated with high costs and limited supply of these materials, is reduced and, therefore, overall energy input into agriculture may be lowered.

Paenibacillus mucilaginosus (syn. *Bacillus mucilaginosus*) is a typical silicate-weathering bacterium characterised as a gram-positive and facultatively anaerobic bacterium with extracellular polysaccharide production (Xiao et al. 2016). Hu et al. (2006) described phosphate and potassium solubilisation ability of *P. mucilaginosus*. According to Xiao et al. (2016), *P. mucilaginosus* can secrete carbonic anhydrase (CA), which catalyses the conversion of CO_2 to carbonate and protons accelerating the acidification by dissolved CO_2. Such effect consequently promotes the microbial conversion of silicate minerals and releases a number of nutritional ions, especially Ca and Mg, from minerals and soil. However, the most well-known mechanism of inorganic nutrient solubilisation, for Ca-associated P in particular, is due to organic acid (OA) production. This was the case in studies by Hu et al. (2006) and Lu et al. (2014), which reported OA production for all tested strains of *P. mucilaginosus*. Moreover, Liu et al. (2006), proposed significant participation of both exopolysacharides and OA produced by *P. mucilaginosus* in the dissolution of silicates. Authors further suggested formation of bacterial-mineral complexes where polysaccharides strongly adsorb the organic acids and formation of an area of organic acids at a high concentration near the minerals (Liu et al. 2006). Several studies reported plant growth-promoting ability of *P. muclaginosus* due to improved K and P nutrition when combined with sudan grass (Basak and Biswas 2009), maize, wheat (Singh et al. 2010), tobacco (Li et al. 2007), cucumber and pepper (Han et al. 2006). Thus, the use of *P. mucilaginosus* may represent a cost-effective and ecological solution, improving the bioavailability of nutrients contained in wood ash. To our knowledge, there is no study which has focused on the combination of *P. mucilaginosus* and ash.

The main objective of this study was to test novel strain *P. mucilaginosus* ABi13 in terms of its influence on maize and on soil solution composition, with particular emphasis on its potential to increase nutrient availability (P in particular) from wood ash in the soil-plant system. We tested the effect of *P. mucilaginosus* ABi13 on maize plant parameters and soil solution composition using a microcosm approach with natural soil reflecting in vivo P-limiting conditions. In order to understand and sufficiently predict the possible influence of the tested strain, a rhizospheric microcosm was developed with emphasis on low-cost and easy-to-use methods. Nutrient-poor, acidic soil with low cation-exchange capacity (CEC) was chosen in order to simulate highly limiting conditions. In such conditions, the influence of PGPR is expected to be maximized as: i) soil buffering capacity is low and, therefore, potentially solubilised nutrients are likely to be taken up by the plant and not to be adsorbed on soil particles; ii) plant growth is limited by nutrient deficiencies and even a small increase in soil-nutrient availability is likely to result in better growth performance of the plant.

Material and methods

Experimental design

The experiment was conducted using soil microcosms, each containing 5 kg dry wt of soil and placed in an outdoor precipitation-controlled vegetation hall. Each microcosm was made from a 6 L polypropylene pot ($h = 20.5$ cm, $d_{top} = 22$ cm, $d_{bottom} = 18.5$ cm). Inner space of each microcosm was vertically divided into two compartments by nylon (PA 6.6) membrane with a 30-μm pore size (SEFAR, Thal, Switzerland). The root zone compartment (70% of inner volume, 3.5 kg of soil) was sown with maize and the bulk zone compartment (30% of inner volume, 1.5 kg of soil) was without plants. The membrane was used to provide comparable conditions between both compartments, mainly in terms of water saturation level, water residence time and consequently redox potential, factors which are known to have a strong influence on soil solution properties. Simple segregation of planted and non-planted pots may lead to bias and incomparable results for carboxylate concentrations in soil solution (Mercl et al. 2017). One Rhizon MOM suction cup (Rhizosphere Research Products, Wageningen, Netherlands) was installed vertically in the centre of each zone. The Rhizons had a 10-cm porous section with a 0.12–0.18 μm pore size. Schematic representation of the microcosm is shown in Fig. 1.

Experimental soil (Haplic Cambisol) was collected one week prior to sowing from a field (plow depth, 0–20 cm) near the city of Žamberk, Czech Republic (50°8′38.102 N, 16°30′51.351 E). Soil was air dried, passed through a 10-mm stainless steel sieve and manually homogenised prior to the experiment. Wood ash originated from the combustion of wood chips in a fluidised bed reactor (15 MWt) and is described in more detail elsewhere (Mercl et al. 2016). In this study, wood ash treatment is denoted W, whereas control treatment (plain soil with no wood ash added) is denoted C. In the case of W treatment, 24 mg P was supplied in form of wood ash by thorough mixing of corresponding amount of ash with soil. This dose of P resulted in theoretical application rate of 7 tons of wood ash per hectare. The basic characteristics of experimental soil and ash are given in Table 1. Results of P fractionation are shown in Table S1.

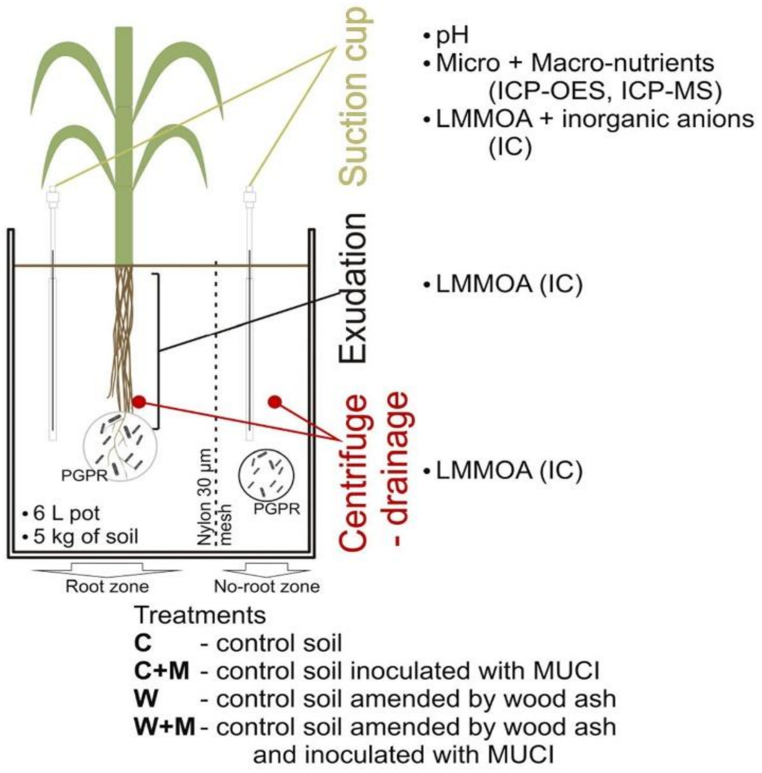


Fig. 1 Schematic representation of the microcosm and description of individual treatments. ICP-OES inductively coupled plasma optical emission spectrometry; ICP-MS inductively coupled plasma mass spectrometer; IC ion-exchange chromatography; PGPR plant growth-promoting rhizobacterium

Table 1 Physicochemical properties of the experimental soil and ash

Characteristics	Experimental soil		Wood ash[a]	
Textural class	Silt loam		–	
Clay (%)	13		–	
Silt (%)	56		–	
Sand (%)	31		–	
CEC (mmol kg^{-1})	74.9 ± 3.7		125 ± 1.20	
pH_{CaCl2}	5.20 ± 0.01		11.2 ± 0	
TC (%)	1.90 ± 0.01		8.00 ± 0.7	
DOC (mg kg^{-1})	63.6 ± 2.0		–	
CO_3^{2-} (%)	0.10 ± 0.05		4.23 ± 0.18	
	Pseudototal (aqua regia)	Available (Mehlich 3)	Total (XRF)	Available (CH_3COOH)
N (%)	0.19 ± 0.01†	55.9 ± 3.82††	0.02 ± 0.01	–
P (mg kg^{-1})	551 ± 26.7	22.0 ± 0.02	10,195 ± 50.0	315 ± 1.00
K (mg kg^{-1})	3802 ± 169	59.6 ± 0.36	58,938 ± 170	6607 ± 571
Mg (mg kg^{-1})	2838 ± 50.5	27.7 ± 0.28	17,478 ± 280	3990 ± 97.0
Ca (mg kg^{-1})	1722 ± 114	1418 ± 8.31	117,789 ± 200	40,824 ± 174
S (mg kg^{-1})	193 ± 16.4	10.3 ± 0.13	15,816 ± 16.0	5313 ± 129
Fe (mg kg^{-1})	20,682 ± 84.6	178 ± 0.63	38,339 ± 84.0	10.8 ± 1.77
Mn (mg kg^{-1})	939 ± 50.1	70.4 ± 0.51	15,292 ± 61.6	1119 ± 13.2
Zn (mg kg^{-1})	53.5 ± 0.69	1.84 ± 0.02	1154 ± 98.0	47.0 ± 1.50
Cu (mg kg^{-1})	7.24 ± 0.13	1.12 ± 0.01	98.8 ± 6.48	0.13 ± 0.04
Al (mg kg^{-1})	21,835 ± 122	1098 ± 0.26	57,028 ± 200	14.4 ± 1.42

Shown values represent arithmetic mean ± standard deviation (SD)

[a] Previously described in more detail by Mercl et al. (2016)

† Total contents of N are given in % and were determined using CNS analyser

†† Available N contents are given in mg kg^{-1} as a sum of NH_4^+ and NO_3^- extractable in 0.01 M $CaCl_2$ (determined by means of colorimetry)

Seven untreated maize seeds (organic variety Colisee; KWS Saat, Germany) were sown in the root zone compartment and plant numbers were thinned to five after germination. Immediately after sowing, both compartments were inoculated with *P. mucilaginosus* ABi13 (MUCI). Tap water (200 mL), instead of inoculum, was applied in the case of control (non-inoculated) treatments (C and W). Treatments involving MUCI inoculation are denoted C + M and W + M in this study.

Each treatment was replicated four times. During the course of the experiment, microcosms were maintained at 60 ± 5% of maximum water-holding capacity (MWHC) by irrigation with demineralized water. MWHC was controlled gravimetrically once every two days.

Bacterial strain *Paenibacillus mucilaginosus* ABi13

Tested strain *Paenibacillus mucilaginosus* Abi13 was isolated from the rhizosphere of wheat growing in Alpine foothills area in Germany and was cultivated in liquid Aleksandrov medium (Hu et al. 2006) at 30 °C. Strain was identified as *Paenibacillus mucilaginosus* by 16S rDNA gene sequencing using universal primers 27F (AGAGTTTGATCMTGGCTCAG), 1492R (TACGGYTACCTTGTTACGACTT) and the resulting PCR fragment was sequenced by Sanger method (Sanger et al. 1977). The sequence was compared with NCBI-Database and matched 100% with *Paenibacillus mucilaginosus*. The strain was grown in a bioreactor at 30 °C in a complex media until complete sporulation. A pure spore product (MUCI) was formulated by membrane-separation of spores from broth culture and subsequent washing out of possible residues of broth and metabolites and was provided by ABiTEP (Berlin, Germany) as a liquid suspension containing living spores of *P. mucilaginosus* ABi13 at a concentration of 1.0×10^9 CFU/mL. MUCI suspension was diluted with a tap water and 200 mL of inoculum was applied directly on seeds in the planting holes resulting in a concentration of 1×10^{10} CFU/kg of soil.

Soil solution sampling

Two methods were used to collect soil solution, namely suction cup and centrifuge drainage method. Using suction cups, soil solution was sampled twice during the experiment (at 14 and 28 days after emergence; DAE) and was used for analysis of available nutrients. Four hours prior to sampling, microcosms were irrigated with a precise amount of demineralized water to reach 60% MWHC and soil solution was collected using 20-mL syringes (B. Braun, Germany) as described elsewhere (Mercl et al. 2017). Immediately after collection, pH of soil solution was measured using a Sentron SI400 pH meter with ISFET electrode (Sentron Europe BV, Leek, Netherlands). Aliquots of collected solution (5 mL) were separately frozen (−42 °C) and kept for subsequent NO_3^- and NH_4^+ analysis. Solutions collected by suction cup method are denoted as root zone solution and no-root zone soil solution.

LMMOA concentrations in rhizospheric and bulk soil solutions were determined at the end of the experiment (28 DAE). These solutions were obtained using a centrifuge drainage method. Immediately after collection of suction cup solutions, individual compartments of microcosms were carefully dismantled. Rhizospheric soil was collected by careful shaking of the whole root system to remove non-adhering soil (this was discarded) and subsequent gentle brushing of roots to remove root-adhering soil which was analysed. Special emphasis was taken to minimize any damage of the roots. Collected soil was thoroughly checked for tiny roots which were then removed with tweezers. Bulk soil was collected from bulk zone compartment by taking representative samples, avoiding both the upper (3-cm) layer and the bottom (3-cm) layer of soil. Approximately 20 g of fresh collected soil was placed in Falcon Maxi-Spin Filter Tubes (Ciro Manufacturing Corp., Deerfield Beach, USA) with a 0.45-μm pore size nylon membrane and was immediately centrifuged at 4 °C for 30 min. No storage of freshly collected soil took place since it is known that LMMOA concentrations are strongly influenced by sample storage (Mimmo et al. 2008). As rhizospheric soil samples inevitably contained root hairs, the relative centrifugal force (RCF) was set to 2500 *g* to avoid symplastic sap leaching and therefore root cell rupture, as reported by Yu et al. (1999). After centrifuging, supernatant was transferred to a 0.5-mL vial; 10 μL of 99.9% (*v*/v) methanol (Lach-ner, Czech Republic) was added (final methanol concentration 2% v/v) in order to prevent any microbial degradation. Samples were then frozen at −42 °C and kept for subsequent analysis.

Collection of exuded LMMOAs

Immediately after collection of the rhizospheric soil, the plant root system was briefly rinsed with a gentle stream of demineralized water and carefully placed for 30 min in a 0.5 L glass beaker covered with aluminium foil and filled with 1 mM $CaCl_2$ solution. During this period, stabilization of root cell membranes by Ca^{2+} is provided and protoplast of mechanically-damaged root cells is washed out into the solution (Neumann and Römheld 2001). After 30 min of stabilization, roots were removed from the solution, gently rinsed with demineralized water and transferred into a new 250 mL beaker filled with a fresh 1 mM $CaCl_2$ solution. Plants were left to exude for 1 h. Plants were then removed, divided into shoots and roots and dried at 60 °C to a constant weight. The exudate solution was stirred briefly and 10-mL aliquots were immediately filtrated through a nylon syringe filter (0.2 μm pore size), frozen at −42 °C after addition of 200 μL of 99.9% (*v*/v) methanol and kept for further analysis.

Analytical procedures

Dried plant biomass was milled and digested with concentrated HNO_3 (65% v/v; Analytika) and H_2O_2 (30% v/v; Analytika) in an Ethos 1 microwave-assisted wet-digestion system (MLS, Leutkirch, Germany). Nutrient concentrations (P, S, Mg, Ca, Fe, Mn, Zn, B, Cu) were then determined by inductive coupled plasma-optical emission spectrometry (ICP-OES; Agilent 720, Agilent Technologies Inc., Santa Clara, CA). Concentrations of K only were determined using flame atomic absorption spectrometry (F-AAS; Varian AA285S, Varian Australia, Mulgrave) (Száková et al. 2013). The standard reference material used was 1515 Apple Leaves (NIST, Gaithersburg, USA).

For the determination of total C and N, a CHNS Vario MACRO cube analyser was used (Elementar Analysensysteme GmbH, Hanau, Germany). Carbonate content was determined using the volumetric calcimeter method (Loeppert and Suarez 1996). Cation-exchange capacity was determined according to Gillman (1979). Pseudototal contents of nutrients in soil were determined by ICP–OES after microwave-assisted aqua regia extraction, as described by Száková et al. (2013). Available fractions of nutrients in soil were determined by extraction of samples in Mehlich 3 solution (Mehlich 1984). Content of available N (NH_4^+ and NO_3^-) in soil was determined using a continuous flow colorimetric analyser (SAN plus System, Skalar Analytical, Breda, Netherlands) after 2 h extraction with 0.01 M $CaCl_2$ (1:10 *w/v*). Concentrations of available nutrients in ash were measured using ICP-OES after extraction for 16 h in CH_3COOH (1:40 w/v). Soil and ash pH values were determined after extraction with 0.01 M $CaCl_2$ at a ratio of 1:2.5 (*w/v*) (VDULFA 1991), using a Sentron SI400 pH meter with ISFET electrode (Sentron Europe BV, Leek, Netherlands).

Concentrations of nutrients (P, K, S, Mg and Ca) in soil solution (root zone and no-root zone solutions sampled with Rhizons) were determined by ICP–OES. Concentrations of P in these solutions were usually below or close to the detection limit (DL) of ICP–OES (0.05 mg L^{-1}). Therefore, P concentrations in all soil solution samples were determined by inductively coupled plasma mass spectrometer (ICP–MS, 7700×; Agilent Technologies Inc., USA). Nitrate, nitrite and LMMOA concentrations in soil solutions and/or exudate samples were determined by means of ion-exchange chromatography with suppressed conductivity, using an ion chromatograph ICS 1600 (Dionex, Sunnyvale, CA) equipped with IonPac AS11-HC (Dionex) guard and analytical columns. The eluent composition was 1 to 35.2 mM KOH with a 1 to 65 min gradient; flow rate was set to 1 mL min^{-1}. Quality control and assurance of ion chromatography analysis are described by Mercl et al. (2017). Concentrations of NH_4^+ in soil solution were determined by means of ion-exchange chromatography with suppressed conductivity. The ion chromatograph ICS 90 (Dionex) equipped with IonPac CS16 (Dionex) guard and analytical columns was used. The eluent composition was 38.0 mM methanesulfonic acid and flow rate was set to 1 mL min^{-1}. To suppress eluent conductivity, the CMMS 300–4 mm suppressor (Dionex) and 0.103 M tetrabutylammonium hydroxide reagent was used. The limit of detection was 0.046 mg L^{-1}.

Soil DNA extraction and Illumina MiSeq sequencing

DNA was extracted from the soil samples collected at the end of the experiment. Prior to DNA extraction, water content of air-dried soil (0.4 g) was firstly adjusted to 20% by adding sterile PCR-grade water and soil was incubated at 4 °C for 30 min (Clark and Hirsch 2008). The method of DNA extraction is described in Sagova-Mareckova et al. (2008). The method is based on bead-beating and phenol/chloroform extraction. The samples are purified by incubation with cetyl trimethylammonium bromide followed by chloroform extraction and incubation with $CaCl_2$, and finally cleaned with GeneClean Turbo kit (MP Biomedicals, Santa Ana, CA, USA).

From the DNA samples, a fragment of the bacterial 16S rRNA gene including the variable region V4 was amplified by PCR using universal primers with overhang adapters CS1-515F (5’-ACACTGACGACATGGTTCTACAGTGCCAGCMGCCGCGGTAA-3′) and CS2-806R 5’-TACGGTAGCAGAGACTTGGTCTGGACTACHVGGGTWTCTAAT-3′) (Caporaso et al. 2011). Construction of amplicon libraries and sequencing using MiSeq sequencer (Illumina, San Diego, USA) were done at the DNA Services Facility, Research Resources Center, University of Illinois (Chicago, USA). The resulting paired sequence reads were merged, filtered, aligned using reference alignment from the Silva database (Quast et al. 2013), and chimera checked using integrated Vsearch tool (Rognes et al. 2016) according to the MiSeq standard operation procedure (Miseq SOP, February 2018; Kozich et al. 2013) in Mothur v. 1.39.5 software (Schloss et al. 2009). A taxonomical assignment of sequence libraries was performed in Mothur using the recreated SEED database subset of Silva Small Subunit rRNA Database, release 132 (Yilmaz et al. 2014) adapted for use in Mothur (https://mothur.org/w/images/7/71/Silva.seed_v132.tgz) as the reference database. Sequences of plastids, mitochondria, and those not classified in the domain Bacteria were discarded. The sequence library was clustered into OTUs using the Uparse pipeline in Usearch v10.0.240 software (Edgar 2013), and the OTU table was further processed using tools implemented in the Mothur software. Distance matrices describing the differences in community composition between individual samples were calculated using the Yue-Clayton theta calculator (Yue and Clayton 2005). Maximum-likelihood phylogram of the OTU representative sequences was constructed using FastTree 2 (Price et al. 2010). The Illumina MiSeq 16S rRNA gene amplicon sequences have been deposited in the NCBI Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) as BioProject ID PRJNA448787.

Statistics and data analyses

The normality of the data was checked using the Shapiro-Wilk test. Data for individual LMMOAs concentrations in rhizospheric and bulk soil solution did not follow normal distribution when comparing mean values ($n = 16$) and the comparison of means was conducted using the non-parametric Mann-Whitney Rank Sum test. STATISTICA 12 (StatSoft, Inc., Tulsa, OK) and SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA) were used for statistical analyses, such as correlation and analysis of variance (ANOVA), and for preparation of figures. Statistically significant differences (post-hoc Tukey's honest significant difference test – HSD) are shown at the 95.0% confidence level.

For the determination of the most limiting nutrient, index values for individual nutrients were calculated using nutrient concentrations in shoot biomass based on the Diagnosis and Recommendation Integrated System (DRIS) (Beaufils 1973). Data published by Elwali et al. (1985) were used as reference parameters. Concentrations of N, P, K, S, Mg and Ca were used for the calculations. Shoot tissue contents of Fe, Mn, Zn, B and Cu were within sufficiency ranges in all treatments and are therefore not shown in this study. No significant differences between treatments were found in soil solution concentrations of NH_4^+ and NO_2^- and, therefore, these results are not shown.

Results

Nutritional status and uptake of nutrients

The application of wood ash (WA) significantly increased the yield of both shoot and root biomass (Table 2). Shoots with wood ash treatment (W) were increased by 33% compared to control (C) and the roots increased by 17%. The influence of MUCI on the biomass yield was inconsistent and varied between treatments. No significant difference in yield from C was found in C + M while a significant decrease in both

Table 2 Biomass yield and nutrient concentrations in individual plant parts at 28 DAE

Plant part		Yield (g pot^{-1})	N (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})	S (mg g^{-1})	Mg (mg g^{-1})	Ca (mg g^{-1})
Shoots	C	8.18 ± 0.30a	16.6 ± 0.38b	0.99 ± 0.03ab	16.3 ± 0.41b	1.26 ± 0.02b	2.53 ± 0.09b	7.66 ± 0.29bc
	C + M	7.79 ± 0.41a	11.3 ± 0.77a	0.91 ± 0.04a	13.8 ± 0.78a	0.93 ± 0.02a	2.42 ± 0.12b	7.79 ± 0.46c
	W	10.9 ± 0.88b	14.8 ± 1.21b	1.00 ± 0.05b	17.7 ± 1.28b	1.19 ± 0.13b	2.35 ± 0.15b	6.62 ± 0.40a
	W + M	7.05 ± 0.45a	9.73 ± 0.27a	1.25 ± 0.02c	23.0 ± 0.41c	0.98 ± 0.04a	2.00 ± 0.01a	6.91 ± 0.14ab
F-value		36.6***	51.4***	46.6***	70.4***	15.7***	13.0***	8.20**
Nutrient sufficiency ranges†			27.6–35.0	2.50 – 4.00	17.1–25.0	1.50–4.00††	2.10 – 6.00	2.10–10.0
Roots	C	7.51 ± 0.67a	8.93 ± 0.31c	0.54 ± 0.05ab	3.83 ± 0.24a	0.85 ± 0.03a	1.14 ± 0.10a	5.50 ± 0.30b
	C + M	7.66 ± 0.38ab	6.83 ± 0.32ab	0.54 ± 0.07ab	4.80 ± 0.54a	0.86 ± 0.03a	1.09 ± 0.11a	3.86 ± 0.21a
	W	8.77 ± 0.74b	7.72 ± 1.23bc	0.52 ± 0.03a	4.92 ± 0.40a	1.45 ± 0.12b	1.25 ± 0.13ab	5.48 ± 0.61b
	W + M	6.81 ± 0.26a	5.78 ± 0.28a	0.68 ± 0.08b	7.44 ± 0.99b	1.90 ± 0.22c	1.51 ± 0.14b	4.71 ± 0.46ab
F-value		8.71**	11.9***	4.32*	19.2***	44.4***	7.29**	10.1**

Values shown represent arithmetic mean ± SD ($n = 4$); different letters indicate significant difference (Tukey's HSD test; $p < 0.05$) between treatments in individual plant parts; all results are given on a dry basis

C control; C + M control inoculated with MUCI; W wood ash treatment; W + M wood ash treatment inoculated with MUCI; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

† Nutrient sufficiency ranges for maize ear leaf taken at silk according to Jones et al. (1990)

†† Sufficiency range for maize biomass content in early growth according to Campbell and Plank (2000)

shoot and root yields after MUCI inoculation was found in the case of W + M compared to W (Table 2).

Phosphorus concentration in biomass was not influenced by WA application, but WA significantly increased the total P content. No significant effect of MUCI on biomass P concentration and subsequently on P content was found in plain soil treatment (C + M) (Table 2; Table S2). However, application of MUCI on ash-treated soil (W + M) significantly increased P concentration in both shoots and roots, but the P content in shoots was significantly decreased. Phosphorus concentrations in shoots were generally lower than the sufficiency range with all treatments (Table 2). Highest P concentration in shoots was found with W + M treatment (1.25 mg g^{-1}), while the lowest value was with C + M (0.91 mg g^{-1}). Phosphorus was identified by DRIS to be the most deficient nutrient in C, C + M and W treatments in contrast to W + M, whereas N was determined as the most limiting one (Table 3).

Wood ash had no significant influence on N concentration in shoots or roots, but significantly increased N content in shoots (Table 2; Table S2). Application of MUCI significantly decreased N concentration in both shoots and roots with both treatments and this reduction led to a decreased shoot and root N contents with C + M and W + M treatments. In general, N concentration in shoot biomass in this experiment ranged between 9.73 mg g^{-1} and 16.6 mg g^{-1} (Table 2). All treatments therefore suffered from N deficiency.

Biomass concentration of K remained the same in W compared to C, but increased K content in shoots and roots was evident after WA addition. Application of MUCI did not affect K content in roots, but had a significant effect on shoots where it resulted in a decreased content with both treatments. Concentration of K in shoot biomass was generally close to the lower level of sufficiency. Only in the case of C + M did K concentration fall slightly more below the sufficiency limit and could be considered as deficient with a value 13.8 mg g^{-1}.

Sulphur nutritional status of maize plants was significantly improved by WA; WA application resulted in increased S concentration in shoots and roots, as well as S content in these parts. The effect of MUCI on biomass S concentration differed between treatments and plant parts. A significant decrease in S concentration in shoot biomass after MUCI application was found with both C + M and W + M treatments. Conversely, the S concentration in roots was increased by MUCI in the case of W + M treatment but no effect was found in C + M treatment. Sulphur concentrations in shoot biomass ranged between 0.93 mg g^{-1} and 1.26 mg g^{-1} in this experiment. These values are lower than the sufficiency range (Table 2).

Finally, Mg and Ca content in shoots and roots were increased by WA application. Application of MUCI resulted in lower shoot Mg concentration in W + M treatment, and subsequent decrease in Mg content and lower shoot Ca content in C + M without any effect on the content of Ca in shoots. Concentrations of Mg and Ca were within the sufficiency ranges (Table 2).

Soil solution properties and nutrient concentrations

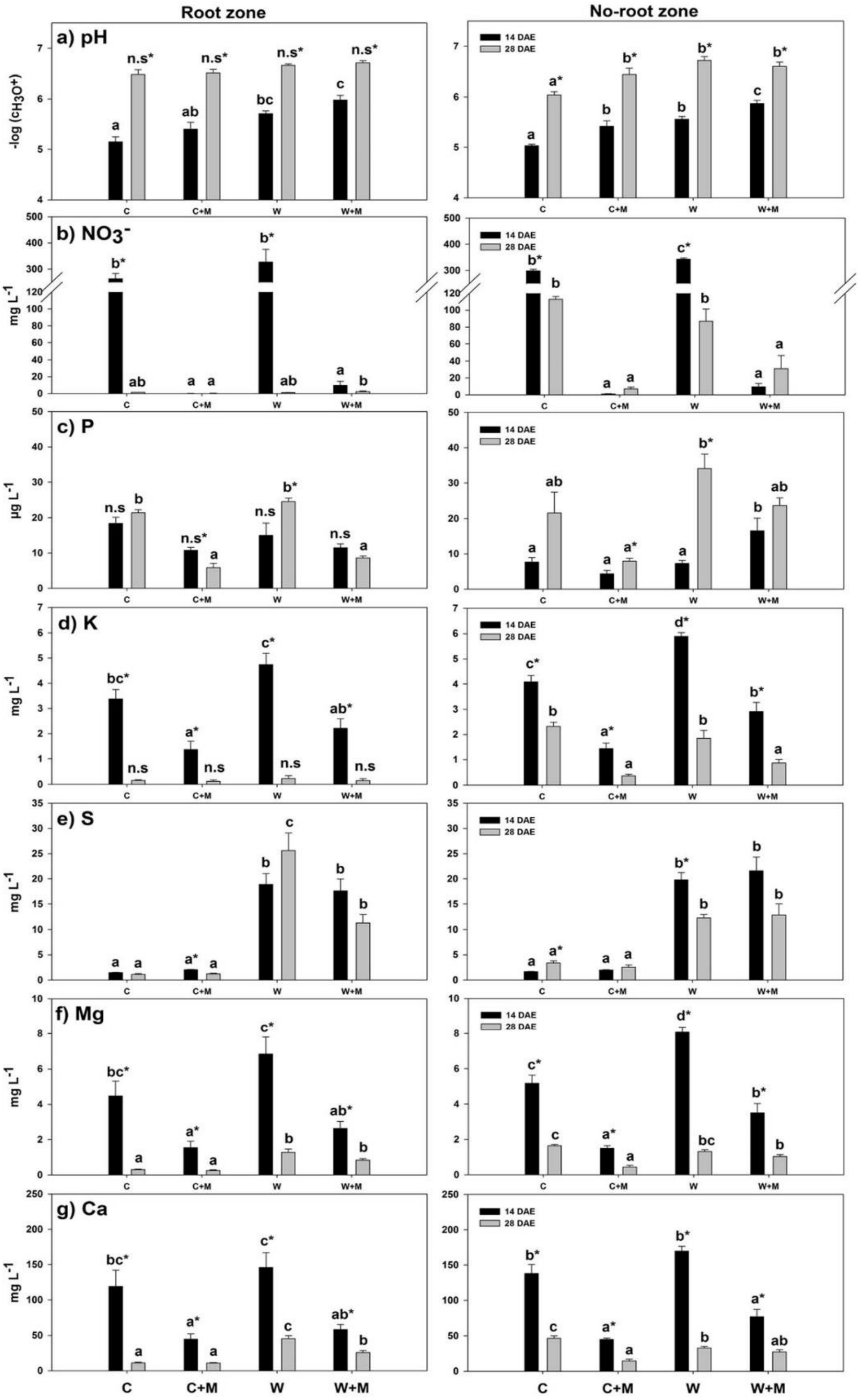
Soil solution pH was in the range 5.03–6.72 and increased approximately by 1 unit over time (14 DAE – 28 DAE) with all treatments (Fig. 2; Table S3). Solution pH was significantly increased by wood ash application in both zones at 14 DAE (by 0.56 and 0.53 in root and no-root zones, respectively). The effect of wood ash on solution pH disappeared in the root zone at 28 DAE but was still significant in the no-root zone. Concentrations of NO_3^- showed a decreasing trend over time in both zones. Wood ash slightly affected NO_3^- concentrations in soil solution, but a significant increase was found only in the no-root zone during the first sampling

Table 3 Nutrient DRIS index values for individual treatments

Treatment	N_{index}	P_{index}	K_{index}	S_{index}	Mg_{index}	Ca_{index}
C	-11	−56	12	−17	25	46
C + M	−31	−51	13	−30	35	64
W	−16	−49	20	−18	24	39
W + M	−49	−32	43	−31	20	48

C control; C + M control inoculated with MUCI; W wood ash treatment; W + M wood ash treatment inoculated with MUCI

Fig. 2 Soil solution properties. Data shown represent arithmetic ▶ mean ($n = 4$); error bars indicate standard error of the mean; different letters above bars indicate significant differences (Tukey's HSD; $p < 0.05$) between treatments for individual zone and sampling time separately; asterisks above bars indicate significant differences (Tukey's HSD; $p < 0.05$) between sampling times for individual treatments separately; F-values of ANOVA are shown in Table S3 n.s not significant; DAE days after emergency; C control; C + M control inoculated with MUCI; W wood ash treatment; W + M wood ash treatment inoculated with MUCI


Root zone
No-root zone
a) pH
-log (cH3O+)
b) NO3-
mg L-1
c) P
µg L-1
d) K
mg L-1
e) S
mg L-1
f) Mg
mg L-1
g) Ca
mg L-1
14 DAE
28 DAE
C
C+M
W
W+M

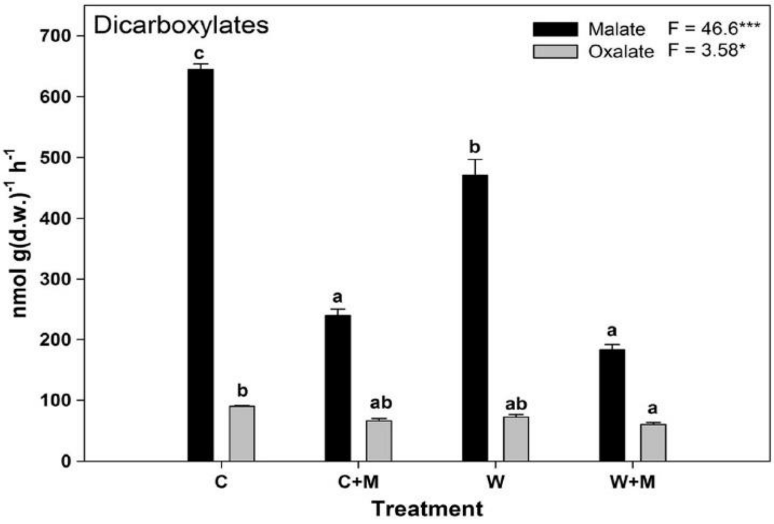


Fig. 3 Exudation rates of individual dicarboxylates at 28 DAE. Data shown represent arithmetic mean ($n = 4$); error bars indicate standard error of the mean; different letters above bars indicate significant differences (Tukey's HSD; $p < 0.05$) between treatments for individual LMMOA separately; C control; C + M control inoculated with MUCI; W wood ash treatment; W + M wood ash treatment inoculated with MUCI; *** $p < 0.001$; * $p < 0.05$

period. Application of wood ash did not affect P concentrations in soil solution within this experiment. Wood ash did increase K concentrations in solution but this effect was significant only in the no-root zone at 14 DAE. Concentrations of S were also increased by WA both at the first and at the second sampling period in both zones. At 14 DAE, concentrations of Mg were increased by wood ash application only in the no-root zone, while the increase became significant in the root zone over time (28 DAE). Finally, wood ash did not influence Ca concentrations in soil solution at 14 DAE, but changes appeared over time.

Application of MUCI significantly increased pH in the no-root zone with both treatments at 14 DAE. In the latter stage of the experiment, the significant increase in pH was recorded only for C + M treatment (Fig. 2). Effects of MUCI on P concentrations in soil solution were relatively inconsistent. At 14 DAE, MUCI affected only soil solution of W + M treatment in no-root zone where a significant increase of 9.24 μg P L^{-1} was found compared to W. No effect of MUCI was obtained for the same treatment at 28 DAE, while P concentrations decreased in root zone after MUCI application at 28 DAE. Application of MUCI resulted in a significant decrease in NO_3^- in both zones of soil solution at 14 DAE, but its effect at 28 DAE was significant only in the no-root zone. Potassium concentrations in solution were lowered with MUCI treatments in both solution types at 14 DAE. The effect of MUCI on S in soil solution was evident only at 28 DAE with W + M treatment when a decrease of 14.3 mg L^{-1} was recorded. The effect of MUCI on Mg concentrations in both zones was visible and significantly lower Mg concentrations were detected at 14 DAE. A significant decrease in Ca concentrations in solution at 14 DAE was found with all MUCI treatments, whereas this effect remained significant at 28 DAE in root zone of W + M.

Low molecular mass organic acids

Wood ash application had no significant influence on the exudation rates of oxalate, but those of malate were significantly decreased by WA application (Fig. 3). This led to the decreased total sum of exuded dicarboxylates (Table 4). Similar to malate, exudation rates of isocitrate

Table 4 Rate of total amount of carboxylates (nmol g(d.w.)$^{-1}$ h^{-1}) exuded at 28 DAE

Treatment	Dicarboxylates	Tricarboxylates
C	734 ± 37.1c	1978 ± 361
C + M	305 ± 38.9a	1258 ± 292
W	542 ± 111b	1585 ± 315
W + M	243 ± 39.3a	1738 ± 340
F-value	48.7***	3.38$^{n.s}$

Values shown represent arithmetic mean ($n = 4$) ± SD; different letters indicate significant differences (Tukey's HSD; $p < 0.05$) between soil treatments for each LMMOA group

C control; C + M control inoculated with MUCI; W wood ash treatment; W + M wood ash treatment inoculated with MUCI; *** $p < 0.001$; $^{n.s}$ not significant at $p < 0.05$

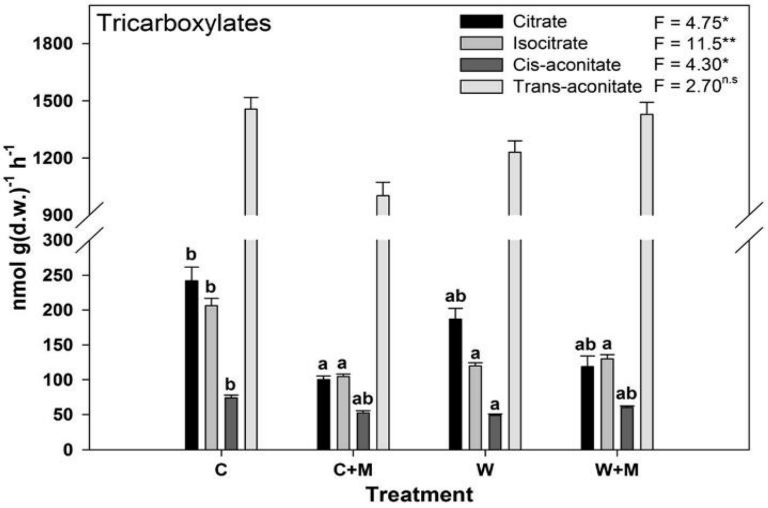


Fig. 4 Exudation rates of individual tricarboxylates at 28 DAE. Data shown represent arithmetic mean ($n = 4$); error bars indicate standard error of the mean; different letters above bars indicate significant differences (Tukey's HSD, $p < 0.05$) between treatments for individual LMMOA separately; C control; C + M control inoculated with MUCI; W wood ash treatment; W + M wood ash treatment inoculated with MUCI; ** $p < 0.01$; * $p < 0.05$; $^{n.s}$ not significant at $p < 0.05$

and cis-aconitate were also suppressed in W compared to C (Fig. 4). Two-way ANOVA showed significant and homogenous effect of wood ash on the exudation rates of malate and isocitrate and consequently on the lower total exuded rate of dicarboxylates (Table S4).

Application of MUCI resulted in a significant decrease of malate exudation rate with both C + M and W + M treatments and consequently in lower total amount of dicarboxylates exuded (Fig. 3; Table 4). The response in terms of exudation of tricarboxylates to MUCI application was different between C and W. In the case of ash-treated soil, total amount of tricarboxylates remained unchanged after MUCI application (Table 4) and no changes in the exuded amount of individual tricarboxylic LMMOAs were detected (Fig. 4). However, exudation rates of citrate and isocitrate were significantly lower when MUCI was applied to plain soil. According to two-way ANOVA, MUCI had significant and consistent lowering effect on the exudation rates of malate, oxalate and citrate (Table S4). In the case of isocitrate, cis- and trans-aconitate and, consequently, the rate of total amount of tricarboxylates, the interactions between the effects of MUCI and WA were observed (Table S4), suggesting a difference in MUCI effects between plain and ash-treated soil.

Concentrations of LMMOAs were determined in rhizospheric and bulk soil solutions at 28 DAE. Significantly higher concentrations (Mann-Whitney Rank Sum test) were generally found in rhizospheric than bulk soil solutions (Table S4; Table S5). Only acetate showed similar concentrations between both solution types. In addition, more types of LMMOA were found in the rhizospheric soil solution (Table S5). Isobutyrate, maleate and isocitrate were below the DL (DL = 0.005, 0.007 and 0.011 mg L^{-1}, respectively) in bulk soil solutions, but were found in rhizospheric ones. No differences in individual LMMOA concentrations between all treatments were found in bulk soil solutions (Table S4). In the case of rhizospheric soil solution, WA showed decreasing influence (two-way ANOVA) on the concentrations of acetate. In the same solution type, MUCI had a significant influence on the concentrations of acetate, formate, malate and, consequently, on the concentrations of total monocarboxylates (Table S4).

Survival of MUCI in soil

We obtained 803,196 high quality bacterial sequences divided to 3821 OTUs on the 3% similarity level. *Paenibacillaceae* were represented by 17,911 sequences divided to 126 OTUs. In the NMDS plot, *Paenibacillaceae* were separated by treatments of both, wood ash and MUCI additions (Fig. S1). The known sequence of MUCI belonged to OTU 37, which was higher by two orders of magnitude in the inoculated treatments (C + M; W + M) compared to the control. Above that bacterial communities were affected by the treatments. In particular, OTUs from *Actinobacteria* were upregulated with the 20 most affected OTUs (Table S6).

Discussion

Verification of the experimental and methodological approaches

Simplified soil microcosms were used in this experiment in order to study rhizospheric processes after PGPR inoculation of maize. The choice of proper growth system and exudates sampling technique still remains challenging since all known approaches have some advantages and drawbacks (Neumann and Römheld 2001; Fitz et al. 2006; Oburger et al. 2013). The soil microcosms were used for following reasons: i) experimental soil underwent only minimal disturbance since it was air dried and sieved through 10 mm sieve. Such procedure should keep soil structure, aggregates and natural microbial communities relatively unchanged; ii) root architecture is also expected to be similar to field conditions, at least in early growth stages since plants were soil-grown and microcosms were placed in outdoor conditions with natural sunlight and temperature. These parameters, when different from natural ones, may strongly influence the rate and composition of root exudates, which, in turn may have strong impact on the structure of rhizoplane and rhizosphere microbiome as well as PGPR colonization and vice versa (Nguyen 2003; Schulze and Pöschel 2004; Lakshmanan et al. 2014; Giagnoni et al. 2016). However, when quantification of LMMOA exudation rates is needed, it is impossible to remove intact root system from soil. Damage of root hairs is inevitable even when maximum effort is taken during roots removal. Exudation rates determined in our study were generally in the same magnitude as reported in published literature (Gaume et al. 2001; Oburger et al. 2013) suggesting minimal damage of the roots.

Sampling for LMMOA determination in soil solution has been subject to other problems. Rapid biodegradation (Neumann and Römheld 2001), adsorption on the suction cup material and spatial resolution of suction cups (Puschenreiter et al. 2005), distinct LMMOA concentrations between methods of sampling (Shen and Hoffland 2007) or changes in LMMOA extractability caused by sample storage (Mimmo et al. 2008) need to be taken into account when interpreting the results. Even though Rhizons used in this study were previously tested for LMMOA sampling from soil solution (Mercl et al. 2017), their spatial resolution is low compared to micro-suction cups (Göttlein et al. 1996; Puschenreiter et al. 2005) and sample collected by suction cup represents an average soil solution concentration over the whole sampling time (Shen and Hoffland 2007). It is notable that in this study LMMOA concentrations in soil solution sampled by suction cups were much lower than in solution obtained by centrifuge drainage and the maximum correlation coefficient (r) between these methods was $r = 0.5$; $p = 0.03$ (for oxalate in bulk soil; data not shown). Despite high root density in root-zone compartment, individual LMMOA concentrations in suction cup solutions were not statistically different between root zone and no-root zone (data not shown). Lack of differences for suction cup solution between rhizospheric and bulk soil solution was previously reported by Oburger et al. (2013) who sampled soil solution using micro-suction cups in mm scale distance from roots. However, these results are not in agreement with the generally accepted concept of rhizosphere where higher carbon concentrations are expected close to the roots due to rhizodeposition (Nguyen 2003; Sauer et al. 2006; Hinsinger et al. 2009; Jones et al. 2009). The centrifugation-drainage method showed better distinction between rhizosphere and bulk soil solution since the concentrations of almost all determined carboxylates were higher in rhizospheric soil solution (Table S5). We hypothesise that the superior performance of the centrifugation-drainage method is likely to be a result of collection of different fractions of pore water (e.g. micropore water), which are not collected by suction cups because of lower suction tensions (Di Bonito et al. 2008). However, one cannot rule out the possibility that higher concentrations of LMMOAs in soil solution obtained by centrifugation-drainage method were caused by disruption of microbial cells even though such phenomenon is generally reported for RCFs higher than 5000 *g* (Peterson et al. 2012).

Suction cup solution was used in our study for the interpretation of plant-available nutrients and pH, as was previously suggested and tested by Argo et al. (1997) and Rais et al. (2006). In general, initial pH of soil solution corresponded well with soil pH determined by $CaCl_2$ extraction (Table 1; Fig. 2) and it increased over time most probably because of predominant NO_3^- uptake by plants (Mistrik and Ullrich 1996; Brimecombe et al. 2007). The shift in pH led to increased P concentrations in soil solution over time (Fig. 2); however, this phenomenon was masked in root zone and was detectable only in no-root zone solution. We assume that the decrease in concentrations of NO_3^-, and subsequent increase in pH in no-root solution was caused by diffusive transport of soil solution from no-root to root zone compartment.

The effect of wood ash

The positive impact of wood ash on crop yield has been widely reported (Erich 1991; Erich and Ohno 1992; Insam and Knap 2011; Ochecová et al. 2017), its agronomic efficiency was shown to be relatively high (Brod et al. 2015) but its influence on crops may vary among soils (Mercl et al. 2016). As we found significant increase in maize yield (Table 2), the combination between soil and rate of wood ash application used in this experiment can be concluded as suitable approach for the soil fertility improvement. According to Nkana et al. (1998), a positive effect of WA on the biomass yield is mainly caused by Ca and K supplementation. In our experiment, the application of WA resulted in increased biomass uptake of all studied nutrients (N, P, K, S, Mg and Ca). The higher uptakes of K, S, Mg and Ca can be easily explained by increased concentrations of these nutrients in soil solution after WA addition (Fig. 2). This may be also partly true for N as significantly higher NO_3^- concentrations in no-root zone soil solution were found at the early stage of the experiment (Fig. 2). Since WA contained a negligible amount of N (Table 1), increased NO_3^- concentrations in soil solution should be related rather to N mineralisation and/or enhanced rate of nitrification as reported by several studies (Park et al. 2004; Patterson et al. 2004; Odlare and Pell 2009). However, maize plants in our experiment were limited mainly by P as determined by both, nutrient sufficiency ranges (Table 2) and calculated DRIS index values (Table 3). One would expect that higher biomass yield in W compared to C as well as higher P uptake was therefore caused by supplementation of P-rich ash. However, results of soil solution P (Fig. 2) cannot confirm this since there was no significant increase of solution P in W compared to C treatment. The lack of difference in P concentrations in soil solution may be caused by sorption and precipitation reactions in soil or may be masked by intensive and continuous uptake by plants since P was still the most limiting nutrient in W treatment (Table 3). A similar trend was reported by Li et al. (2007) who found higher P and K uptake by plants but no increase in soil available P or K content. Such phenomenon may also lead to the suggestion that these nutrients were taken up by plants not directly from soil solution but likely from “biofilm cover” as proposed by several authors (Seneviratne and Jayasinghearachchi 2005; Liu et al. 2006; Bogino et al. 2013). Our suggestions are partly confirmed by the results of root exudation (Figs. 3 and 4). Increased citrate and malate exudation rate from roots in maize has been reported by Gaume et al. (2001) to be the adaptation to P-limiting conditions. Our results showed a significantly lower rate of malate and isocitrate exudation in W compared to C which may, therefore, indicate higher supply of P to plants. However, Lyu et al. (2016) reported rather root morphological than physiological response of maize to P-limiting conditions. In order to study the influence of individual treatments on LMMOA concentrations in soil solution, rhizospheric and bulk soil solutions were analysed. It is known that once carboxylates are released from root to the rhizosphere, they may be quickly utilised and/or transformed by the soil microbial community or may be adsorbed onto soil particles. Oburger et al. (2009) suggested that sorption is a major regulator of bioavailability of di- and tri-carboxylates. A recent study of Gunina et al. (2017) showed the carbon oxidation state to be a dominant factor governing half-life of low-molecular-weight organic substances in soil solution. Moreover, it has been suggested by Oburger et al. (2013) that the majority of LMMOA in soil solution sampled from the close vicinity of the root (1–3 mm) are not of plant origin. In our study, two-way ANOVA revealed an influence (decreasing trend) of WA application on the acetate concentrations in rhizospheric soil solution (Table S4). Since no effect was observed in bulk soil solution, the direct effect of WA on shifts in microbial metabolism is unlikely. We hypothesise that the lower concentration of acetate in rhizospheric soil solution was an indirect result of suppressed carboxylate rhizodeposition due to better P supply to maize plants. It is noteworthy that WA significantly decreased carboxylate C flux from maize plants to soil (Table 4), but only with a little effect on carboxylate concentration in rhizospheric soil solution (Table S4; Table S5).

The effect of *Paenibacillus mucilaginosus* ABi13

The inoculation was successful and MUCI was detectable in soil even after 35 days from the application as seen from the differences in counts of OTU 37 between the treatments. However, it seems that the inoculated strain as well as ash supplementation changed the bacterial community so the observed effect on soil chemistry and plant performance might have been partly a result of more complex bacterial interactions (Panke-Buisse et al. 2015).

Bacterial inoculation has been reported to have better stimulatory effects on plant growth in nutrient deficient (Egamberdiyeva 2007), unfertile, stressed soils or in soils with poor microbial biomass and activity (Strigul and Kravchenko 2006; Fliessbach et al. 2009; Mäder et al. 2011). According to several studies (Krey et al. 2011; Krey et al. 2013; Mosimann et al. 2017), positive effects of P-solubilising bacteria on maize growth and nutrition are to be expected in P-deficient soils, but it cannot be taken for granted that inoculation with well-colonising and persistent PGPR will lead to a positive effect on the yield (Mosimann et al. 2017). The wood ash-amended soil (W) and control soil (C) and in our experiment can, therefore, be considered as suitable for PGPR use, since with both treatments maize plants exhibited P deficiency (Tables 2 and 3). However, application of MUCI had no beneficial effect on maize growth when applied in plain soil (C + M), possibly due to inefficient P solubilisation since P remained to be the most limiting nutrient, even after inoculation (Table 3). On the one hand, such an effect could be expected as the soil was strongly acidic (pH_{CaCl2} 5.2) and, therefore, a considerable amount of P was present as precipitated Fe- and Al-phosphates, or was sorbed on Fe- and Al-oxides (Hinsinger 2001). Potential localised simple acidification induced by MUCI, as reported by Hu et al. (2006), Liu et al. (2006) and Lu et al. (2014), would thus be expected to have lower efficiency as the solubility of these phosphates decreases with decreasing pH (Lindsay 1979; Bashan et al. 2013). On the other hand, organic acids formed may act through other mechanisms, such as ligand exchange and occupation of P sorption sites and/or ligand-promoted dissolution of Fe- or Al-oxides and subsequent desorption of P into soil solution (Jones et al. 2003; Oburger et al. 2011). However, to mediate significant desorption of P, relatively high carboxylate concentrations are required (Neumann and Römheld 2001). Such "hotspots" could be theoretically possible, as was hypothesised by Liu et al. (2006), for the combinatory carboxylate and polysaccharide excretion by MUCI. More favourable conditions for microbial-induced P dissolution were expected with W + M treatment, as the soil was supplied with WA containing a considerable amount of acid-soluble P (Table 1; Table S1). In this case, we found significantly higher P concentrations in soil solution, but only at 14 DAE in no-root zone (Fig. 2). We attribute the fact that no significantly different P concentrations were found in root zone due to the continuous P uptake by maize roots. However, part of P solubilized by MUCI could be hidden in "biofilm cover" and therefore, not sampled by suction cups. Nevertheless, inoculation of MUCI to W + M shifted the nutritional status of maize in favour of N deficiency (Table 3), which consequently led to lower biomass yield (Table 2) and N uptake (Table S2). Soil solution analysis (Fig. 2) revealed that the application of MUCI lowered NO_3^- concentrations in solution irrespective of the treatment. It is questionable whether N was utilized by MUCI into organic forms, denitrified and lost from the system or simply sorbed onto exopolysacharides produced by MUCI, since we found no differences in NO_2^- or NH_4^+ concentrations in soil solution between treatments. Interestingly, MUCI also decreased solution concentrations of K, Mg and Ca (Fig. 2). Tang et al. (2014) studied exopolysaccharides produced by *P. mucilaginosus* as a possible bioflocculant in waste water treatment. Authors showed that the production of polysaccharides was enhanced by the addition of Mg^{2+}, Ca^{2+} and Fe^{3+} ions into the culture medium and they hypothesised a metabolic reaction of *P. mucilaginosus* to these ions. Moreover, these polysaccharides contained hydroxyl, carboxyl and phosphate functional groups, making them a possible sorbent for mentioned ions since divalent cation bridging of polysaccharides is reported to be the main mechanism of bioflocculation (Sobeck and Higgins 2002). Nevertheless, as soil conditions were not K, Mg or Ca limiting, the decreased concentrations of these ions in soil solution induced by MUCI were unlikely to affect growth of maize. As mentioned above, inhibition of maize growth in W + M treatment was related to N deficiency. Lower N status of plants probably led to lower exudation rates of malate in C + M and W + M treatments (Fig. 3) since carboxylate exudation of maize is known to be affected by nitrate supply due to involvement of carboxylates in nitrate reduction in roots (Neumann and Römheld 2001). This is partly confirmed by Pearson correlation analysis between exudation rates of individual carboxylates and nutrient concentrations in shoot biomass. This analysis revealed strong correlation for malate ($r = 0.94$; $p < 0.001$), oxalate ($r = 0.70$; $p < 0.01$), citrate ($r = 0.72$; $p < 0.01$) and isocitrate ($r = 0.58$; $p < 0.05$), particularly with N concentration in shoots. As a consequence, the effect of MUCI on the concentrations of acetate, formate and malate in rhizospheric soil solution was noticeable (Table S4). As mentioned above, lower concentrations of carboxylates in the rhizosphere may, in turn, decrease P acquisition by roots. This effect could be theoretically overcome by increased N supply but such hypothesis needs be tested in future research.

Conclusions

Wood ash, as a recycled fertiliser product, represents an important source of mineral nutrients which are, however, of low plant availability as demonstrated in this experiment. Application of *P. mucilaginosus* ABi13 led to changes in soil microbiome from which *Actinobacteria* were mostly affected. Inoculation of *P. mucilaginosus* in untreated, nutrient-poor, acidic soil had no significant influence on biomass yield of maize due to inefficient mobilisation of soil P. When soil was amended by addition of wood ash, increased P concentrations in soil solutions were detected after *P. mucilaginosus* inoculation. In this case, however, the growth of maize was inhibited due to significant decrease in NO_3^- concentrations in soil solution induced by *P. mucilaginosus*. As a consequence, the rate of LMMOA rhizodeposition in maize was lowered but decreased LMMOA exudation rates resulted in negligible concentration changes in rhizospheric soil solution sampled using centrifuge drainage method. To the best of our knowledge, this is the first report of increased P solubility from wood ash amended soil by PGPR. However, the mechanism by which *P. mucilaginosus* ABi13 immobilises N needs to be elucidated in future research in order to optimise N fertilisation strategies. Moreover, *P. mucilaginosus* ABi13 could be theoretically used for prevention of K, Ca, Mg and particularly NO_3^- leaching from soils after further tests. This study demonstrated the importance of plant nutritional status on the final outcome of PGPR inoculation and contributes to our understanding of interactions between introduced PGPR, soil microbiome and plants. Lower LMMOA rhizodeposition induced by PGPR may be clearly of ecological significance.

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11) Soil carbon transformation in long-term field experiments with different fertilization treatments

Přeměny půdního uhlíku v dlouhodobých polních pokusech s různými systémy hnojení

Přiložená studie řeší potenciální chování živin a různých systému hnojení v širších souvislostech. Je zaměřena na přeměny uhlíku, které mají klíčovou roli i v metabolismu fosforu a síry. Udává se, že fosfor v organických vazbách tvoří často více než polovinu celkového obsahu P a u síry je to dokonce přes 90 %. Dále je půdní organická hmota zdrojem C pro mikrobiální aktivitu, podílí se na stabilitě půdních částic, vodním režimu půdy apod. V článku byly hodnoceny půdy (černozem a luvizem) z dlouhodobých přesných polních pokusů s následujícími variantami hnojení: 1) nehnojená kontrola, 2) čistírenské kaly, 3) chlévský hnůj a 4) minerální NPK. Pro stanovení uhlíku byly využity následující extrakční postupy: 1) výluh 0,01 mol/l $CaCl_2$, 2) extrakce horkou vodou, 3) uhlík mikrobiální biomasy a 4) celkový organický uhlík. Cílem bylo stanovit změny uvedených forem uhlíku v závislosti na rozdílném organickém i minerálním hnojení, půdním typu a půdně-klimatických podmínkách. Z výsledků vyplývá, že k poklesu obsahu organického uhlíku došlo pouze u kontrolní nehnojené varianty. Naproti tomu varianta hnojená minerálními hnojivy vykazovala nárůst obsahu organického uhlíku po 21 letech trvání pokusu. Příčinou je pravděpodobně zařazení dvou obilnin v osevním postupu. Hnojení NPK podpořilo oproti kontrole výnosy včetně produkce strniště a vedlo tak k akumulaci organické hmoty. To potvrzují i výsledky Wipps (1990), podle kterých je 30-60 % fotosyntetické asimoilace translokováno do kořenů. Aplikace čistírenských kalů a hnoje podle předpokladů podpořila nárůst obsahu organického uhlíku v půdě, přičemž rozdíly mezi těmito variantami byly statisticky neprůkazné. Při hodnocení reaktivních forem uhlíku (C mikrobiální biomasy, C extrahovatelný horkou vodou nebo $CaCl_2$) nebyly zaznamenány statisticky průkazné rozdíly mezi sledovanými variantami, avšak na obou stanovištích byly patrné tendence k nejnižšímu obsahu u kontrolní varianty. Dále zde varianta hnůj zpravidla dosahovala vyšších hodnot ve srovnání s čistírenskými kaly. To koresponduje s výsledky Scherer et al. (2011). V pokusech se dále projevil klíčový vliv půdně-klimatických podmínek na transformace C v půdě.

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Soil carbon transformation in long-term field experiments with different fertilization treatments

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ABSTRACT

Balík J., Černý J., Kulhánek M., Sedlář O. (2018): Soil carbon transformation in long-term field experiments with different fertilization treatments. Plant Soil Environ., 64: 578-586.

Soil carbon transformation was observed in long-term stationary field experiments (longer than 20 years) at two sites with different soil-climatic conditions (Luvisol, Chernozem). The following crops were rotated within the trial: row crops (potatoes or maize)-winter wheat-spring barley. All three crops were grown each year. Four different fertilization treatments were used: (a) no fertilizer (control); (b) sewage sludge (9.383 t dry matter/ha/3 years); (c) farmyard manure (15.818 t dry matter/ha/3 years); (d) mineral NPK fertilization (330 kg N, 90 kg P, 300 kg K/ha/3 years). At the Luvisol site, the control treatment showed a tendency to decrease organic carbon (C_{org}) in topsoil. At organic fertilization treatments the content of C_{org} increased: sewage sludge – +15.0% (Luvisol) and +21.8% (Chernozem), farmyard manure – +19.0% (Luvisol) and +15.9% (Chernozem). At the NPK fertilization, the increase was +4.8% (Luvisol) and +4.7% (Chernozem). The increased C_{org} content was also associated with an increase of microbial biomass carbon (C_{mic}) and extractable organic carbon (0.01 mol/L $CaCl_2$ and hot water extraction). The ratio of C_{mic} in C_{org} was within the range 0.93–1.37%.

Keywords: farmyard manure; microbial biomass; organic matter; sewage sludge

Soil fertility care comprises mainly maintenance of soil organic matter; that is not only its amount but especially its quality. Soil organic matter is a key factor determining biological, chemical and physical properties of soils. Among the most important carbon (C) sources there are plant roots and post-harvest residues, as well as traditional organic fertilizers such as farmyard manure, slurry and compost. An alternative source of carbon in soil may be sewage sludges. In past, they contained high amounts of risk elements and negative impacts of sludges on soil quality were recorded. Brookes and McGrath (1984) reported a 50% decrease of soil microbial biomass in treatments with sludges as compared to manure. Smith (1991) assigned a soil microbial biomass decrease definitely to the negative impact of risk element in sludges. The content of microbial biomass carbon (C_{mic}) is one of the important indicators of soil organic matter transformation (Brookes et al. 1985, Mueller et al. 1998). Soil organic matter (SOM) consists of primary organic matter (POM) and humus. According to Körschens et al. (1990) SOM may be divided into 2 fractions. One is inert (stabile) and closely correlates with the content of clay particles, the other fraction is potentially mineralizable and it may be denominated as active organic carbon. For its extraction, Körschens et al. (1990) used hot water (hot water soluble carbon – C_{HWS}). A very easily mineralizable fraction is 0.01 mol/L

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$CaCl_2$-extractable organic carbon (C_{DOC}) (Nedvěd et al. 2008). To study soil carbon transformation is very difficult and it requires long-term field experiments.

The aim of this study was to determine changes in the content of soil organic matter and extractable carbon fractions in soils at different organic and mineral fertilization treatments at sites with different soil-climatic conditions.

MATERIAL AND METHODS

The effect of fertilization on grain yield of winter wheat and spring barley was observed in precise long-term field trials. These trials were established in 1996 at two sites of the Czech Republic with different soil-climatic conditions: Červený Újezd and Praha-Suchdol (Table 1). Within the trials, three crops were rotated in the following order: potatoes, winter wheat, spring barley. Because of the agrotechnical conditions of the Červený Újezd site, potatoes as the experimental crop were replaced by silage maize. The trial comprised 4 treatments: (1) no fertilization (control); (2) sewage sludge (SS); (3) farmyard manure (FYM); (4) NPK in mineral fertilizers (NPK). Organic fertilizers – sewage sludge and farmyard manure – were always applied in autumn (October) to potatoes (maize). Mineral phosphorus and potassium fertilizers were applied to each crop in autumn; mineral nitrogen fertilizers were applied to potatoes (maize) and spring barley in spring prior to the crop establishment. In the case of winter wheat, the nitrogen dose was divided into halves; the first one was applied as regenerative fertilization, the second one as productive fertilization (Table 2). The dose of nitrogen was 140 kg N/ha for wheat and 70 kg N/ha for spring barley. The NPK treatment of winter wheat and spring barley included phosphorus at a rate of 30 kg P/ha (triple super phosphate) and potassium at a rate of 100 kg K/ha (60% potassium chloride). Average characteristics of organic fertilizers and their dry matter application rates are shown in Table 3. The sewage sludge used for application at both experimental sites originated from one wastewater treatment plant during all experimental years.

During the 3 years (2014/2016), soil samples (depth 30 cm) were taken for determination of contents of organic carbon, microbial biomass carbon and other extractable forms of carbon. The monitoring was always carried out on blocks of wheat and potatoes (maize). Soils were sam-

Table 1. Experimental sites characteristics

Site		Červený Újezd	Suchdol
Location		50°4'22"N, 14°10'19"E	50°7'40"N, 14°22'33"E
Altitude (m a.s.l.)		410	286
Mean annual temperature (°C)		7.7	9.1
Mean annual precipitation (mm)		493	495
Soil type		Luvisol	Chernozem
Soil texture		loam	loam
pH_{CaCl_2}		6.5	7.5
Organic carbon (%)		1.168	1.486
Cation exchange capacity ($mmol_+$/kg)		145	230
P	Mehlich III (mg/kg)	100	91
K		80	230
Mg		110	240
Ca		3600	9000

Table 2. Application rates of nutrients (kg/ha) – (3-year cycle)

Treatment	Potatoes/maize			Wheat			Barley		
	N	P	K	N	P	K	N	P	K
Control	–	–	–	–	–	–	–	–	–
Sewage sludge	330[1]	201[2]	55[2]	0	0	0	0	0	0
Farmyard manure	330[1]	118[2]	374[2]	0	0	0	0	0	0
NPK[3]	120	30	100	140	30	100	70	30	100

[1]Nitrogen as the total nitrogen in organic fertilizers; [2]Average yearly dose depends on the nutrient content in organic fertilizers (Table 3); [3]Mineral fertilizers: N – calcium ammonium nitrate (27% N); P – triple super phosphate (21% P); K – potassium chloride (50% K)

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Table 3. Average characteristics of organic fertilizers and their dry matter (DM) application rates

	Dose (t/ha/3 year)	DM content (%)	Nitrogen content (% DM)	Carbon content (% DM)
Sewage sludge	9.383	31.08	3.52	25.75
Farmyard manure	15.818	30.76	2.09	27.86

pled in each crop since the beginning of spring (March/April) in intervals of 2–3 weeks, and the last samples were taken after the harvest of the crop. Eight soil samplings were taken for each crop during one experimental year.

Extractable organic carbon

0.01 mol/L $CaCl_2$ extraction. The extraction agent 0.01 mol/L $CaCl_2$ was used (1:10, w/v). The content of C_{DOC} was determined in fresh soil samples by segmental flow-analysis using the infrared detection on a Skalar^{plus}Ssystem (Skalar, Breda, Netherlands).

Hot water extraction. Extraction using hot water (Körschens et al. 1990) was used for assessment of extractable soil organic carbon. Soil samples were dried at 40°C and extracted with water (1:5, w/v). Suspension was boiled for one hour. The C_{HWS} was determined by a segmental flow analysis using the infrared detection on a Skalar^{plus}System (Skalar, Breda, Netherlands).

Microbial biomass C. Microbial biomass C was estimated in fresh soil samples by the fumigation-extraction method after pre-extraction (Brookes et al. 1985, Mueller et al. 1998). It was calculated as a difference in C content in fumigated and non-fumigated sample (E_C) using the k_{EC} coefficient (microbial biomass C = $E_C : k_{EC}$). The value of $k_{EC} = 0.45$ was used to calculate microbial biomass C (Mueller et al. 1998).

Total organic carbon. The content of total organic carbon in dry samples of soils, in farmyard manure and in sewage sludge was determined using oxidation on a carbon/nitrogen/sulphur analyser (Elementar Vario Macro, Elementar Analysensysteme, Hanau-Frankfurt am Main, Germany).

Statistical analysis. The results were assessed using the ANOVA statistical analysis. To evaluate the obtained results, the Statistica programme (StatSoft, Tulsa, USA) was used.

RESULTS AND DISCUSSION

The amount of plant biomass affects the individual carbon fractions in the soil, therefore the results of the average yields of crops grown on both sites were evaluated. Overall yield results are shown in Figure 1. To evaluate the results, values for all crops including the yield of straw at cereals obtained for the whole trial period of 20 years were related to the control treatment (control = 100%). Of course, there were significant differences among individual years; the results were influenced by weather conditions in individual

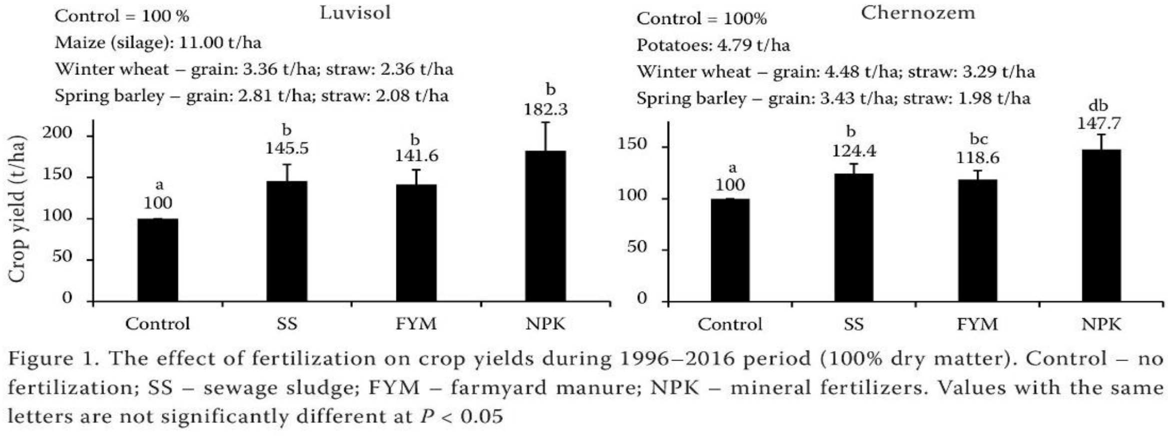

Figure 1. The effect of fertilization on crop yields during 1996–2016 period (100% dry matter). Control – no fertilization; SS – sewage sludge; FYM – farmyard manure; NPK – mineral fertilizers. Values with the same letters are not significantly different at $P < 0.05$

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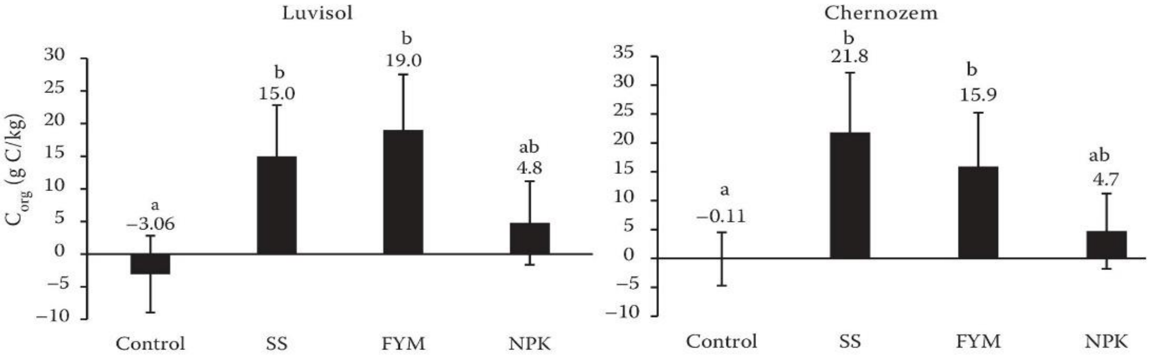

Figure 2. The relative changes of organic carbon (C_{org}) contents in soils (%) during 1996–2016 period. Control – no fertilization; SS – sewage sludge; FYM – farmyard manure; NPK – mineral fertilizers. Values with the same letters are not significantly different at $P < 0.05$

years, which increased variability of the whole data set. Significantly higher yields were obtained in mineral NPK fertilization treatments. The total N dose for a 3-year period (at crop rotation) was the same at all fertilization treatments (330 kg N/ha). Yet, at the NPK treatment a positive influence of relatively even distribution to individual crops was observed, which resulted in increased yields especially in barley and partly wheat. Organically fertilized plots did not vary significantly, however, at sludge treatment a tendency to increase yields was observed. Fertilization efficiency was higher at less fertile Luvisol sites, compared to Chernozem. Soil fertility potential at both sites could not be fully utilized due to the lack of precipitation during the growing period. The differences between control and other fertilization treatments were more pronounced with increasing length of the experimental period, mainly at Luvisols. In the first ten years (1996/2006) the average increase at fertilized plots at both sites was 133.1% compared to control; in 2007/2016 it was 153.6%. The ratio of grain and straw in wheat was 1:0.70 (Luvisol) and 1:0.73 (Chernozem), whereas in barley it was 1:0.74 (Luvisol) and 1:0.58 (Chernozem).

Organic carbon content. Changes in the organic carbon content in the topsoil are shown in Figure 2. The changes were calculated in relation to the initial state as follows:

$$\Delta C_{org} = [(C_{org.n} - C_{org.i})/C_{org.i})] \times 100$$

Where: $C_{org.n}$ – total C_{org} (g C/kg) at our sampling time (2014/2016; $C_{org.i}$ – initial C_{org} content in 1996 (Johnson et al. 2014).

This evaluation was done at plots with wheat, potatoes and maize and it represents average values for a 3-year period.

The figures show a decrease of C_{org} content at the control plots, by 3.06% at Luvisol and by 0.12% at Chernozem. The crop rotation included 2 cereals and 1 row crop, i.e. crops with relatively low input of C compounds into soil. There was no perennial forage crop used in the experiment. The changes were more marked at less fertile Luvisols in contrast to Chernozem. The results also show that inappropriate fertilization systems may cause degradation even of fertile soils, such as Luvisol. Mineral fertilization treatment increased C_{org} content by 4.80% (Luvisol) and by 4.70% (Chernozem). Significantly higher yields of the aboveground biomass (Luvisol by 82.3%, Chernozem by 47.7%) and thus also higher amounts of plant roots and root exudates contributed to an increase of C_{org} in soil. As reported by Lynch and Wipps (1990), 30–60% of the net photosynthesis production is allocated in roots; out of this amount, a substantial part is released in form of organic compounds (rhizodeposition) and carbon dioxide into the rhizosphere. Out of the total amount of carbon accumulated in roots, rhizodeposition covers 4–70%. Körschens et al. (2013) evaluated long-term trials in Europe (with various lengths of 16–80 years) and observed an average increase of C_{org} in topsoil by 10% at mineral fertilization treatments. Lower values in our experiments are probably caused by a shorter trial length. Literature often brings contradictory data on the influence of mineral (mainly N) fertilization on the changes

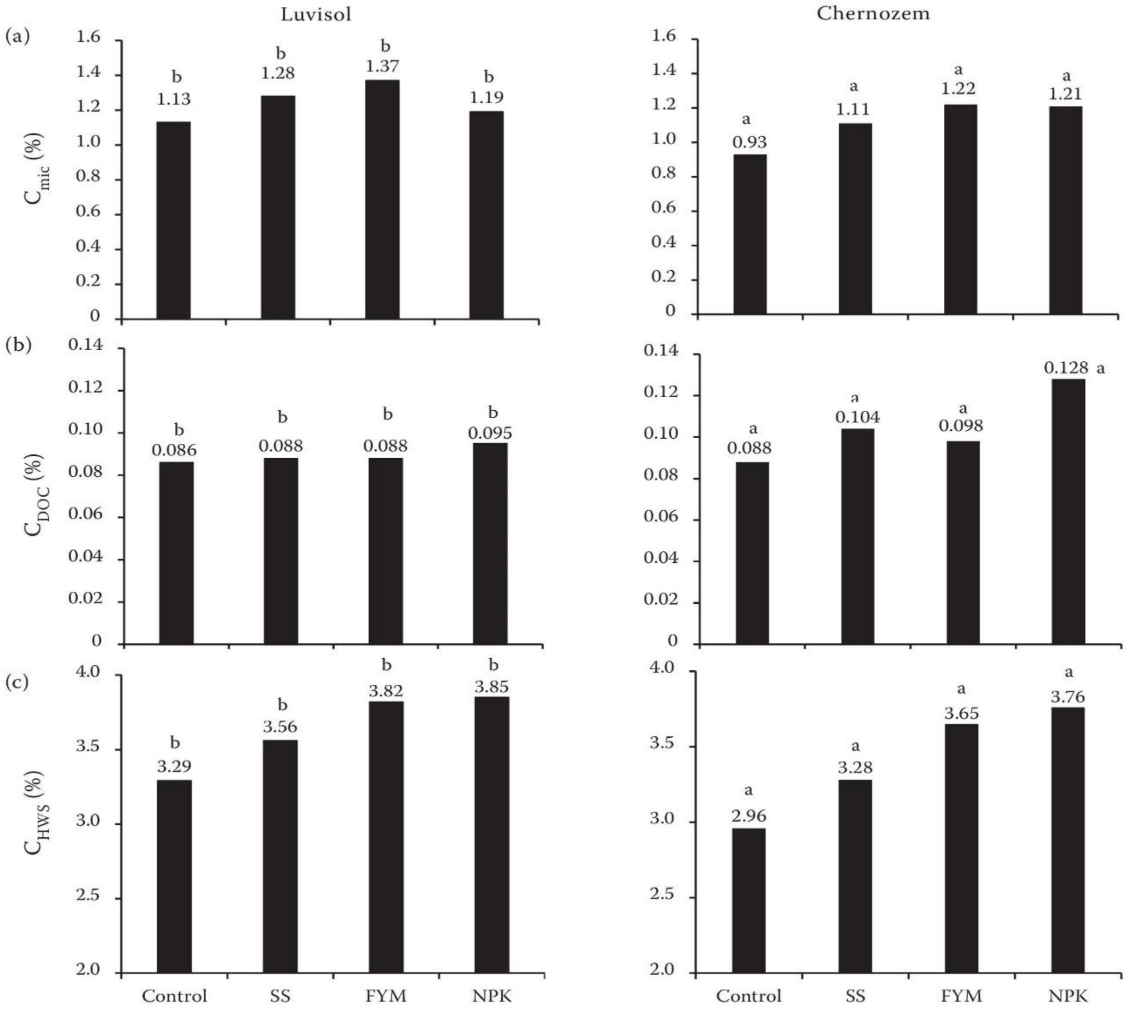

Figure 3. The portion of (a) microbial biomass carbon (C_{mic}); (b) $CaCl_2$-extractable organic carbon (C_{DOC}) and (c) hot water soluble carbon (C_{HWS}) in organic carbon (C_{org}). Control – no fertilization; SS – sewage sludge; FYM – farmyard manure; NPK – mineral fertilizers. Values with the same letters are not significantly different at $P < 0.05$

of C_{org} content in the soil. This is caused by site-specific soil-climatic conditions (e.g. pH value), crops grown, and form and dosage of N fertilization. For instance, Shahbaz et al. (2017) reported a C_{org} increase by 12–14% compared to the initial state at treatments with intensive nitrogen fertilization in long-term trials (32 years).

Organic fertilization treatments in this experiment resulted in a significant increase of C_{org} content in topsoil. The average dose of fresh manure was 51.42 t/ha/3 years, which corresponds to the agronomic recommendations for a sowing method of this type. Precise annual calculations suggest that in the experimental period, 30 850 kg C/ha was supplied into soil. At comparison with the carbon content in topsoil (depth 30 cm), carbon supplied by farmyard manure stands for 58.7% of the total amount of carbon in topsoil (Luvisol) and 46.1% (Chernozem). The increase of C_{org} in topsoil by 19% (Luvisol) and by 15.9% (Chernozem) thus corresponds to the fertilization intensity. Besides the organic fertilization, the increase is supported

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also by higher yield of biomass yield, including roots and root exudates. The absence of mineral fertilization and thus lower mineralization of soil organic matter might also have played a role.

Sewage sludge rate used in the experiment significantly exceeds the limits set for the sludge application on the agricultural soils in the Czech legislation. The allowed rate is 5 t dry matter/ha/3 years. This limit was surpassed almost 2 times. Meanwhile, the supplied amount of carbon was significantly lower compared to manure and totalled 16 910 kg C/ha. Compared to the carbon content in the topsoil it was 32.1% (Luvisol) and 25.3% (Chernozem). Although the rate of carbon supplied by sewage sludge was lower compared to manure, the changes in the C_{org} content in topsoil were at the same level, in case of sludge at Chernozem even higher. This might be due to supplied sludge mineralization or an increase of the root biomass, which corresponds to the fact that in the sludge treatment the yields of above-ground biomass were higher compared to the manure treatment.

Microbial biomass carbon content. In a 3-year period, the content of microbial biomass C (C_{mic}) was observed. Figure 3a shows relations (in %) to total C_{org}. The ratio of C_{mic} in our experiments was 0.93–1.37% and it was lower compared to Scherer et al. (2011), who reported 1.7–3.3% in long-term trials with sludge, manure and composts. Fierer et al. (2009) published even lower values than those in our study, namely 0.6–1.1%. On the contrary, Rasmussem and Collins (1991) reported the values of 2.3–2.9%. All these values show the influence of specific soil-climatic conditions as well as other factors, such as crop rotation, mineral and organic fertilization intensity, etc. Median values at control were 121 mg C_{mic}/kg (Luvisol) and 149 mg C_{mic}/kg (Chernozem) – Figure 4a,b. Also at other treatments, the C_{mic} values at the Luvisol site were by 15–25% lower compared to Chernozem, which corresponds to naturally lower soil fertility of Luvisol. The highest values were reported after the application of manure (Figure 4a,b). Černý et al. (2008) reported the C_{mic} values obtained in the same experiment on Chernozem for a period from its establishment (1996) till 2005. The authors determined a difference between control and sludge/manure treatment as 11% and 12%, respectively. Differences obtained in our experiment are more significant 21.5% (sludge) and 18.1% (manure). It confirms the fact that even at fertile sites, soil fertility degradation may progress if the soils are left unfertilized in the long term or unbalanced crop rotation is used. As reported by Fliessbach et al. (2007), high input of organic matter into soil results in an increased content of C_{mic}. Our data confirm these results for sludge and manure at both sites. The ratio of C_{mic} in C_{org} is higher at the farmyard manure application compared to sewage sludge (Figure 3a). Similarly, Scherer et al. (2011) also reported a significantly higher C_{mic} ratio at the manure treatment than at sludge. Compared to our trials, in their experiments with sludge significantly higher carbon amounts were supplied than with manure. Sewage sludge in our experiment was the same throughout the whole trial period and it contained significantly lower concentrations of risk elements than the limits set by legislation. At the same time, the amount of sludge used significantly overpassed the permissible dosage. However, neither a significant increase of the total risk elements concentrations at the sludge treatment, nor their concentrations in mobile soil fractions were recorded; hence, it may be presumed that their activity did not affect the C_{mic} content in soil. It must be also highlighted that at the sludge treatment, significantly less carbon was supplied compared to manure, which is probably the main reason of differing content of C_{mic}. Similarly, Johansson et al. (1999) did not observe a negative effect of high sludge rates application (8 t dry matter/ha/2 years) on soil microorganisms in the long-term trials.

The NPK values approximately by 10% higher than control confirm a positive effect of mineral fertilization (Figure 4a,b). It is interesting that the 10% difference is in good agreement with the results obtained by Geisseler and Scow (2014), who evaluated 64 long-term trials all over the world (107 data sets) and found an average increase of C_{mic} by 15.1% at treatments with mineral nitrogen fertilization compared to the control. Significance of the effect was related to the pH change. If the pH decreased to ≤ 5 no positive effect was observed whereas at higher values it was almost always positive. The positive effect was in correlation with the length of the trials, especially if the trial period was longer than 20 years. In our experiment, the value of pH_{CaCl_2} at the NPK treatment was 6.16 (Luvisol) and 7.23 (Chernozem). Also Fierer and Jackson (2006) in their extensive studies confirm a significant influence of soil reaction on the amount,

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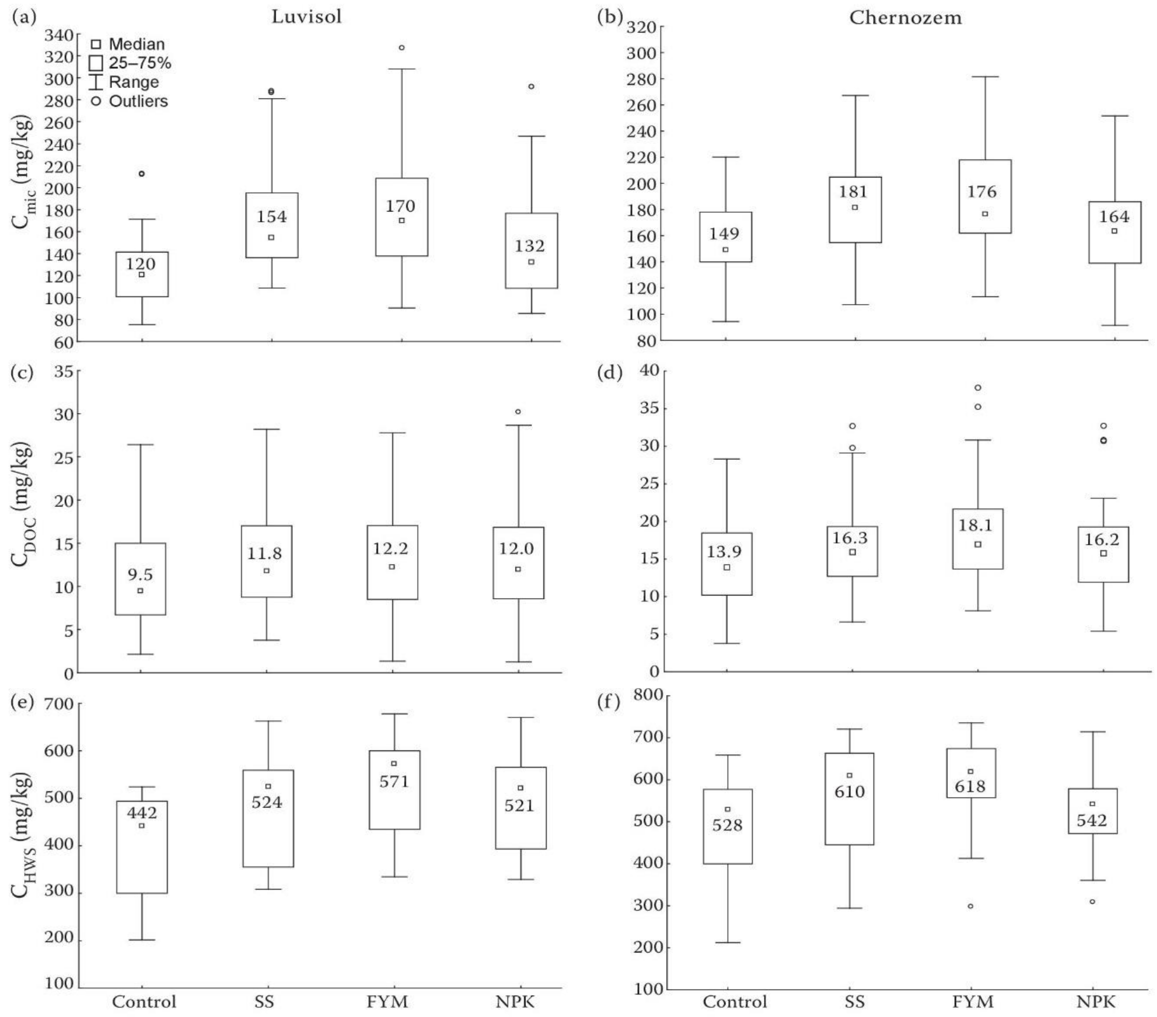

Figure 4. The content of (a,b) microbial biomass carbon (C_{mic}) (mg/kg); (c,d) $CaCl_2$-extractable organic carbon (C_{DOC}) and (e,f) hot water soluble carbon (C_{HWS}) Control – no fertilization; SS – sewage sludge; FYM – farmyard manure; NPK – mineral fertilizers

composition and activity of soil microbial biomass. Differences were observed especially at pH < 5. Also in the experiments carried out by Kallenbach and Grandy (2011) the content of C_{mic} at fertilization treatments increased by 9% compared to control. Similarly, a C_{mic} increase was observed in long-term trials in Sweden (Ultuna) at treatment with calcium nitrate compared to control and ammonium sulphate treatment (Marstorp et al. 2000). A significant increase of the aboveground biomass growth at the NPK treatment positively influenced the content of C_{mic} in our trials. It is supported also by the results of Börjesson et al. (2016) who studied the influence of cultivated crop on the content of C_{mic}. They found that after 13 years of maize monoculture, C_{mic} comprised 10–33% carbon from maize, i.e. from roots and root exudates. The authors further stated that this value was influenced by the level and quality of organic and mineral fertilization.

Extractable carbon fractions. A very weak extraction agent (0.01 mol/L $CaCl_2$) corresponds to

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low values of the released carbon (Figure 4c,d). With respect to the treatment and site, it was 0.084% to 0.128% of C_{org} (Figure 3b). At hot water extraction, a slightly mineralizable fraction should be released (Körshens et al. 1990). Though this fraction has not been precisely described, yet, it is presumed that it comprises part of the microbial biomass and simple organic compounds hydrolysable in hot water (Schulz 1997). In hot water extraction, 2.96% to 3.85% of C_{org} were mobilized (Figure 3c). Median values are in intervals 442–571 mg C_{HWS}/kg (Luvisol) and 542–610 mg C_{HWS}/kg (Chernozem) (Figure 4e,f). Šimon et al. (2013) in their study reported values lower by ca. 1/3. In their experiments, in accordance with our results, significantly higher values were also obtained at the manure application and NPK fertilization. Bolinder et al. (2007) supposed that ca. 75% are root exudates and 25% is the ratio of stubble. Residues with low C:N ratio decompose faster than those with a higher ratio, for instance plant roots (Cotrufo et al. 2013). Roots thus contribute to more stable C_{org} fraction in soils. Based on the prerequisite of a smaller amount of root exudates and amount of roots at control, the ratio of stabile forms of organic substances should be the highest in this treatment. In our study, the lowest ratio of 0.01 mol/L $CaCl_2$ and hot water extractable C (C_{DOC}) (C_{HWS}) in C_{org} was recorded at both sites. Differences compared to other treatments are nevertheless small, which is due to a variability of plots at individual sites and relatively short trial period.

Root exudates are an important source of labile carbon for soil microorganisms (Luo et al. 2014). The chemical composition of exudates is as follows: sugars 50–70%, carboxylic acids 20–30%, amino acids 10–20% (de Graaff et al. 2010). Root exudates stimulate fast growth of soil microbes, which may lead to increased mineralization of original organic compounds, a so called priming effect (Merino et al. 2015). Intensive mineral fertilization may thus result in increased C_{org} in soil, but at the same time in an increase of more labile organic fractions (Shahbaz et al. 2017). Also clay fractions that sorb these low-molecular organic compounds have a great impact on the whole process. The results are thus influenced by specific soil-climatic conditions. In our experiments, the NPK treatment gives the highest ratio of C_{DOC} and C_{HWS} in C_{org} (Figure 3b,c); however, these differences are not significant. It is not possible to prove that the NPK fertilization increases the ratio of mobile carbon fraction at the expense of stabile fractions, probably due to a short experimental period, relatively low intensity of nitrogen fertilization and quality of soils at experimental sites (Luvisol and Chernozem).

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12) Mehlich 3 extractant used for the evaluation of wheat-available phosphorus and zinc in calcareous soils

Vyhodnocení obsahu přístupného fosforu a zinku u pšenice pěstované na karbonátových půdách se zaměřením na možnost využití metody Mehlich 3

Je prokázáno, že se stoupajícím podílem vápníku v půdě klesá dostupnost fosforu a zinku (Kizilgoz 2016). Na karbonátových půdách je tak zvýšené riziko nedostatku těchto živin. Dále zde může docházet k antagonismu obou prvků, kdy při vysokých koncentracích fosforu dochází ke zhoršenému příjmu zinku a naopak (Nikolic et al. 2016). Metoda Mehlich 3 byla ověřena jako vhodné extrakční činidlo i pro půdy s hodnotou pH přesahující 6.5 (Wang et al. 2004). Cílem této studie bylo definovat optimální poměr P:Zn v karbonátových půdách při použití činidla Mehlich 3 s přihlédnutím na optimální poměr P:Zn v nadzemní hmotě a indexu využití fosforu (PNI) pšenicí ozimou. Za tímto účelem bylo provedeno sledování na široké škále karbonátových půd přímo v provozních podmínkách doplněné nádobovými pokusy. Korelační analýza mezi poměrem P:Zn v půdě a v nadzemní biomase pšenice ukázala v nádobových pokusech těsné vzájemné vztahy. To je v souladu s výsledky Karaman et al. (2006). Naopak vztahy poměru P:Zn v půdě k PNI byly pouze slabé. Z praktického hlediska jsou mnohem významnější výsledky polních pokusů. Zde se potvrdily výsledky nádobových pokusů, to znamená velmi silné až silné korelace mezi poměrem P:Zn v půdě a P:Zn v rostlině. Nejtěsnější vztah mezi těmito poměry byl zjištěn ve vegetační fázi počátku dlouživého růstu (BBCH 30-32), kde korelační koeficient činil 0,68. Na základě výsledků bylo možno určit pravděpodobné poměry P:Zn v nadzemní hmotě rostlin na základě půdních analýz. Například při dosažení poměru P:Zn v půdě 11:1 se dá ve dlouživé fázi růstu pšenice očekávat poměr P:Zn 140:1. Podobně lze postupovat i opačně – naměříme-li v nadzemní hmotě pšenice (BBCH 30-32) poměr P:Zn 130:1, dá se v půdě s vysokou pravděpodobností očekávat poměr P:Zn 9,3:1. Dosažené výsledky tak umožní s vysokou spolehlivostí odhadnout poměry P:Zn pouze na základě jedné analýzy (buď půdy, nebo nadzemní hmoty rostlin).

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Mehlich 3 extractant used for the evaluation of wheat-available phosphorus and zinc in calcareous soils

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ABSTRACT

Sedlář O., Balík J., Kulhánek M., Černý J., Kos M. (2018): Mehlich 3 extractant used for the evaluation of wheat-available phosphorus and zinc in calcareous soils. Plant Soil Environ., 64: 53–57.

Relation between wheat (*Triticum aestivum*) nutritional status determined at the beginning of stem elongation and during anthesis, respectively, and available content of phosphorus (P-M3) and zinc (Zn-M3) determined by the Mehlich 3 extractant was studied. Both one-year pot experiment with spring wheat and two-year on-farm trials with winter wheat were run on various calcareous soils (pH values of 7.18–7.94, median 7.80, P-M3 1–289 ppm, median 54, and Zn-M3 2–14 ppm, median 4), in the Czech Republic (Central Europe). Phosphorus nutrition index (ratio of phosphorus concentration in shoot biomass to critical phosphorus concentration – P_c) was calculated using the Belanger et al.'s model: $P_c = -0.677 + 0.221N - 0.00292N(2)$, where both phosphorus and nitrogen concentrations were expressed in g/kg shoot dry matter. Unlike phosphorus concentration in shoot biomass, phosphorus nutrition index significantly correlated with P-M3 content in soil. Optimal values of the phosphorus nutrition index were recorded if P-M3 was 51–68 ppm. Zinc concentration in shoot biomass more strongly correlated with P:Zn ratio (M3) in soil compared to Zn-M3 content in soil. P:Zn ratio in shoot biomass of 130:1 did not lead to phosphorus deficiency and corresponded to P:Zn (M3) ratio in soil of 9.3:1–14.3:1.

Keywords: biofortification; bioavailability; carbonate; soil test; zinc deficiency; soil test

Phosphorus (P) fertilization significantly affects grain yield of wheat plants (Zhou et al. 2017). Concurrently, both P and zinc (Zn) nutrition of wheat is reduced by increased soil Ca^{2+} content (Kizilgoz 2016). The Mehlich 3 (M3) method is suitable for the determination of available zinc content even in soils with pH > 6.5 (Wang et al. 2004). Furthermore, the Mehlich 3 method provides the advantage of multielement analysis (Iatrou et al. 2014). Zbíral (2016) expressed critical values of Zn-M3 content in soil. However, Zn concentration in cereal grains depends on available phosphorus content in soil (Nikolic et al. 2016) because the excess P rate decreases Zn uptake by plants (Konieczny and Kowalska 2016, Nikolic et al. 2016). Subclinical Zn deficiency is widespread (Rosado 2003). Zn bioavailability is often lower in vegetarian diets mainly due to their low Zn and high phytic acid contents (Kristensen et al. 2006). A latent Zn deficiency in wheat grain poses a high risk for grain quality relevant to human health in regions where wheat bread is a staple food (Nikolic et al. 2016). Therefore, high levels of P supply should be avoided, and Zn application to high-P soils should be considered (Kizilgoz and Sakin 2015). Karaman et al. (2006) stated, that an important factor for maximum Zn utilization by wheat plants is a site-specific P:Zn ratio in high-P soils (Karaman et al. 2006). However, the P:Zn ratio has not been determined in the Mehlich 3 extractant simultaneously for both elements so far. Furthermore, optimal values of P:Zn in soil remain to express.

Jones et al. (2005) stated that adequate soil P levels can substantially offset the impact of drought

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on growth and yield of barley. This is especially important because of frequent drought occurrence and higher temperatures during the growing season in the conditions of changing climate in Central Europe as reported by Neugschwandtner et al. (2015) and Křen et al. (2015). Sensitivity to Zn deficiency stress also becomes more pronounced when plants are drought-stressed (Bagci et al. 2007). Remobilization of Zn from older tissues is then critical for Zn accumulation in wheat grain (Kutman et al. 2012).

The aim of this study was to define the optimal P:Zn ratio in calcareous soils using the Mehlich 3 multielement extractant. The optimal P:Zn ratio in soil is bound to respect optimal P:Zn ratio in shoot biomass and simultaneously avoid reduced P uptake by wheat plants. All these criteria are essential prerequisites for biofortification of wheat with Zn.

MATERIAL AND METHODS

A pot experiment was conducted in a greenhouse in the year 2015 with spring wheat of cv. Chamsin on 37 different calcareous soils (pH > 7.1). Each soil had three repetitions (pots). Four germinated seeds were sown in each pot filled with 400 g of soil with the moisture of 60% of field capacity. Each pot was thinned to two plants nine days after emergence. A dose of 0.1 g N/kg soil (NH_4NO_3 solution) and 0.1 g K/kg soil (KCl solution) was applied two times during a vegetation period: two weeks after emergence and six weeks after emergence. The experiment was harvested when most plants reached a beginning of anthesis (61 BBCH). Results of the pot experiment were accompanied by on-farm trials realized at 43 plots with calcareous soils in various regions of the Czech Republic. Sampling of shoot biomass of winter wheat in on-farm trials was done at the beginning of stem elongation (30–31 BBCH) and during anthesis (61–69 BBCH). Sampling of soils was done at the beginning of stem elongation of winter wheat to a depth of 30 cm. Soils studied in both pot experiment and on-farm trials achieved pH values of 7.18–7.94 (median 7.80), P-M3 of 1.0–289.0 ppm (median 54.0) and Zn-M3 of 2–14 ppm (median 4).

Both P and Zn content in soil in the Mehlich 3 extractant, 1:10 w/v, and in shoot biomass were determined using the inductively coupled plasma with optical emission spectroscopy (ICP-OES Varian Vista Pro, Melbourne, Australia). Available P content in soil was also determined in 0.5 mol/L $NaHCO_3$ solution, pH 8.5 (Olsen et al. 1954) by segmented flow colorimetric analysis (Skalar San plus System, Breda, the Netherlands).

Due to the connection between phosphorus nutrition index and plant nitrogen (N) status, only wheat samples with nitrogen nutrition index (NNI) calculated according to Ziadi et al. (2010) of NNI = 0.84–1.30 (median 1.12) were assessed in the pot experiment. The nitrogen nutrition index was expressed as a ratio of the nitrogen concentration measured in shoot biomass to critical nitrogen concentration (N_c) calculated according to the model of Ziadi et al. (2010):

$$N_c = 38.5(DM)^{-0.58} \qquad (1)$$

In on-farm trials, only wheat plants with N concentration in shoot biomass ranging around 4.1% at the beginning of stem elongation (Balkcom and Burmester 2015) and 1.8% during anthesis (Lopez-Bellido et al. 2004) were assessed, which should indicate N optimal conditions. Total nitrogen concentration in shoot biomass of wheat plants was determined by the Kjeldahl method (Gerhardt Vapodest 50s, Königswinter, Germany).

Plant P status at the beginning of stem elongation as well as during anthesis were assessed using the calculation of shoot critical P concentration (P_c) according to the model of Belanger et al. (2015):

$$P_c = -0.677 + 0.221N - 0.00292N^2 \qquad (2)$$

with both P and N concentrations expressed in g/kg of shoot dry matter (DM). Analogously to nitrogen nutrition index calculated by e.g. Ziadi et al. (2010), phosphorus nutrition index (PNI) was calculated as a ratio of measured total P concentration in shoot biomass – P_m (g/kg) and the critical phosphorus concentration in shoot biomass – P_c:

$$PNI = \frac{P_m}{P_c} \qquad (3)$$

Values of PNI equal to or greater than 1.0 indicated that the crop is in situation of non-limiting P, while the values lower than 1.0 would indicate P deficiency.

A statistical analysis of the data was carried out using the Statistica 13 (Dell Inc., Reston, USA). Spearman's rank correlations were used to analyse relations among variables studied in the pot experiment; each variable consists of 111 cases. A probability value of 0.05 or less ($P \leq 0.05$) was taken to be statistically significant. Correlations among the selected variables in on-farm trials were studied using a linear regression analysis.

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Table 1. Spearman's rank correlations between the traits determined in pot experiment

	P-M3	Zn-M3	P-Olsen	P plant	Zn plant	PNI	P:Zn soil	P:Zn plant	Shoot DM
Zn-M3	0.42**								
P-Olsen	0.75**	0.35**							
P plant	0.18	0.20	0.34*						
Zn plant	–0.21*	0.38**	–0.25*	0.46**					
PNI	0.48**	0.20	0.60**	0.88**	0.21				
P:Zn soil	0.69**	–0.27*	0.49**	–0.04	–0.58**	0.28*			
P:Zn plant	0.39**	–0.26*	0.52**	0.24*	–0.70**	0.46**	0.63**		
Shoot DM	0.78**	0.41**	0.74**	0.00	–0.32*	0.28*	0.54**	0.36**	
% shoot DM	0.49**	0.12	0.41**	–0.36**	–0.52**	–0.05	0.49**	0.26*	0.73**

The *r*-values marked with asterisks are significant at the levels of significance $^*P < 0.05$ and $^{**}P < 0.001$. M3 – Mehlich 3; PNI – phosphorus nutrition index; DM – dry matter

RESULTS AND DISCUSSION

Although Olsen P method is usually used for determination of available soil P in calcareous soils (Iatrou et al. 2014), a strong correlation was recorded between P-M3 content and P-Olsen content in soil in the pot experiment (Table 1), which is in accordance with the findings of Iatrou et al. (2014). Therefore, in our study, the content of available P in soil was determined solely using the Mehlich 3 extractant.

No significant correlation was recorded between the P-M3 content in soil and P concentration in shoot biomass of wheat plants (Table 1). In contrast, the P-M3 content significantly correlated with phosphorus nutrition index. The phosphorus nutrition index can be then considered as a more reliable indicator of wheat P status on calcareous soils compared to the P concentration in shoot biomass during anthesis. Bélanger et al. (2015) recommend this predictive model of critical P concentration to quantify the degree of P deficiency during the wheat growing season mostly for high shoot N concentrations, which is in accordance with our results because nitrogen nutrition index ranged between the values of NNI = 0.84–1.30 (median 1.12). Simultaneously, a very strong correlation ($r > 0.80$) was recorded between the phosphorus concentration in shoot biomass and PNI.

The phosphorus nutrition index correlated significantly both with the P-M3 content in soil and P:Zn ratio in shoot biomass. A strong correlation was also found between the P:Zn ratio in shoot biomass and P:Zn ratio in soil, which is in accordance with the results of Karaman et al. (2006). However, the P:Zn ratio in soil weakly correlated with the PNI. Therefore, the P:Zn ratio in soil itself cannot be used as an indicator of available P content in soil. As a result, relations of P-M3 content in soil vs. PNI, P:Zn ratio in shoot biomass vs. PNI and P:Zn (M3) ratio in soil vs. P:Zn ratio in shoot biomass of winter wheat plants were further studied in on-farm trials (Figures 1–3).

A strong correlation was recorded between the PNI determined at both growth stages and P-M3 con-

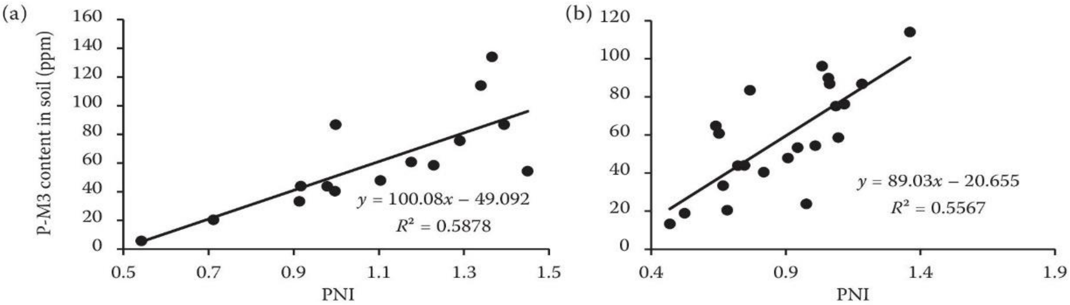

Figure 1. Correlation between P-M3 content in soil and phosphorus nutrition index (PNI) of winter wheat (a) at the beginning of stem elongation and (b) during anthesis, respectively, in on-farm trials

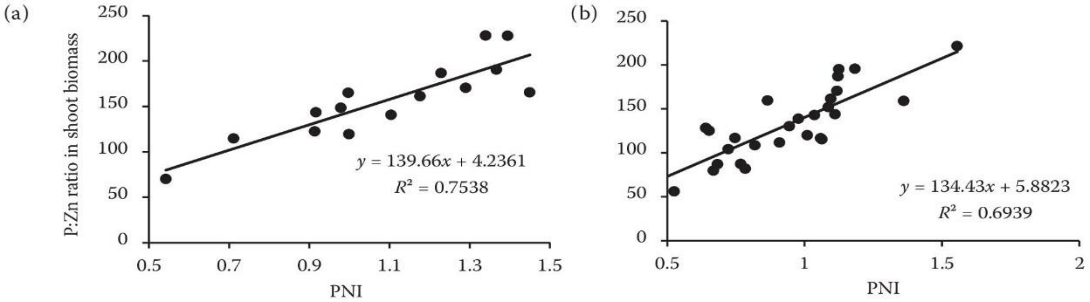

Figure 2. Correlation between phosphorus nutrition index (PNI) of winter wheat and P:Zn in shoot biomass of winter wheat (a) at the beginning of stem elongation and (b) during anthesis, respectively, in on-farm trials

tent in on-farm trials with winter wheat (Figure 1). According to regression equations, optimal plant phosphorus status, i.e. PNI = 1, corresponded to P-M3 content of 51 ppm and 68 ppm at the beginning of stem elongation and during anthesis, respectively. This result is in accordance with the findings of Buondonno et al. (1992), who expressed optimal P-M3 content in calcareous soils in Italy as 37–77 ppm. Buondonno et al. (1992) determined P-deficient soil if the P-M3 content is lower than 30 mg P/kg. After fitting into the regression equations in Figure 1, the P-M3 content of 30 ppm corresponded to PNI = 0.79 and PNI = 0.57 at the beginning of stem elongation and during anthesis, respectively. These values of phosphorus nutrition index indicate P deficit in plants.

Zinc concentration in shoot biomass correlated weakly with Zn-M3 content in soil (Table 1), which is in accordance with the results of Junus and Cox (1987). However, the Zn concentration in shoot biomass correlated moderately with P:Zn (M3) in soil in the pot experiment.

Mai et al. (2011) stated that P:Zn ratio in shoot biomass of the wheat grown 30 days with addition of Zn into nutrient solution was at the level of ca. 120:1 which approximates to the ratio of 140:1 stated by Dennis (1971) as necessary for balanced P and Zn nutrition of wheat plants. As follows from a very strong linear correlation showed in Figure 2, P:Zn ratio of 140:1 in shoot biomass of winter wheat at the beginning of stem elongation and during anthesis corresponded to PNI = 0.97 and PNI = 1.0, respectively. The P:Zn ratio in shoot biomass of 120:1 corresponded to phosphorus nutrition index determined at the beginning of stem elongation and during anthesis of PNI = 0.82 and 0.85, respectively. Compromisingly, the P:Zn ratio in shoot biomass of 130:1 corresponded to the phosphorus nutrition index determined at the beginning of stem elongation and during anthesis of PNI = 0.90 and 0.92, respectively, indicating a sufficient phosphorus supply of winter wheat.

A correlation between P:Zn (M3) ratio in soil in on-farm trials and P:Zn ratio in shoot biomass of

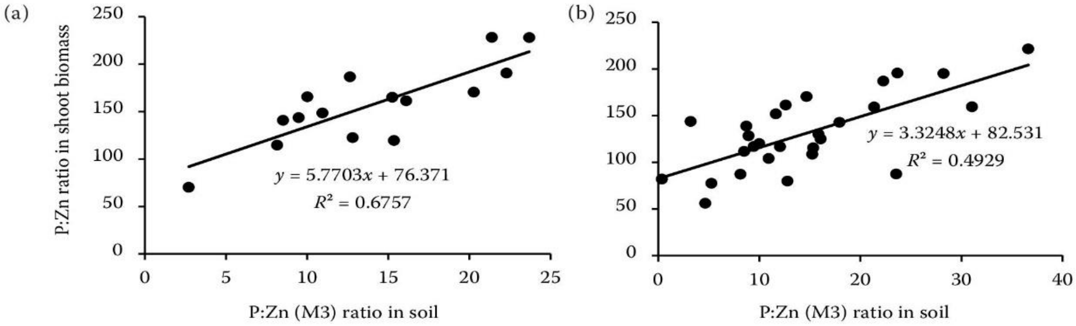

Figure 3. Correlation between P:Zn (M3) ratio in soil and P:Zn in shoot biomass of winter wheat (a) at the beginning of stem elongation and (b) during anthesis, respectively, in on-farm trials

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winter wheat at the beginning of stem elongation and during anthesis was very strong and strong, respectively (Figure 3), which complies with the findings of Karaman et al. (2006). According to the regression equation, P:Zn ratio in shoot biomass of winter wheat at the beginning of stem elongation and during anthesis of 140:1 corresponded to P:Zn (M3) ratio in soil of 11.0:1 and 17.3:1, respectively. However, the P:Zn ratio in shoot biomass of winter wheat at the beginning of stem elongation and during anthesis of 130:1 corresponded to P:Zn (M3) ratio in soil of 9.3:1 and 14.3:1, respectively.

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13) Use of active microorganisms in crop production – a review

Využití aktivních mikroorganismů v rostlinné produkci – literární přehled

Tento literární přehled byl zpracován s cílem získat přehled o aktuálních možnostech využití zejména mikrobiálních biostimulantů v rostlinné produkci, to vše se zaměřením na roli biostimulantů při tvorbě výnosu a při mobilizaci živin z hůře dostupných forem. Vycházeli jsme z faktu, že zejména fosfor je v současné době doplňován do půdy především z neobnovitelných zdrojů. Nejnovější poznatky ukazují, že hlavní neobnovitelné zdroje mletých fosfátů budou vyčerpány za 50-100 let. Jednou z cest jak šetřit tyto zdroje je zvýšit mobilizaci P z nepřístupných forem pomocí biostimulantů. Přestože již na trhu existuje celá řada mikrobiálních i jiných biostimulantů, mechanismy jejich působení zůstávají zatím do značné míry nepopsané. Z celé řady laboratorních testů i nádobových pokusů vyplývá, že je zde velký potenciál různých mikroorganismů v oblasti uvolňování živin z hůře přístupných forem z půdy i z aplikovaných hnojiv. To paradoxně představuje riziko v nadhodnocení jejich účinnosti v zemědělské praxi. Důkazem jsou výsledky z polních pokusů, kde ještě pozitivní role biostimulantů prakticky nebyla prokázána. Ukazuje se tak, že biostimulanty zatím představují mnohem větší potenciál využití pro skleníkové provozy ve srovnání s běžnými polními. V níže uvedeném přehledu jsou zahrnuty výsledky laboratorních, nádobových i polních pokusů. Jako příklad lze uvést pozitivní vliv houby *Trichoderma harzianum* na výnos brambor ve skleníkových podmínkách (Buysens et al. 2016), dále vyšší výnos zrna pšenice při aplikaci *Penicillium bilaiae* v polních pokusech (Ram et al. 2015). Více úspěšných pokusů bylo provedeno s bakteriálními přípravky – např. aplikace aplikace *Pseudomonas sp.* vedla ke zvýšení výnosu kukuřice (Kifle et Laing 2016, Gholami et al. 2009) a ječmene (Fröhlich et al. 2012) v polních pokusech. *Bacillus subtilis* prokázal v polních pokusech pozitivní vliv na růst různých rostlin (Altuhaish et Tjahjoleksono 2014, Turan et al. 2014) a aplikace *Rhizophagus intraradices* měla pozitivní vliv na růst rostlin rajčat (Mohamed et al. 2016). I přes tyto první pozitivní výsledky z reálných podmínek však podrobný dlouhodobý výzkum v této oblasti stále chybí.

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Review Article Open Access

Use of Active Microrganisms in Crop Production – A Review

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Abstract

Phosphorus, other elements and natural resources are scarce, and so it is necessary to find alternative strategy to increase availability of nutrients for plants. One possible way could be application of so-called bioeffectors (BE) which should improve the mobilisation of nutrients (especially phosphorus) from less available forms in soil, improve plant growth and contribute to mycorrhiza development. BEs are commercially supplied products which contain active substances (live microorganisms and active natural compounds). BEs can be used in organic agriculture, because their application represents no risk for the environment. Several studies and experiments are focused on impact of bioeffectors' application and their active compounds on plants. Experiments were performed under different conditions (field, pot, greenhouse), on various testing plants and on various bioeffectors. These BEs have been used as a fertilizer, fungicide or molluscicide and they were applied either to soil, seed or leaf. Application should increase growth of root system and above-ground part of plants and also nutrient uptake. These products are developed for a wide variety of crops (e.g. maize, wheat, tomatoes, rape, spinach, grass, ornamentals). This review summarizes the most recent knowledge in this scientific field.

Keywords: Bioeffector; Microoragnisms; Soil; Nutrients; Crop production

Introduction

Most of the nutrients found in soil are in for plants inaccessible forms, therefore our society and crop production depend on commercially produced fertilizers. Even commercially produced fertilizers used in agriculture are produced from natural nutrient resources and as such are limited in availability. The most limited nutrient for plant production and agriculture is phosphorus with its natural reserves estimated for fifty years. For these reasons, it is necessary to find an alternative strategy for future generations that would help in better availability and use of plant nutrients in the application of lower input of commercially/industrially supplied products and would also be environmentally friendly.

Phosphorus (P) is an essential, non-renewable nutrient for plant development and growth. Plants acquire P from soil solution as orthophosphate anions. However, orthophosphate is very reactive and may be immobilised through precipitation or adsorption, making P highly insoluble and unavailable to plants. The majority of P fertilizers are currently derived from rock phosphate, which is predicted to become increasingly scarce in the future. Research and development on the efficient use of other available sources of P is therefore crucial [1-3]. Phosphorus deficiency is one of the major limiting factors for decreased agricultural production [4]. Due to a growing world population it is expected that demand for food and feed will increase. Limited availability of productive agricultural land and increasing dependance on mineral fertilizers make it necessary to develop alternative strategies for plant nutrition [5,6]. BEs can contribute, depending on soil and climate conditions, to overcome limitations in the availability of nutrients. These compounds contain microorganisms such as bacteria or fungi and active natural substances (extracts from soil, compost or seaweeds, microbial residues, plant extracts). These products are developed for a wide variety of crops (e.g. crops, grass, ornamentals, grass). Their effective use should cause the mobilisation of nutrients from less bioavailable forms in soil [5] and further support root growth [7,8] and mycorrhiza development [9]. Microorganisms may play an important role in enhancing availability of P to plants and have been proven to enhance uptake directly by extending the root system (e.g. mycorrhizal associations), increasing mobilisation of orthophosphate from soil organic and inorganic phosphorus, and stimulating root growth [1].

Mycorrhiza is highly effective in absorbing nutrients from the soil, especially for nitrogen and phosphorus. Nitrogen and phosphorus are often limited in supply and fungal hyphae are able to absorb these nutrients more efficiently and from greater area of soil than the roots, which leads to increased plant growth. This causes mutually beneficial linkage between plants and fungi, the sugars (organic carbon) formed during photosynthesis are transported to the roots and the fungi are taken and the nutrients are absorbed by fungal hyphae from the soil and are transported into plants [10,11]. Arbuscular mycorrhizal fungi colonise most agricultural species (exceptions include *Brassica* spp., and *Lupinus* spp.) and play an important role in the phosphorus nutrition of many farming systems worldwide, especially on soils with low available phosphorus [3].

Literature Review

Examples of plant strategies for phosphorus obtaining:

a) Growth of roots

b) Root exudates (acidic phenolics)

c) Mycorrhiza

d) Cooperation with microorganisms (P-solubilization).

One alternative strategy in plant production can be use of unmycorrhizal organism's P mobilizing nutrients, which should help to increased nutrient availability for plants. These substances are so-called bioeffectors.

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Bioeffectors

In the last two decades, increased interest in sustainable agricultural practices has seen the growing development and use of commercial microbial inoculants for increasing crop productivity and resource use efficiency. Microbial inoculants mainly include free-living bacteria, fungi and arbuscular mycorrhizal fungi [12]. Development of the BEs increases due to the potential use of these substances in organic farming and also because of the limited natural resources of nutrients [13].

These products are divided into three main groups, according to which of active substances or microorganisms they contain. BEs addressed comprise fungal strains of *Trichoderma*, *Penicillium* as well as bacterial strains of *Bacillus*, *Pseudomonades*, *PaeniBacillus* and *Rhizophagus* with well-characterized root growth promoting and nutrient-solubilising potential. Natural extraction products of seaweed, compost and plant extracts, as well as their purified active compounds with protective potential against biotic and abiotic stresses are also tested in various combinations [5].

Fungal bioeffectors: As mentioned above BEs can be divided into two main groups namely fungal and bacterial. Several fungal representatives have been selected and described further in this section and in Table 1. There are selected bacteria and their impact on crop production.

***Trichoderma ssp.*:** The genus *Trichoderma* spp. are wild filamentous fungi occurring in most soil types and different habitats. *Trichoderma* is a fungal genus that includes species that are currently being used as BEs or as biofertilizers [14,15]. The *Trichoderma* is known for producing enzymes and antibiotics. These species are attributed to a variety of physiological, antifungal and insecticidal effects. It acts against a broad spectrum of plant pathogens. These fungi increase plant growth and development, but also development of root system [7,8,13,16,17]. It has also been observed that selected *Trichoderma* strains can improve plant nutrients' uptake [18]. Increased growth occurs due to its strong anti-pathogenic activity, biosynthesis of hormones, improving nutrient uptake from the soil, root development by increasing metabolism rate of carbohydrates and increased photosynthesis [13]. The main hydrolytic enzymes secreted by the fungus are proteases, chitinases and endochitinases [16]. Chitinase are produced by e.g. bacteria, algae, fungi, plants, insects, nematodes, molluscides, vertebrates, including man and certain viruses [19].

***Trichoderma harzianum*:** *T. harzianum* is wild filamentous fungus that occurs in soil. *Trichoderma* belongs to fungi that includes species that are currently being used as biological control agents or as biofertilizers [14,15]. It has also been observed that selected *Trichoderma* strains can improve plant nutrient uptake [18]. Buysens et al. [20] used *T. harzianum* in study on potato were conducted in a greenhouse or *in vitro* conditions. Experiments were conducted at two sites in Belgium 2009-2012. The objective of this study was to investigate the impact on potato yield of the co-inoculation of *R. irregularis* (strain MUCL 41833) and *T. harzianum* (strain MUCL 29707) applied to a cover crop (*Medicago sativa*) preceding potato planting or to potato at planting. In both trials we observed that the most advantageous agricultural practice to increase potato yield was the inoculation of a preceding cover crop with both microorganisms. Inoculation with beneficial microorganisms increased potato tuber weight in both trials compared to the non-inoculated treatments. This was mainly attributed to improved arbuscular mycorrhizal fungi colonization of potato plants. The inoculation via cover crop seems a more efficient strategy as compared to the direct inoculation at potato plantation. However, difference between these strategies on potato production may not be solely attributed to Arbuscular mycorrhizal fungi colonization rates but could also be due to higher N availability, but it was not tested. Gupta et al. [21] conducted a study and pots experiment focused on the non-target effects of a microbial consortium comprising three selected bioinoculants: *Bacillus megaterium* (strain MTCC 453), *Pseudomonas fluorescens* (strain MTCC 9768) and *Trichoderma harzianum* (strain MTCC 801), on the resident as well as active microbial community structure in pigeon pea (*Cajanus cajan*) rhizosphere. The treatment was found to result in a significant increase in shoot length (1.2-fold), root length (1.3-fold), dry mass (2.4-fold) and grain yield (2.5-fold) of pigeon pear plants with the application of microbial consortium over control plants. The use of chemical fertilizers also led to improvement in plant parameters over control but upto a lesser extent than that with the microbial consortium. The performance of the consortium was found to be about 1.2-fold better than the recommended dose of chemical fertilizers in terms of grain yield. Ahmad et al. [22] conducted a pot experiment with *Brassica juncea* (var. Varuna) respectively focused on influence of soil salinity on brassica after application of *T. harzianum*. Stress caused by soil salinity causes the plants smaller and slower growth, change of plant physical and biochemical properties and decrease in yields of biomass. Results showed that the seedling plants were treated with *T. harzianum* were significantly more resistant to stress conditions caused by salinity, compared to untreated plants.

***Penicillium bilaii*:** Microorganism *P. bilaii* is a soil fungus that lives in symbiosis with plant roots and has been shown to increase the dissolution and absorption of phosphorus in certain crops [1,23]. Some *Penicillium* species can also release fixed phosphorus (P) in the soil and make it available to growing plants. Compared with other nutrients, P is the least mobile and available to plants in most soils. P-solubilizing fungi play an important role in the global phosphorus cycle and can supply P to plants in an environmentally friendly and sustainable

Fungi	Experimental conditions	Effect on the plant	References
Trichoderma spp.	Laboratory conditions	Improve growth and seed production of soybean	Paradiso et al. [79]
	Laboratory conditions	Growth promoter of cowpea	Chagas et al. [74]
Trichoderma harzianum	Pot experiment	Improve germination and seedling growth of wheat	El-Gremi et al. [13]
	Greenhouse and laboratory conditions	Increase potato yield	Buysens et al. [20]
	Pot experiment	Increase shoot and root length, dry mass and grain yield of Pigeon pea	Gupta et al. [21]
	Pot experiment	Increase growth of *Brassica juncea*	Ahmad et al. [22]
	Pot experiment	Increased root length, growth and shoot dry weight in *Brassica nigra* and melon	Galletti et al. [7]
Penicillium bilaii	Rhizobox experiment	Increase root length of maize	Gomez-Munoz et al. [1]
	Field conditions	Increase grain yield of wheat	Ram et al. [2]
	Field conditions	Increase root length and P-content in root of pea	Vessey and Heisinger [26]
	Plot experiment	Increase yield of alfalfa	Beckie et al. [28]

Table 1: BEs as promoting fungi of crop production.

Bacteria	Experimental conditions	Effect on the plant	References
Pseudomonas spp.	Laboratory, greenhouse and field conditions	Increased germination, shoot and root length, grain yield of maize	Kifle and Laing [30]
	Field conditions	Increased grain yield and straw weight of barley	Fröhlich et al. [34]
	Pot experiment and field conditions	Improves germination, growthh parameters and yield of maize	Gholami et al. [75], Nezarat and Gholami [78]
	Laboratory conditions	Growth stimulation of tomato plants	Gravel at al. [76]
Pseudomonas jessenii	Greenhouse and field conditions	Increase yield and shoot dry weight of chickpea	Valverde et al. [35]
	Greenhouse conditions	Increase growth of tomato	
Bacillus amyloliquesfaciens	Laboratory conditions	Increase root and shoot growth of rice	He et al. [41]
Bacillus subtilis	Field conditions	Increase macro and micro nutrient absorption, growth and plant production	Altuhaish et al. [54]
	Field conditions	Increase fresh and dry shoot and root weight	Turan et al. [55]
Paenibacillus mucilaginosus	Pot experiment	Improve growth of trifoliate orange seedlings	Wang et al. [56]
Rhizophagus intraradices	Greenhouse conditions	Increase the plant growth, number of leaves, plant height, shoot and root length and weight of tea	Sharma and Kayang [80]
	Field conditions	Increase growth of tomato	Mohamed et al. [62]

Table 2: BEs promoting bacteria of crop production.

manner. *P. bilaii* is used as a seed inoculant to improve P efficiency in a variety of crops such as wheat, maize, rape, bean, soya, legumes and alfalafa. This soil fungus is able to solubilize mineral phosphates and enhance plant uptake of phosphate [1,3,24]. Three mechanisms are involved by P-solubilising microorganisms: acidification of the soil, release of organic acid anions and release of phosphomonoesterase and phytase [1]. Cunningham and Kuiack [25] demonstrated that the major acidic metabolites produced by *P. bilaii* are oxalic and citric acid and so *P. bilaii* may increase the availability of phosphate to the plant by releasing organic acids. Gomez-Munoz et al. [1] conducted rhizobox experiments with maize, which was grown for 27 days in rhizoboxes enabling studies of root growth in addition to plant and soil parameters. In this experiment inoculated *P. bilaii* (strain ATCC 20851) either at the seed or the sewage sludge patch. At early growth stages, *P. bilaii* inoculation of seeds increased maize shoot length. However, at the end of experiment, the effect had ceased. Root growth was increased by seed *P. bilaii* inoculation alone and in combination with sewage sludge, whereas patch inoculation was less effective. Colonization studies performed at harvest showed that *P. bilaii* could not be detected in the maize rhizosphere but stayed at the place of inoculation. *P. bilaii* did not colonise the rhizosphere extensively but merely stayed at the place of inoculation. At the end of this experiment inoculation of *P. bilaii* showed no effect on shoot length or shoot biomass. Inoculation of sewage sludge with *P. bilaii* did not result in an increase in phosphorus uptake and thus proved to be less effective than seed inoculation. These findings confirm that *P. bilaii* application can promote root growth, increasing potential plant adsorptive capacity. While, in this study, the higher root development did not result in an increased P uptake, presumably due to severe limitations in the soil nutrient content, it remains an open question. Ram et al. [2] were conducted field experiments during 2009-2011 to evaluate the effect of seed inoculation with Penicillium bilaii on wheat at different rates of phosphorus fertilizer on P content in leaves and grain yield of irrigated wheat in India. The study showed potential of using *P. bilaii* as bio-inoculants along with 50% of recommended P fertilizer dose that produced wheat yield similar to 100% P when no *P. bilaii* was used. However, more such long-term studies are needed on different soil types varying in P availability, pH and P fixation capacity. Karamanos et al. [23] conducted a serie of 47 experiments with spring wheat. Experiments were carried out in the three prairie provinces in 1989 and 1995 and included the application of *P. bilaii*. Of the 47 trials was found the reaction to the P-fertilizers in 33 cases. These effects can not be attributed to the concentration of P in the soil, soil organic matter, texture or weather conditions and are considered a random event. Effect on the intake of phosphorus was only P-fertilizers. Vessey and Heisinger [26] describes experiments on pea (*Pisum sativum*) that were established at two locations in Canada. Inoculation of this organism in combination with a phosphorus fertilizer caused a prolongation of root length and increased the phosphorus content in the roots compared to the control which has been performed by phosphorus fertilizer. Gulden and Vessey [27] mainly focused on observation of formation of root hairs in pea after inoculation *P. bilaii*. The experiment was based on the application of the microorganism and P-fertilizer. In this experiment, the effects were investigated by *P. bilaii* on growth and morphology of the root of the pea grown in three different quantities delivered phosphorus (0, 1, 10 mg l^{-1}). The proportion of root hair was significantly higher in pea inoculated *P. bilaii* compared with control plants. Different quantities of supplied phosphorus did not affect the proportion of root hairs or their length. Root hairs in pea, which were inoculated *P. bilaii* were on average 33.3% higher than for uninoculated plants. Beckie et al. [28] used the *P. bilaii* for inoculation alfalfa in combination with P-fertilizers and the results of the experiments show that the greatest response to inoculation occurred at the beginning of the growing season. In the year following vaccination yield of vaccinated alfalfa grown on average by 3% compared to uninoculated plants (Table 1).

Bacterial bioeffectors: Several promising bacterial representatives have been selected and described further in this section and in Table 2. There are selected fungi and their impact on crop production.

***Pseudomonas spp.*:** *Pseudomonas* sp. is ubiquitous in agricultural soils, well adapted to growing in the rhizosphere. *Pseudomonas* well suited as biocontrol and growth-promoting agents [29]. These bacteria are a component biofertilizers, which use along with mineral fertilizers may serve as an effective approach for enhancing the crop nutrient requirements, thereby leading to the sustainable crop production. Biofertilizers consist of beneficial microbes, which form colonies in soils and promote plant growth by increasing nutrient availability when applied as a seed dressing or on plant surfaces. These microorganisms can enhance the availability of deficient or immobile nutrients in soils after solubilizing their mineral forms. For example, *Pseudomonas putida* can promote plant growth by P-solubilization, biological nitrogen fixation, availability of trace elements such as Fe and Zn and the production of plant growth regulators. Use of *P. putida* has improved the growth and yield of various crops such as bean, pea, rice, tomato and wheat. Therefore, use of this bacteria has been suggested as a sustainable solution for improving crop production. Factor *P. putida* either alone or in combination with addition of phosphorus improved the plant growth, plant uptake (N, P, K) and antioxidative activity [4]. Laboratory, greenhouse, and field experiments were conducted at University of KwaZulu-Natal, Pietermaritzburg, in the 2010/2012 seasons to study the effects of eight strains of diazotrophic bacteria on the growth and yield of maize. Maize seeds were treated with *Bacillus megaterium*, *Pseudomonas* sp. (strains B5, A3, A6, A61), *Burkholderia ambifaria*, *Enterobacter cloacae* and *Pantoea ananatis*, aiming to stimulate plant growth, and maintain or increase yields while reducing the need for N fertilization. All the diazotrophic bacteria increased germination of maize seed, and *Pseudomonas* sp. (B5) and *B. megaterium* significantly increased shoot length. *Pseudomonas* sp. (B5) and *Pseudomonas* sp. (A3) very significantly increased root length and seed vigor index. Seed treatments with selected diazotrophs resulted in increases in seed germination, but they caused no significant increases in grain yield, dry weight, plant height and chlorophyll content when compared to the untreated control. This may have been due to high competition from the indigenous soil microflora, given that success of microbial inoculation depends on the colonization and competitive ability of the inoculants. Plant roots exudates, colonization of roots by other bacteria, and soil health may also influence the efficiency of bacterial inoculations [30-32] conducted the positive effect of seed inoculation with diazotrophic bacteria on shoot dry weight and yield of maize has been reported by many researchers, for example Kifle and Laing [30]. The most closely related bacteria are *Pseudomonas fluorescens*. Knot et al. [33] reported the fact that application of *Pseudomonas* sp. increases germination of *Poa pratensis* seeds in laboratory conditions, especially 2-4 years old seeds. Also Fröhlich et al. [34] researched the positive effects of this product in growing barley. When *Pseudomonas* used in field conditions grain yield and weight of the straw increased. Also in the greenhouse conditions plants showed greater yield and better growth. Yusran et al. [9] reported that application of Proradix and RhizoVital (individually or in combination) into soil in pot trial led to improved state of tomato roots. They were healthy and showed significantly higher colonization by arbuscular mycorrhizal fungi.

***Pseudomonas jessenii*:** *P. jessenii* is a fluorescent, gram-negative bacteria and this bacteria was applied in two regions of Spain, Castilla y Leon and Andalucia was conducted study by Valverde et al. [35] with aim to find useful biofertilizers for staple grain-legumes, chickpea. In this study were made pot, greenhouse and field experiments, where was tested single and dual inoculations or in combination with phosphate fertilizer on chickpea growth. Under greenhouse conditions, plants inoculated with *P. jessenii* (strain PS06) yielded a shoot dry weight 14% greater than the uninoculated control treatment, but it was not correlated with shoot P contents. Dual inoculation of *P. jessenii* with *Mesorhizobium ciceri* resulted in a decrease in shoot dry weight with respect to the single M. ciceri inoculation. Under field conditions, plants inoculated with *M. ciceri*, in single or dual inoculation, produced higher nodule fresh weight, nodule number and shoot N content than the other treatments. Inoculation with *P. jessenii* had no significant effect on plant growth. However, the co- inoculation treatment ranked the highest in seed yield (52% greater than the uninoculated control treatment) and nodule fresh weight. These data suggest that *P. jessenii* can act synergistically with *M. ciceri* in promoting chickpea growth. Eltlbany and Smalla [36] conducted a study in which the effect was observed adding *Pseudomonas jessenii* (strain RU 47) and *Bacillus amyloliquefaciens* (strain FZB42) on the growth of plants in an environment of naturally occurring bacteria and fungal colonies on rhizosphere as well as in the surrounding soil with tomato and corn plants. A greenhouse experiment was conducted with two different kinds of plants (tomato and maize). The experiment consisted of three variants (control, *P. jessenii* and *B. amyloliquefaciens*), and each variant had four repetitions. Parameters evaluated were plant growth. *P. jessenii* increased the growth of tomato plants compared to control, while *B. amyloliquefaciens* increased the growth of maize plants. It was found that the both microorganisms was clearly influenced by rhizosphere bacterial composition.

***Bacillus amyloliquefaciens*:** *B. amyloliquefaciens* is gram-positive, aerobic, and endospore-forming bacteria, which have been both widely used as producers of commercial chemicals in industry [37-39], and beneficial agents for plant growth promotion and suppression of soil-borne diseases in agriculture. *B. amyloliquefaciens* produces many metabolites such as e.g. enzymes (chitinase, peroxidases and proteases), casein, elastin, gelatin, starch, nitrites, esculin and arbutin, phosphatases, adenine, cellulose, guanine, hypoxanthine, pectin, testosterone, tyrosine, and many types of antibiotics (eg. bacillomycins, fengycin, difficidin) and other substances [39-42]. Production of antibiotic inhibiting growth of fungal pathogens [13]. Proteins secreted by *B. amyloliquefaciens* (strain FZB42) protects plants against disease by eliciting innate immunity [43]. Furthermore Lagerlöf et al. [40], Talboys et al. [44], Fan et al. [45], Burkett-Cadena et al. [46] report that the *B. amyloliquefaciens* promotes plant growth, based primarily on the production of secondary metabolites suppressing competing microbial pathogens and the diseases occurring in the rhizosphere of plants. It also encourages root development and improves seed germination. It was found that lactic acid is the main component of maize root exudates, and that these acid and other root exudates are a source of carbon and energy for the *B. amyloliquefaciens*. Due to these properties, are often *B. amyloliquefaciens* used as a "bio-fertilizer" and as means of biological protection in agriculture. The bacteria also reduce the influence of abiotic stress conditions at the plant, such as drought, salinity or lack of nutrients in the plant [39-41,47,48]. He et al. [41] dealt in their study with influence of *B. amyloliquefaciens* inoculation on the growth of rice plants under stress conditions caused by salinity for 30 days. This study was based on the assumption that the use of microorganisms provides an alternative technology to improve the ability of stress tolerance in plants. Results of laboratory experiments have shown that the inoculated plants in comparison with the control plants, better growth of the above-ground parts of plants, but also parts of the root. Stimulating root growth and the effective root surface is important for a better water and nutrients uptake, which is the most important tool for coping with stress. Healthy, strong and large enough root system plays an important role in maintaining optimum growth and development under stress conditions. Analysis of this study showed, besides other things, that the presence of deaminase in bacteria mitigates the effect of salt on chlorophyll, thus supporting the growth of plants under stressful conditions caused by salinity was largely credited deaminase activity, which bacteria produce.

***Bacillus subtilis*:** *B. subtilis* is a ubiquitous gram-positive bacterium commonly found in water, soil, air, and decaying plant residues. However, the primary occurrence of bacteria found in soil [49,50]. The bacteria produce endospores, which enable it to endure and overcome extreme temperatures and dry periods. *B. subtilis* produces a series of proteases and another enzyme. This bacterium is considered a benign organism, as it has no properties that cause disease nor is pathogenic or toxigenic for humans, animals or plants [50,51]. *B. subtilis* can be used as part of a fertilizer usable in organic farming which is applied to a crop seed or directly into soil where colonize the rhizosphere. Although reports on extensive positive effects of this bacteria to the plant (growth, yield, disease resistance) have been published, these positive effects are not yet sufficiently verified [52]. Brutti et al. [53]

conducted study and used of plant growth-promoting rhizobacteria in tomato production. Before sowing, the micro-organisms were inoculated into the substrate. Tomato seedlings were grown using two different substrates. The first substrate was composed of 70% peat and 30% perlite by volume. And a second substrate with 20% peat, 20% perlite and 60% compost by volume, both inoculated with *Bacillus subtilis* or *Pseudomonas fluorescens* or Bioroot, which is a commercial product containing *B. subtilis*, *P. fluorescens*, *Trichoderma harzianum*, yeast, algae and Nocardia. Inoculation improved the leaf area, shoot dry weight, root dry weight, radical contact area, volume of roots and root forks compared with the control without inoculation. And so, inoculation can be recommended as an alternative to tomato seedling growers' dependence on synthetic agrochemicals. Because of low soil fertility is caused by continue crop and using chemical fertilizer. Altuhaish et al. [54] conducted field experiment and the aim of this research was to investigate the effect of biofertilizer, which contain *B. subtilis* dried by different methods and exposed to different period of storage on nutrient, growth and productivity of tomato plant grown under the field conditions. The result showed that viability of bacterium tended to decline during storagebut did not significantly reduce the effect on growth and production of plant. Application of biofertilizer increased total macro and micro nutrient absorption, vegetative growth and plant production. This research suggested that application of biofertilizer improve growth and production and there was no different effect between 0 and 3 months storage of biofertilizers on plant growth. A greenhouse experiment was conducted by Turan et al. [55] to observe the effects of *Bacillus megaterium* (strain TV-91C), *Pantoea agglomerans* (strain RK-92), and *B. subtilis* (strain TV-17C) inoculation on the growth, nutrient, and hormone content of cabbage seedlings. The seeds of cabbage were incubated two hours at 28 degrees C. The highest concentrations for N and P were recorded in *B. megaterium*, while in *B. subtilis* for Ca, Na, and Fe and in *P. agglomerans* for K, Mg, and Mn. The hormone content of cabbage seedlings was significantly affected by application of microorganisms treatments. *B. subtilis* decreased the abscisic acid content compared to the other treatments. Inoculation increased fresh and dry shoot and root weight, stem diameter, seedling height, chlorophyll reading values, and leaf area of cabbage seedlings compared with the control. Highest fresh and dry shoot and root dry weight, stem diameter, seedling height, and chlorophyll reading values of cabbage seedlings were obtained from *B. megaterium* and following *P. agglomerans* and *B. subtilis*.

***PaeniBacillus mucilaginosus*:** *P. mucilaginosus* is a bacterium which has been widely used in agriculture since 1990 as a biological fertilizer. These bacteria take part on the biogeochemical cycle of potassium, phosphorus and other elements. It is able to degrade insoluble soil minerals releasing nutrient ions (potassium and water-soluble phosphorus), useful for plants [55-59]. *P. mucilaginosus* is typical silicate bacteria, has long been used as a biofertilizer in agriculture and has recently shown potential in bioleaching and wastewater engineering [60]. *P. mucilaginosus* is often used in biological fertilizers for its ability of phosphorus and potassium mineralization, and also for the ability of nitrogen fixation [61]. Wang et al. [56] researched the effects of combined inoculation with arbuscular mycorrhizal fungi (*Rhizophagus intraradices*) and plant growth promoting rhizobacteria (*PaeniBacillus mucilaginosus*) on the growth of citrus seedlings under phosphorus deficient conditions have not been extensively studied. A pot experiment was performed to compare growth, root morphology, and other physiological variables in trifoliate orange (*Poncirus trifoliata*) seedlings that had been inoculated with *Rhizophagus intraradices*, *PaeniBacillus mucilaginosus* or both. Root length were also considerably improved by inoculation with dual inoculation however, taproot length was notably reduced by mycorrhizal inoculation. At treatment with zero phosphorus level, seedlings inoculated with a combination of *R. intraradices* and *P. mucilaginosus* yielded the greatest leaf chlorophyll concentrations and fine root activity, in comparison to those had either not been inoculated at all, or inoculated with just one of them. Combined inoculation increased plant height, stem diameter, shoot dry weight, and root dry weight. In addition, total N and P concentrations and uptake in seedlings were substantially improved both by individual and combined inoculation.

***Rhizophagus intraradices*:** *R. intraradices* is an arbuscular mycorrhizal fungus used as a soil inoculant in agriculture and horticulture. Mohamed et al. [62] realized project, which has investigated the early growth rate and establishment of cherry tomato plants as a model system inoculated with *R. irregularis*. After one month of growth, the number of leaves of mycorrhizal tomato seedlings was significantly increased and the height was approximately doubled in response to inoculation compared with non-inoculatedtomatoes. Colonna et al. [12] realized experiment, which had the aim was to assess the effect of two commercial inoculants containing arbuscular mycorrhizal fungi alone or arbuscular mycorrhizal fungi in combination with plant growth promoting bacteria (*Rhizophagus intraradices*) on yield components and quality of artichoke (*Cynara cardunculus* subsp. scolymus). Overall, inoculation of arbuscular mycorrhizal fungi or dual inoculation arbuscular mycorrhizal fungi and *R.intraradices* could be considered an effective and sustainable tool to improve yield components with less pronounced positive effects on quality of artichoke. Very often various plant components and extracts are added to the active microorganisms. One of the most widely used ingredients is seaweed. Next chapter describes in detail most commonly used seaweed species.

***Algae extracts*:** Algae extracts are used in crop production as an alternative to conventionally use fertilizers and plant protection. These components have several functions for plant: protection against a broad spectrum of plant diseases and pests, support of plant metabolism, enzyme production, food for positive organisms.

Extracts from seaweed can be a component of the so-called biostimulants, which can enhance the growth, yield, and quality of crops. Algal biostimulant provide added benefit to plants when applied by foliar spray or drenching. Seaweed extracts have been widely used as amendments in crop production systems due to the presence of a number of plant-growth-stimulating compounds. Extract is rich for many nutrients and other substances such as amino acids, vitamins, cytokinins, and auxin and abscisic acid like growth promoting substances and have been reported to stimulate the growth and yield of plants [63], enhance tolerance to environment stress [64], increase nutrient uptake from soil [65], enhance antioxidant properties, and increase activity against broad range of pathogenic viral, bacterial, and fungal diseases and enhanced resistance to insect attack [65,66]. The most known and used algae is *Ascophyllum nodosum*.

***Ascophyllum nodosum*:** *A. nodosum* is a brown seaweed, which is a rich source of phenolic compounds with antioxidant and antimicrobial properties. Algae is a good source of bioactive agents such as laminarin, sulfated polysaccharides, carotenoids, vitamins, minerals and polyphenols [67]. Extracts from seaweed *Ascophyllum nodosum* are intended for the specific plant organs (leaves and roots). Utilization is actual in food production in different regions of the world through their positive effect when applied into the soil, if necessary reduction of harmful bacteria, fungi, insects and parasites [68].

Discussion

From the agricultural industry perspective, they are considered as alternative organic fertilizers to conventional agrochemicals, new generation of competitive fertilizers and growth stimulants [69]. Rioux et al. [70] reported that extract from the seaweed *A. nodosum* and the chemical composition of these algae includes a high percentage of ash, proteins, lipids, polysaccharides, antioxidants, minerals and inorganic salts absorbed from seawater. Furthermore Michalak et al. [71] and Rayirath et al. [72] published that the extract of brown seaweed *A. nodosum* increases the resistance of plants against environmental influences (stress factors), such as drought, salinity and frost. Furthermore Kadam et al. [67] conducted, that *A. nodosum* is also used as fertilizer in the agriculture. Brown algae is a rich source of biologically active compounds, such as polysaccharides, peptides, omega-3 fatty acids, carotenoids, phenolic compounds, vitamins and minerals. One of many important polysacharides is laminarin, which is contained at 0% to 35% in Algae dry matter [67,69,71]. *A. nodosum* enhanced the growth of field crops, fruit crops and vegetable crops. These studies reported also an improved vegetative growth, chlorophyll content, fruit yield, sugar content and resistance against leaf and soil borne pathogens [69]. Michalak et al. [71] researched the influence of supercritical algal extracts on the growth and development of winter wheat (variety Akteur). As a raw material for the supercritical fluid extraction, the biomass of microalga *Spirulina plantensis*, brown seaweed - *Ascophyllum nodosum* and *Baltic green macroalgae* was used. It was found that the tested biostimulants did not influence statistically significantly the plant height, length of ear, and shank length. Crop height was similar in all the treated and the untreated plots. There were no significant differences in ear-bearing culms' and barren culms' number between the treated and the untreated plots. Tandon and Dubey [65] conducted study, where used formulation with is extracted from *A. nodosum* in soybean under field conditions. They investigated the appropriate dose of formulation in combination with NPK fertilizers and its effects on chlorophyll content, number of trifoliate leaves, number of pods, number of nodules, root length, yield, and other parameters under field conditions in soybean. Biozyme application greatly influenced number of trifoliate leaves, leaf area, and leaf area index. Also total chlorophyll content and total number of nodules per plant was significantly influenced after application. Conclusion of this study was, that use of biostimulants extracted from *A. nodosum* may optimize the use of chemical fertilizers, thereby reducing the impact of environment pollution and increasing the soil fertility. The use of such biostimulants must be combined with all available modern agronomic practices and it is one of the possible alternative strategies in agriculture, in the future with aim at maximizing the potential of a crop plant to boost crop production, crop quality. Sen et al. [73] used *A. nodosum* (granule or liquid sprays) in field experiments with wheat in combination NPK fertilizers. The application of two liquid sprayings in combination with fertilizers increases in the grain and straw yields, respectively, compared to the control more than 10% [74-80]. Liquid spraying of the seaweed extract stimulates metabolic processes in the leaf and helps the plant exploit nutrients in the leaf. Considerable proportion of photosynthesis is carried out by bacteria on the leaf surface and application of liquid sprays is activated by the liquid spray and the rate of photosynthesis increases as a consequence.

Conclusion

There have been severals studies conducted in research of lack of nutrients and bioeffectors application. Some authors report positive impact of bioeffectors application on plant. Other authors do not identify with it because they do not have enough results and confirming conclusions. Studies and experiments were performed under different conditions, with diferent preparation and their active ingredients with also different parameters observed. It is therefore important to further develop these alternative plant nutrition strategies in the future.

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14) Fractionation of sulfur in beech (*Fagus sylvatica*) forests soils in relation to distance from the stem base as useful tool for modelling S biogeochemistry

Frakcionace síry v bukových (*Fagus sylvatica*) lesních půdách v závislosti na vzdálenosti od kmene jako vhodný nástroj pro modelování bio-geochemie půdní síry

Tento článek se zabývá pohybem síry v jiných než zemědělských půdách, konkrétně v lesních půdách. Byl vypracován ve spolupráci s týmem univerzity BOKU Wien. Vychází z teorie, že stanovení různých frakcí síry v různých vzdálenostech od kmene stromu (buk lesní, *Fagus sylvatica*) a následné vyhodnocení výsledků umožní lépe pochopit chování síry v lesních půdách. Cílem práce bylo vytvoření obecného modelu popisujícího biogeochemické procesy síry, odehrávající se v prostředí bukových porostů. Za tímto účelem byla zvolena 2 kontrastní stanoviště: Flysch jako představitel vysokého pH a obsahu přístupných živin a Molasse typické nízkým pH a chudé na živiny. Půdní vzorky byly odebírány v různých vzdálenostech od kmene buků, tj. 55 cm směrem nahoru do svahu a 27, 55, 100, 150 a 300 cm dolů ze svahu, kde byl očekáván vyšší pohyb S. Vzorky byly rozděleny i horizontálně s přihlédnutím na půdní profil (0-3, 3-10, 10-20, 20-30 a 30-50 cm). Kromě základních parametrů (pH, oxidy železa a hliníku) byly stanoveny různé frakce síry v NH_4Cl, $NH_4H_2PO_4$, HCl, estersulfátová a celková síra. Z těchto údajů bylo možno vypočítat podíl přístupné, adsorbované, okludované (tj. minerální) a organické S včetně síry přímo vázané na uhlík. Výsledky ukazují na vysokou variabilitu některých frakcí S mezi jednotlivými stanovišti i v jejich rámci. Na stanovišti Flysch byly podíly z celkové S následující: 3-6 % okamžitě přístupné; 2-12 % potenciálně přístupné, 0-8 % okludované a 81-95 % organické. Na stanovišti Molasse to bylo 1-6 % okamžitě přístupné, 1-60 % potenciálně přístupné, 0-8 % okludované a 37-95 % organické. Hlavním poznatkem studie byl fakt, že dochází k signifikantním změnám v obsahu S jak se vzdáleností od kmene, tak s hloubkou odebraného vzorku. Prezentovaná data jsou v souladu s literaturou poukazující na negativní bilanci síry v půdě (Berger et al. 2009a, b, Berger et al. 2016, Berger et Muras 2016) v důsledku vyšších ztrát (mineralizací, desorpcí a následným vyplavením) než vstupů a studie může být dále použita pro tvorbu modelů umožňujících predikci chování S v půdě.

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ORIGINAL ARTICLE

Fractionation of sulfur (S) in beech (*Fagus sylvatica*) forest soils in relation to distance from the stem base as useful tool for modeling S biogeochemistry

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Abstract The investigation of the fractionation of S compounds in forest soils is a powerful tool for interpreting S dynamics and S biogeochemistry in forest ecosystems. Beech stands on high pH (nutrient-rich) sites on Flysch and on low pH (nutrient-poor) sites on Molasse were selected for testing the influence of stemflow, which represents a high input of water and dissolved elements to the soil, on spatial patterns of sulfur (S) fractions. Soil cores were taken at six distances from a beech stem per site at 55 cm uphill and at 27, 55, 100, 150 and 300 cm downhill from the stem. The cores were divided into the mineral soil horizons 0–3, 3–10, 10–20, 20–30 and 30–50 cm. Soil samples were characterized for pH, C_{org}, pedogenic Al and Fe oxides and S fractions. Sequential extraction by NH_4Cl, $NH_4H_2PO_4$ and HCl yielded readily available sulfate-S (*RAS*), adsorbed sulfate-S (*AS*) and HCl-soluble sulfate-S (*HCS*). Organic sulfur (*OS*) was estimated as the difference between total sulfur (*ToS*) and inorganic sulfur (*RAS* + *AS* + *HCS*). Organic sulfur was further divided into ester sulfate-S (*ES*, HI-reduction) and carbon bonded sulfur (*CS*). On Flysch, *RAS* represented 3–6%, *AS* 2–12%, *HCS* 0–8% and *OS* 81–95% of *ToS*. On Molasse, *RAS* amounted 1–6%, *AS* 1–60%, *HCS* 0–8% and *OS* 37–95% of *ToS*. Spatial S distribution patterns with respect to the distance from the tree stem base could be clearly observed at all investigated sites. The presented data is a contribution to current reports on negative input–output S budgets of forest watersheds, suggesting that mineralization of *OS* on nutrient rich soils and desorption of historic *AS* on nutrient-poor soils are the dominant S sources, which have to be considered in future modeling of sulfur.

Keywords *Fagus sylvatica* · Sequential extraction · Soil acidification · Stemflow · Sulfur biogeochemistry

Abbreviations

RAS	Readily available sulfate-S
AS	Adsorbed sulfate-S
HCS	HCl-soluble sulfate-S
OS	Organic sulfur
ToS	Total sulfur
ES	Ester sulfate-S
CS	Carbon bonded sulfur
HIS	HI-reducible sulfur
PES	Phosphate-extractable sulfate-S
HCE	HCl-extractable sulfate-S

Introduction

Due to the combustion of fossil fuels, European sulfur (S) emissions and deposition increased steadily since the industrial revolution in the middle of the nineteenth century. As a consequence, sulfur deposition in forested ecosystems of north and central Europe peaked in the early 1980s, reaching, in certain cases, loads of more than 100 kg S ha^{-1} $year^{-1}$

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(Prechtel et al. 2001). Legislation to reduce acidifying emissions has taken place at an international level, and, e.g., in Austria, SO_2 emissions declined by 77% from 1990 (75,000 t $year^{-1}$) to 2013 (17,000 t $year^{-1}$; Umweltbundesamt 2015). Throughfall (plus stemflow) fluxes in beech (*Fagus sylvatica*) forests of eastern Austria declined from 23 to 6 kg S ha^{-1} $year^{-1}$ from 1984 to 2013 (Berger and Muras 2016).

Release of previously stored S delays the recovery of pH of soils and surface waters, because sulfate is the only S output in the leachate. Leaching of sulfate is associated with loss of base cations and soil acidification. Reuss and Johnson (1986) called the time prior the peak of S deposition, when soils adsorbed S, a grace period, since base cations remained available for plant uptake and sulfate adsorption neutralized deposited acids resulting from the replacement of $–OH^-$ groups on the soil exchanger surface by SO_4^{2-}. Sulfate adsorption/desorption is a concentration-dependent process, meaning that the amount of adsorbed sulfate increased with increasing sulfate soil solution concentrations in the past but desorption is expected in the future due to declining S deposition and associated decreased sulfate soil solution concentrations. However, a time lag may be expected until this new equilibrium is reached, causing higher sulfate outputs than inputs of forest ecosystems.

In fact, in many regions the chemistry of soils and surface waters has not recovered as expected (Berger et al. 2016; Lawrence et al. 2015). Several calibrated watersheds are currently monitored throughout eastern North America and Europe, reporting net losses of base cations in recent decades as reviewed by Watmough et al. (2005). It is striking that the same authors reported that SO_4^{2-} export exceeded input at 18 out of 21 studied catchments by 6–76 kg SO_4^{2-} ha^{-1} $year^{-1}$. This discrepancy may be explained by net desorption of inorganic S and net mineralization of organic S (Likens et al. 2002).

Inorganic S, determined as the sum of readily available (also termed water-extractable; Morche 2008), adsorbed (also phosphate-extractable; Likens et al. 2002) and carbonate occluded SO_4^{2-} (also HCl-extractable S, Chen et al. 1997) represent in general less than 10% of the total S (Likens et al. 2002; Tisdale et al. 1993; Vannier and Guillet 1994). Organic S compounds represent in general over 90% of the total S in soils (Likens et al. 2002; Tisdale et al. 1993; Vannier and Guillet 1994). The organic S is often divided in two groups: ester sulfates (*ES*; a variety of alkyl-, aryl-, phenol sulfates, etc.) and carbon bonded S (*CS*; amino acids, sulfoxides, etc.; Tabatabai 1996). The mineralization of organic S compounds to SO_4^{2-} depends on the microbial activity, which is affected by many parameters (soil temperature, humidity, pH, use of soil; Morche 2008). Aside the soil microflora, the type of vegetation growing on the soil determines whether *ES* or *CS* will be mineralized (Morche 2008).

Many biological and chemical processes in the soil are pH-dependent. Mineralization of organic S, as a result of high S plant uptake rates in the past, coupled with increasing soil pHs after the end of the acid rain period, provides additional current sulfate sources. The density of net positive surface charges in the soil (humus, clay minerals, Al and Fe oxides and hydroxides) decreases with increasing soil pH (Likens et al. 2002), expecting a further increased desorption rate of sulfate after acid rain had ceased. Precipitation/dissolution processes of aluminum hydroxy sulfate are expected to release sulfate at higher soil pH as well (Prietzel and Hirsch 1998).

Stemflow of beech represents a high input of water and dissolved elements to the forest soil. Due to the particular canopy structure and bark composition stemflow effects are confined to beech trees only and is negligible for other tree species (Berger et al. 2008). Therefore, deposition of acidifying substances (NO_3^-, SO_4^{2-}) has been shown to be significantly higher close to a beech stem than in areas affected by throughfall only (Kazda 1983; Kazda and Glatzel 1984; Koch and Matzner 1993; Sonderegger 1981). Comparison of soil chemistry between the infiltration zone (near the base of the stem) and the "between trees area" in old beech stands by Lindebner (1990) and Rampazzo and Blum (1992) in the "Vienna Woods" proved a significant impact of deposition of atmospheric pollutants: soil acidification, heavy metal accumulation, increased total sulfur contents and loss of the base cations, especially in the infiltration zone. Berger and Muras (2016) estimated that routing 50% of measured stemflow fluxes in 1984 through the soil profile at 27 cm downhill of a beech stem in the "Vienna woods" resulted in a deposition load (throughfall plus stemflow) of 215 kg S ha^{-1} $year^{-1}$ while the input at 300 cm distance from the stem (throughfall only) amounted only 15 kg S ha^{-1} $year^{-1}$. Meanwhile (2013), these loads declined to 30 kg S ha^{-1} $year^{-1}$ (stem base at 27 cm) and 5 kg S ha^{-1} $year^{-1}$ (between trees area at 300 cm distance), respectively. Thus, focusing on the spatial heterogeneity of soil chemistry related to the distance from beech stems enables the study of recovery of differently polluted soil within the same stand. Assuming that increasing soil solution fluxes with decreasing distance from the stem cause a quicker steady state of soil SO_4^{2-} pools in response to currently decreased inputs, studying soil gradients from the base of the stem to "bulk soil" from the between trees area should enable to better characterize soil acidification recovery dynamics (similar to a false chronosequence), as suggested by Berger and Muras (2016).

Sulfur content and the distribution of S into different fractions were investigated in S-deficient podzolic and chernozemic forest soil profiles (Lowe 1965), in spruce forest soils (Vannier and Guillet 1994) and, to a smaller extent, in hardwood forest soils (Likens et al. 2002). All listed S fractions proved to be of importance for S dynamics and

S biogeochemistry (e.g., Chen et al. 1997). As pointed out input–output budgets of sulfate are critical for prediction of soil recovery from acid rain. For that reason the relation between inorganic and organic soil S fractions is crucial which may depend on the applied analytical methods. It is suggested that analyzing the micro-spatial heterogeneity of soil columns downhill of a beech stem enables predictions of soil recovery as a function of historic acid loads and time (Berger and Muras 2016). Hence, we analyzed S fractions at different distances to the stem base of beech at nutrient-rich, high pH (Flysch) and nutrient-poor, low pH (Molasse) sites and hypothesized that high historic S deposition coupled with high water inputs via stemflow result in spatial soil patterns of S fractions. The hypothesis was developed into three specific research questions to address above issues:

1. How are S fractions distributed and related to each other in low pH (nutrient-poor) and high pH (nutrient-rich) forest soils?
2. Are stemflow induced soil changes reflected in spatial patterns of S fractions?
3. Do these patterns match the reported negative S-budgets or recovery from acid rain?

Materials and methods

Study sites

Three study sites on two different bedrocks (Flysch and Molasse) each were selected in pure old-growth beech stands. The sites J, E and W on Flysch (Table 1) are located in the Vienna Woods along a distance gradient from the urban area from south–east to north–west (prevailing wind direction during high-pressure episodes). The impact of atmospheric pollutants in the early 1980s on soil characteristics of these sites was documented by Sonderegger (1981) and Kazda (1983). The beech stands B1, B2 and F on Molasse are located in the "Kobernaußerwald", Upper Austria, and have been extensively studied before (Berger et al. 2006, 2009a, b). The study sites on Molasse are characterized by higher mean annual precipitation (1050 mm) and lower mean annual temperature (7.8 °C) than the sites on Flysch (742 mm, 9.2 °C), as indicated by long term means (1971–2000) of nearby weather stations.

Flysch consists mainly of old tertiary and mesozoic sandstones and clayey marls. Nutrient release from this bedrock is high and, consequently, the prevalent humus forms are mull, indicating quick turnover of the forest litter layer (usually less than 2 cm thickness). All soils of these study sites were classified as stagnic cambisol.

Molasse is composed of tertiary sediments ("Hausruck-Kobernausserwald" gravel), which consist mainly of quartz

Table 1 Forest stand characteristics of pure beech stands at the experimental sites on Flysch and on Molasse

Site	Age years	Stems n ha^{-1}	Timber volume m^3 ha^{-1}	Basal area m^2 ha^{-1}	Tree height m	Elevation m a.s.l.	Slope °	Aspect (from N to E) °	Coordinates (WGS84) N	Coordinates (WGS84) E
Flysch										
Windischhütte (W)	150	305	910	60	34	460	17	280	48°17′04″	16°13′42″
Exelberg (E)	120	248	525	40	29	460	13	143	48°14′42″	16°15′14″
Jubiläumswarte (J)	170	167	525	40	28	430	14	151	48°13′13″	16°15′56″
Molasse										
Bradirn 1 (B1)	90	434	434	45	30	610	11	293	48°05′18″	13°14′14″
Bradirn 2 (B2)	120	245	245	25	29	640	15	248	48°05′11″	13°16′42″
Frauschereck (F)	100	385	384	42	28	690	7	315	48°05′35″	13°18′36″

and other siliceous material (granite, gneiss, hornblende schist, pseudotachylite and colored sandstone). Because of this acidic bedrock with low rates of nutrient release, the dominant soil types are dystric cambisols to podzols. Humus form is acidic moder and the thickness of the forest litter layer varies between 5 and 10 cm, indicating slow turnover and accumulation of nutrients.

Soil sampling and preparation

Soil samples in the vicinity of one beech stem per site were collected in June 2010 (Flysch) and in October 2011 (Molasse). Soil cores were taken with a core sampler of 70 mm diameter to a depth of 50 cm. Two soil cores were sampled at each of the following six distances from one beech stem per site: at 55 cm uphill (further labeled −55), and at 27, 55, 100, 150 and 300 cm downhill from the stem. The cores were divided into the mineral soil horizons 0–3, 3–10, 10–20, 20–30 and 30–50 cm depth and both replicates were mixed. The samples were sieved (mesh size 2 mm), homogenized and oven dried (105 °C, 24 h). Masses of roots (only one diameter class) in the individual soil horizons did not show clear patterns in relation to the distance to the stem base. Hence, uniform S plant uptake rates are assumed within 3 m distance to the stem. Soil pH was measured in $0.01\ \mathrm{mol\ L^{-1}\ CaCl_2}$, according to ISO 10390:2005. Basic soil parameters, relevant for this study, are listed in Table 2. More details on sampling and soil characterization are given by Muras (2012) and Kaliwoda (2015). Organic C was calculated total C (Wösthoff Carmhomat ADG 8, Germany, ÖNORM L1080) minus C_{CaCO3} (Scheibler method: reaction of carbonates with HCl and volumetric determination of emerging CO_2 according to ÖNORM L1084). In general, soils on Molasse contained more organic carbon, were characterized by wider C_{org}/N_{tot} (mineral soil) and C_{org}/S_{tot} ratios (total soil profile), were more acidic, more sandy and less supplied with nutrients than soils on Flysch. Total soil S stocks (S m^{-2} per horizon) were within the same range for all study sites (due to higher bulk densities of loamy to clayey soils on Flysch than of sandy soils on Molasse).

Determination of sulfur fractions

Inorganic S is determined as the sum of readily available (also termed water-extractable; Morche 2008), adsorbed (also phosphate-extractable; Likens et al. 2002) and carbonate occluded SO_4^{2-} (also HCl-extractable S, Chen et al. 1997). A sequential extraction is applied to obtain these three inorganic S fractions (Chen et al. 1997; Kulhanek et al. 2011; Lowe 1965; Morche 2008; Shan et al. 1992).

Table 2 Soil properties (means of six soil profiles at −55, 27, 55, 100, 150 and 300 cm distance to the base of one beech stem per site) at the study sites on Flysch and Molasse. Ranges of pH and exchangeable base saturation in the mineral soil are minimum and maximum values of 30 soil horizons (6 distances × 5 soil depths)

Site	Horizon	C_{org} kg m^{-2}	N_{tot} g m^{-2}	S_{tot} g m^{-2}	C_{org}/N_{tot} ratio	C_{org}/S_{tot} ratio	pH ($CaCl_2$)	Base sat. %
Flysch								
Windischhütte (W)	Forest floor	0.53	16	0.9	32.9	592		
	0–50 cm	7.46	502	73.3	14.9	102	3.2–3.7	63.0–91.7
	Total soil	7.99	518	74.2	15.4	108		
Exelberg (E)	Forest floor	0.47	18	1.3	26.3	363		
	0–50 cm	8.13	667	76.7	12.2	106	3.6–4.3	88.4–99.4
	Total soil	8.60	685	78.0	12.6	110		
Jubiläumswarte (J)	Forest floor	0.51	17	1.3	29.7	395		
	0–50 cm	10.27	967	101.3	10.6	101	3.5–7.0	95.4–100.0
	Total soil	10.78	984	102.6	11.0	105		
Molasse								
Bradirn 1 (B1)	Forest floor	4.84	240	15.4	20.1	314		
	0–50 cm	10.92	568	94.6	19.2	115	2.8–4.4	3.0–14.8
	Total soil	15.76	808	110.0	19.5	143		
Bradirn 2 (B2)	Forest floor	3.92	183	11.4	21.4	344		
	0–50 cm	10.24	585	73.0	17.5	140	2.9–4.3	4.6–13.6
	Total soil	14.16	768	84.4	18.4	168		
Frauschereck (F)	Forest floor	6.14	278	15.2	22.1	404		
	0–50 cm	6.92	377	68.5	18.3	101	2.9–4.3	3.5–15.5
	Total soil	13.06	655	83.7	19.9	156		

Although the extraction processes are not sulfate-specific (it cannot be excluded that hydrophilic organic S compounds are co-extracted), it is not likely that the amount of readily available or adsorbed SO_4^{2-} is overestimated significantly, as demonstrated by Shan et al. (1992). In the case of carbonate occluded SO_4^{2-}, our preliminary measurements have shown that a sulfate-specific measurement, i.e., ion chromatography (IC), is required to not overestimate this fraction, and, consequently, the total inorganic S amount in accordance to Shan et al. (1992).

Organic S can be calculated as the difference between total S and inorganic S. To divide it into *ES* and *CS*, the soil was reduced by HI as described by Johnson and Nishita (1952), modified by Shan et al. (1992) and applied routinely since then (Chen et al. 1997; Kulhanek et al. 2011; Likens et al. 2002; Morche 2008). All reagents, salts and acids applied were of *pro analysis* grade.

Total sulfur (ToS)

The *ToS* was determined according to the ÖNORM L 1096 using LECO SC 444 (LECO Corp., St. Joseph, MI, USA). LECO 302–508 was used as a standard.

Soil sulfur extraction

The extraction method as described by Tabatabai (1996) and Kulhanek et al. (2011) was slightly modified (NH_4^+ instead of Na^+ salt solutions were used in this work at the same anion concentrations as used by Kulhanek et al. 2011) and applied for extraction of soil S fractions. Soil was extracted separately for each fraction (not sequentially). Each extraction was repeated three times. Each extract was filtered through a syringe filter (0.45 µm). Double sub-boiled HNO_3 was used for acidification of extracts to 2% (w/w) HNO_3 prior to analysis. RTS-1 (CCRMP, Ottawa, ON, CA) certified soil reference material was used for method validation. The reference material was certified for both total S content and adsorbed sulfate. The following S fractions were extracted:

Readily available sulfate-S (*RAS*) 1.0 g soil sample was shaken with 6 mL 0.02 mol L^{-1} NH_4Cl solution for 3 h,

Phosphate extractable sulfate-S (*PES*) 1.0 g soil sample was shaken in 6 mL 0.02 mol L^{-1} $NH_4H_2PO_4$ solution for 3 h,

HCl-extractable sulfate-S (*HCE*) 1.0 g soil sample was shaken in 20 mL cold 1 mol L^{-1} HCl for 1 h.

HI-reduction of soil sulfur

Samples from one site per bedrock (W on Flysch and B1 on Molasse), representing the lowest and highest soil S_{tot} stocks according to Table 2, were investigated in more details: W and B1 soil samples were reduced by HI as described by Johnson and Nishita (1952), modified by Shan et al. (1992) and applied routinely since then (Chen et al. 1997; Kulhanek et al. 2011; Likens et al. 2002; Morche 2008).

HI-reducible sulfur (*HIS*) 0.5 g soil sample was reduced using a HI-reducing agent (Shan et al. 1992). Each sample was processed twice.

Sulfur content measurement

Sulfur contents in all extracts were determined (detection limit 0.5 µg L^{-1}) by ICP-MS (Element XR, Thermo Scientific, Waltham, MA, USA) operated in medium mass resolution, applying external calibration and internal normalization (In and Sb, both single element ICP standards). However, given amounts of *HCE* (sulfate-S) throughout the paper were determined (detection limit 0.1 mg L^{-1}) by means of ion-exchange chromatography (IC; ICS 1600, Dionex, USA) with suppressed conductivity, applying the IonPac AS11-HC (Dionex, USA) guard and analytical columns, and the ASRS 300-4 mm suppressor (Dionex, USA). 30 mmol L^{-1} KOH was used as the effluent (flow rate 1 mL min^{-1}).

Calculation of soil S species

Adsorbed sulfate-S (*AS*) mass fraction: $AS = PES - RAS$
HCl-soluble sulfate-S (*HCS*) mass fraction: $HCS = HCE - PES$
Organic sulfur (*OS*) mass fraction: $OS = ToS - HCE$
For soil profile imaging, *OS* was further divided into
Ester sulfate-S (*ES*) mass fraction: $ES = HIS - HCE$
Carbon bonded sulfur (*CS*) mass fraction: $CS = ToS - HIS$

Determination of pedogenic Al and Fe oxides

Pedogenic Al and Fe oxides (comprising oxides, oxyhydroxides, and hydrated oxides) were analyzed for the sites W (Flysch) and B1 (Molasse). The dithionite-soluble Fe (Fe_d) and Al (Al_d) fractions were determined according to Holmgren (1967): 2.0 g soil sample were mixed with 2.0 g $Na_2S_2O_4$ and shaken for 16 h in 100 mL 0.3 mol L^{-1} sodium citrate – 1.0 mol L^{-1} $NaHCO_3$ (4:1) solution. The contents of the oxalate-soluble Fe (Fe_o) and Al (Al_o) were determined according to ÖNORM L 1201: 1.0 g soil sample was shaken in 50 mL 0.2 mol L^{-1} ammonium oxalate – 0.2 mol L^{-1} oxalic acid solution (4:3) for 4 h in the dark. The first 10 mL of the filtered extract were not used for measurement. Contents of Fe and Al in these extracts were analyzed by ICP-OES (ICP-OES, Optima 3000 XL, Perkin Elmer, USA), using external calibration.

Applied statistics

One-way analysis of variance (ANOVA) and Duncan post hoc analyses were applied using IBM SPSS Statistics 21 software (IBM Corporation, Armonk, NY, USA). Mean values of pH, *RAS, AS, HCS, OS* and *ToS* were evaluated with respect to soil depth and distance from a tree stem. ANOVA and the associated multiple range test was used as the data were normally distributed in most cases. In a few cases (*AS* on Molasse at 100 and 150 cm distance, and pH and *ToS* on Flysch at 30–50 and 0–3 cm, respectively), this precondition for ANOVA was fulfilled after a simple 1/square root transformation of the data. All statements on significance throughout the text are based on applied statistics. Bivariate linear correlation matrices were carried out for all combinations of pH, C_{org}, Fe_d, Fe_o, Al_d, Al_o and individual S fractions for the sites W (Flysch) and B1 (Molasse) using IBM SPSS Statistics 21 software.

Results

Tables and figures

Mean soil pH and sulfur mass fractions in soil profiles at −55 and 27, 55, 100, 150 and 300 cm distance to the base of one beech stem per site are presented for individual horizons on (a) Flysch and (b) Molasse in Table 3. The study sites W (Flysch) and B1 (Molasse), for which *HIS* was measured (enabling the calculation of *ES* and *CS*), were selected for graphical illustration of all possible S fractions in the soil (Fig. 1). The relative S distribution at W (Fig. 1a) and B1 (Fig. 1b) matches well to the mean values of the corresponding S fractions of all three sites on Flysch and Molasse, respectively (Table 3). Bivariate coefficients of linear correlations (*R*, Pearson) among pH, C_{org}, Fe_d, Fe_o, Al_d, Al_o and individual S fractions in the soil are given for the sites W and B1 in Table 4. Significant ($p < 0.01$) linear correlations are debated in the "Discussion" section.

Soil pH and C_{org}

The pH values of mean soil profiles were significantly lower on Molasse than on Flysch, except for the profiles at 27 and 55 cm distance (Table 3).

Organic C contents (C_{org}) ranged from 5/21 (30–50 cm depth) to 106/243 (0–3 cm; W/B1, data in mg g^1) and declined significantly with increasing depth (mean contents of all distances per depth were compared by posthoc Duncan multiple range test; $p < 0.05$). The mean ($N = 6$ horizons × 6 soil depth = 30) C_{org} content was significantly ($p < 0.001$) lower at W (20 mg g^{-1}) than at B1 (76 mg g^{-1}). Though there was a clear trend of enriched C_{org} contents in the soil profile at 27 cm on Flysch (W), no significant impact of distance on C_{org} patterns was recorded (data not shown).

Sulfur fractions

On average (mean geometric horizon data of three study sites), the *RAS* fraction represented 3–6%, *AS* 2–12%, *HCS* 0–8% and *OS* 81–95% of *ToS* on Flysch. On Molasse, *RAS* amounted 1–6%, *AS* 1–60%, *HCS* 0–8% and *OS* 37–95% of *ToS*.

The contents of all S fractions were significantly higher on Molasse than on Flysch (see statistics for each average soil profile in Table 3), with the exception of not significant differences for *RAS* (27, 55), *AS* (27) and *HCS* (all distances). On both bedrocks, distance from the stem base had no significant effect on *ToS* and *OS* fractions. However, these fractions declined with increasing soil depth (Table 3).

Significantly higher *RAS* values were found in the topsoil on both bedrocks. The *RAS* maxima were reached at distances around 100 cm from the stem base. The *AS* fraction increased with depth on Molasse, while *AS* tended to decline (not significantly) with depth on Flysch. Average *AS* of the soil profile next to the stem base (27 cm) was significantly reduced on Flysch. A similar behavior was observed on Molasse (not significant differences, see Table 3). The *HCS* fraction was significantly enriched next to the stem base (27 cm) on Molasse. Since 1 mol L^{-1} HCl represents the strongest extractant whithin this study, it is suggested that the generally very low *HCS* contents, measured by IC (0–8% of *ToS;* Table 3) represented the remaining sulfate adsorbed on soil particles that was not exchanged by phosphate.

Pedogenic Al and Fe oxides

Dithionite-soluble Fe (Fe_d) amounted 3.8–6.6 and 5.5–14.4 mg g^{-1} at W and B1, respectively. The mean Fe_d content was significantly ($p < 0.001$) lower at W (4.6 mg g^{-1}) than at B1 (10.8 mg g^{-1}). Oxalate-soluble Fe (Fe_o) amounted 2.3–3.3 and 2.5–14.0 mg g^{-1} at W and B1, respectively. The mean Fe_o content was significantly ($p < 0.001$) lower at W (2.7 mg g^{-1}) than at B1 (9.9 mg g^{-1}). The higher Fe_o/Fe_d ratio at B1 (0.9) than at W (0.6) indicated higher crystallinity and lower amounts of more active forms of Fe (Rampazzo et al. 1999). Duncan multiple range tests showed that the content of the mean soil profile at 27 cm was higher than at 55 cm for Fe_d and higher than at all other distances for Fe_o on Flysch (W), but there was no effect by distance on Molasse (B1; data not shown).

Dithionite-soluble Al (Al_d) amounted 1.1–1.9 and 2.2–9.7 mg g^{-1} at W and B1, respectively. The mean Al_d content was significantly ($p < 0.001$) lower at W (1.4 mg g^{-1}) than at B1 (5.7 mg g^{-1}). Oxalate-soluble Al (Al_o) amounted 0.8–1.2 and 1.6–7.6 mg g^{-1} at W and

Table 3 Mean ($n=3$ sites/trees per bedrock) soil pH ($CaCl_2$) and sulfur mass fractions (µg g^{-1}) in soil profiles at −55 (uphill) and 27, 55, 100, 150 and 300 (downhill) cm distance to the base of one beech stem per site on (a) Flysch and (b) Molasse

(a) Flysch

pH

Horizon	-55			27			55			100			150			300		
0 - 3	4.7	*a*	*A*	4.2	*a*	*A*	4.2	*a*	*A*	4.3	*a*	*A*	4.6	*a*	*A*	4.6	*a*	*A*
3 - 10	4.4	*a*	*A*	3.7	*a*	*A*	3.9	*a*	*A*	4.1	*a*	*A*	4.1	*a*	*A*	4.2	*a*	*A*
10 - 20	4.5	*a*	*A*	3.6	*a*	*A*	3.7	*a*	*A*	3.8	*a*	*A*	3.8	*a*	*A*	4.1	*a*	*A*
20 - 30	4.7	*a*	*A*	3.5	*a*	*A*	3.6	*a*	*A*	3.8	*a*	*A*	3.8	*a*	*A*	4.2	*a*	*A*
30 - 50	4.9	*a*	*A*	3.6	*a*	*A*	4.6	*a*	*A*	4.5	*a*	*A*	4.6	*a*	*A*	4.7	*a*	*A*
Average	4.6	*c*	*	3.7	*a*	*ns*	4	*ab*	*ns*	4.1	*ab*	(*)	4.2	*b*	*	4.4	*bc*	**

Readily available sulfate-S (RAS)

Horizon	-55			27			55			100			150			300		
0 - 3	18	*abc*	*B*	16	*ab*	*B*	22	*bc*	*B*	26	*c*	*B*	20	*bc*	*C*	11	*a*	*B*
3 - 10	11	*ab*	*A*	7	*a*	*A*	13	*b*	*A*	10	*ab*	*A*	12	*ab*	*B*	9	*ab*	*AB*
10 - 20	8	*a*	*A*	6	*a*	*A*	9	*a*	*A*	8	*a*	*A*	8	*a*	*AB*	5	*a*	*A*
20 - 30	7	*a*	*A*	5	*a*	*A*	7	*a*	*A*	6	*a*	*A*	6	*a*	*A*	4	*a*	*A*
30 - 50	6	*a*	*A*	4	*a*	*A*	7	*a*	*A*	6	*a*	*A*	6	*a*	*A*	4	*a*	*A*
Average	10	*a*	(*)	8	*a*	*ns*	12	*a*	*ns*	11	*a*	(*)	10	*a*	*	7	*a*	(*)

Adsorbed sulfate-S (AS)

Horizon	-55			27			55			100			150			300		
0 - 3	12	*ab*	*A*	12	*ab*	*A*	15	*ab*	*A*	18	*b*	*A*	14	*ab*	*A*	10	*a*	*A*
3 - 10	12	*a*	*A*	9	*a*	*A*	14	*a*	*A*	15	*a*	*A*	14	*a*	*A*	10	*a*	*A*
10 - 20	12	*a*	*A*	11	*a*	*A*	15	*a*	*A*	13	*a*	*A*	13	*a*	*A*	10	*a*	*A*
20 - 30	11	*a*	*A*	10	*a*	*A*	16	*a*	*A*	14	*a*	*A*	13	*a*	*A*	10	*a*	*A*
30 - 50	11	*a*	*A*	10	*a*	*A*	13	*a*	*A*	13	*a*	*A*	11	*a*	*A*	9	*a*	*A*
Average	12	*bc*	**	10	*ab*	*ns*	15	*d*	**	15	*d*	*	13	*cd*	*	10	*a*	*

HCl-soluble sulfate-S (HCS)

Horizon	-55			27			55			100			150			300		
0 - 3	0	*a*	*A*	1	*a*	*A*	0	*a*	*A*	0	*a*	*A*	0	*a*	*A*	0	*a*	*A*
3 - 10	0	*a*	*A*	0	*a*	*A*	0	*a*	*A*	5	*a*	*A*	0	*a*	*A*	0	*b*	*A*
10 - 20	11	*a*	*A*	0	*a*	*A*	0	*a*	*A*	3	*a*	*A*	0	*a*	*A*	0	*a*	*A*
20 - 30	0	*a*	*A*	0	*a*	*A*	0	*a*	*A*	0	*a*	*A*	0	*a*	*A*	0	*a*	*A*
30 - 50	1	*a*	*A*	0	*a*	*A*	0	*a*	*A*	0	*a*	*A*	0	*a*	*A*	0	*a*	*A*
Average	2	*a*	*ns*	0	*a*	*ns*	0	*a*	*ns*	2	*a*	*ns*	0	*a*	*ns*	0	*a*	*ns*

Organic S (*OS*)

Horizon	-55			27			55			100			150			300		
0 - 3	344	*a*	*B*	619	*a*	*B*	330	*a*	*B*	400	*a*	*B*	404	*a*	*C*	369	*a*	*B*
3 - 10	260	*a*	*AB*	226	*a*	*A*	224	*a*	*AB*	212	*a*	*A*	226	*a*	*B*	244	*a*	*AB*
10 - 20	173	*a*	*AB*	172	*a*	*A*	144	*a*	*A*	155	*a*	*A*	153	*a*	*AB*	155	*a*	*A*
20 - 30	141	*a*	*A*	150	*a*	*A*	128	*a*	*A*	111	*a*	*A*	119	*a*	*A*	107	*a*	*A*
30 - 50	97	*a*	*A*	138	*a*	*A*	103	*a*	*A*	104	*a*	*A*	116	*a*	*A*	102	*a*	*A*
Average	203	*a*	(*)	261	*a*	(*)	186	*a*	**	196	*a*	(*)	204	*a*	(*)	196	*a*	**

Total S (*ToS*)

Horizon	-55			27			55			100			150			300		
0 - 3	373	*a*	*B*	648	*a*	*B*	367	*a*	*B*	444	*a*	*B*	438	*a*	*C*	391	*a*	*B*
3 - 10	283	*a*	*AB*	242	*a*	*A*	251	*a*	*AB*	242	*a*	*A*	251	*a*	*B*	263	*a*	*AB*
10 - 20	202	*a*	*AB*	190	*a*	*A*	167	*a*	*A*	178	*a*	*A*	174	*a*	*AB*	170	*a*	*A*
20 - 30	159	*a*	*A*	164	*a*	*A*	151	*a*	*A*	131	*a*	*A*	138	*a*	*A*	121	*a*	*A*
30 - 50	115	*a*	*A*	153	*a*	*A*	123	*a*	*A*	123	*a*	*A*	134	*a*	*A*	115	*a*	*A*
Average	227	*a*	*	279	*a*	*	212	*a*	**	224	*a*	*	227	*a*	*	212	*a*	**

Table 3 (continued)

(b) Molasse

pH

Horizon	-55			27			55			100			150			300		
0 - 3	3.1	*a*	*A*	2.9	*a*	*A*	3	*a*	*A*	2.9	*a*	*A*	3	*a*	*A*	2.9	*a*	*A*
3 - 10	3.3	*a*	*AB*	3.1	*a*	*A*	3.3	*a*	*A*	3.1	*a*	*A*	3.1	*a*	*A*	3.1	*a*	*A*
10 - 20	3.7	*a*	*BC*	3.5	*a*	*B*	3.7	*a*	*B*	3.7	*a*	*B*	3.6	*a*	*B*	3.6	*a*	*B*
20 - 30	4	*a*	*C*	3.9	*a*	*C*	4	*a*	*C*	4.1	*a*	*C*	4	*a*	*C*	4	*a*	*C*
30 - 50	4.2	*a*	*C*	4.1	*a*	*C*	4.2	*a*	*C*	4.2	*a*	*C*	4.2	*a*	*C*	4.2	*a*	*C*
Average	3.7	*a*	*	3.5	*a*	*ns*	3.6	*a*	*ns*	3.6	*a*	(*)	3.6	*a*	*	3.6	*a*	**

Readily available sulfate-S (RAS)

Horizon	-55			27			55			100			150			300		
0 - 3	54	*ab*	*B*	41	*ab*	*B*	44	*ab*	*C*	86	*b*	*B*	88	*b*	*B*	29	*a*	*B*
3 - 10	25	*ab*	*AB*	21	*ab*	*AB*	23	*ab*	*B*	37	*ab*	*A*	44	*b*	*A*	17	*a*	*AB*
10 - 20	12	*a*	*A*	9	*a*	*A*	11	*a*	*A*	9	*a*	*A*	14	*a*	*A*	8	*a*	*A*
20 - 30	8	*a*	*A*	4	*a*	*A*	6	*a*	*A*	6	*a*	*A*	6	*a*	*A*	4	*a*	*A*
30 - 50	11	*a*	*A*	3	*a*	*A*	5	*a*	*A*	12	*a*	*A*	8	*a*	*A*	5	*a*	*A*
Average	22	*a*	(*)	16	*a*	*ns*	18	*a*	*ns*	30	*a*	(*)	32	*a*	*	13	*a*	(*)

Adsorbed sulfate-S (AS)

Horizon	-55			27			55			100			150			300		
0 - 3	32	*ab*	*A*	11	*a*	*A*	30	*ab*	*A*	38	*ab*	*AB*	48	*b*	*AB*	13	*a*	*A*
3 - 10	30	*c*	*A*	9	*a*	*A*	28	*bc*	*A*	32	*c*	*A*	29	*bc*	*A*	14	*ab*	*A*
10 - 20	37	*c*	*A*	17	*a*	*A*	33	*c*	*A*	37	*c*	*AB*	31	*bc*	*A*	19	*ab*	*A*
20 - 30	63	*a*	*A*	24	*a*	*A*	49	*a*	*A*	65	*a*	*AB*	56	*a*	*AB*	31	*a*	*AB*
30 - 50	169	*a*	*B*	66	*a*	*A*	119	*a*	*B*	236	*a*	*B*	189	*a*	*B*	63	*a*	*B*
Average	66	*a*	**	26	*a*	*ns*	52	*a*	**	82	*a*	*	71	*a*	*	28	*a*	*

HCl-soluble sulfate-S (HCS)

Horizon	-55			27			55			100			150			300		
0 - 3	0	*a*	*A*	2	*a*	*A*	0	*a*	*A*	0	*a*	*A*	0	*a*	*A*	0	*a*	*A*
3 - 10	0	*a*	*A*	6	*a*	*A*	0	*a*	*A*	0	*a*	*A*	0	*a*	*A*	1	*a*	*A*
10 - 20	0	*a*	*A*	27	*a*	*A*	1	*a*	*A*	0	*a*	*A*	0	*a*	*A*	3	*a*	*A*
20 - 30	0	*a*	*A*	3	*a*	*A*	3	*a*	*A*	4	*a*	*A*	0	*a*	*A*	6	*a*	*A*
30 - 50	0	*a*	*A*	5	*a*	*A*	0	*a*	*A*	0	*a*	*A*	0	*a*	*A*	0	*a*	*A*
Average	0	*a*	*ns*	9	*b*	*ns*	1	*a*	*ns*	1	*a*	*ns*	0	*a*	*ns*	2	*a*	*ns*

Organic S (*OS*)

Horizon	-55			27			55			100			150			300		
0 - 3	954	*a*	*B*	1149	*a*	*B*	710	*a*	*B*	1695	*a*	*B*	1741	*a*	*B*	739	*a*	*B*
3 - 10	461	*a*	*AB*	566	*a*	*A*	400	*a*	*A*	777	*a*	*A*	808	*a*	*A*	549	*a*	*AB*
10 - 20	285	*a*	*A*	340	*a*	*A*	359	*a*	*A*	248	*a*	*A*	321	*a*	*A*	381	*a*	*AB*
20 - 30	227	*a*	*A*	306	*a*	*A*	229	*a*	*A*	135	*a*	*A*	231	*a*	*A*	280	*a*	*A*
30 - 50	169	*a*	*A*	232	*a*	*A*	162	*a*	*A*	80	*a*	*A*	93	*a*	*A*	240	*a*	*A*
Average	419	*a*	(*)	519	*a*	(*)	372	*a*	**	587	*a*	(*)	639	*a*	(*)	438	*a*	**

Total S (*ToS*)

Horizon	-55			27			55			100			150			300		
0 - 3	1039	*a*	*B*	1201	*a*	*B*	785	*a*	*B*	1820	*a*	*B*	1877	*a*	*B*	781	*a*	*B*
3 - 10	516	*a*	*AB*	602	*a*	*A*	451	*a*	*A*	846	*a*	*A*	881	*a*	*A*	581	*a*	*AB*
10 - 20	358	*a*	*A*	389	*a*	*A*	404	*a*	*A*	334	*a*	*A*	377	*a*	*A*	408	*a*	*A*
20 - 30	298	*a*	*A*	285	*a*	*A*	237	*a*	*A*	210	*a*	*A*	298	*a*	*A*	273	*a*	*A*
30 - 50	349	*a*	*A*	284	*a*	*A*	238	*a*	*A*	327	*a*	*A*	304	*a*	*A*	241	*a*	*A*
Average	512	*a*	*	552	*a*	*	423	*a*	**	707	*a*	*	747	*a*	*	456	*a*	**

Comparison of means (one-way ANOVA and Duncan multiple range test; 95% confidence interval): small letters for comparison between distances within a given soil horizon and capital letters for comparison between soil horizons within a given profile (*a* or *A* indicates the lowest mean). Average values ($n=15$), representing mean values of individual soil profiles, were compared between the distances from a tree (small letters) and between Flysch and Molasse (level of significance is shown as: *ns* not significant, $p>0.10$; $^{(*)}p<0.10$; $^{*}p<0.05$; $^{**}p<0.01$; $n=2$ bedrocks × 3 sites × 5 horizons = 30)

B1, respectively. The mean Al_o content was significantly ($p < 0.001$) lower at W (1.0 mg g^{-1}) than at B1 (4.8 mg g^{-1}; data not shown). It must be pointed out that Al_o and Al_d contents did not differ between mean soil depths at W but increased significantly with increasing depth at B1 (Al_d: 0–3, 3–10 < 10–20 < 20–30, 30–50; Al_o: 0–3, 3–10 ≤ 20–30, 30–50; soil horizon ranges in cm).

Discussion

How are S fractions distributed and related to each other in low pH (nutrient-poor) and high pH (nutrient-rich) forest soils?

Inorganic SO_4^{2-} accounts generally for less than 10% of the total S (Tisdale et al. 1993). In the presented study, however, these theoretical limits were exceeded on both investigated bedrocks for mean horizon values (Table 3): the sum of *RAS, AS* and *HCS* reached up to 19 and 63% in some points on Flysch and Molasse, respectively. The *AS* fraction contributed mainly to these high values. According to a simplified S flow diagram of an upland forest ecosystem by Reuss and Johnson (1986), SO_4^{2-} is the only S output in the leachate. In turn, the SO_4^{2-} soil solution pool is connected to two S pools: the adsorbed SO_4^{2-} pool and the organic S pool. In accordance with Reuss and Johnson (1986), we suggest that the observed high inorganic SO_4^{2-} pools exert a strong control on leaching of SO_4^{2-} and associated loss of base cations and soil acidification.

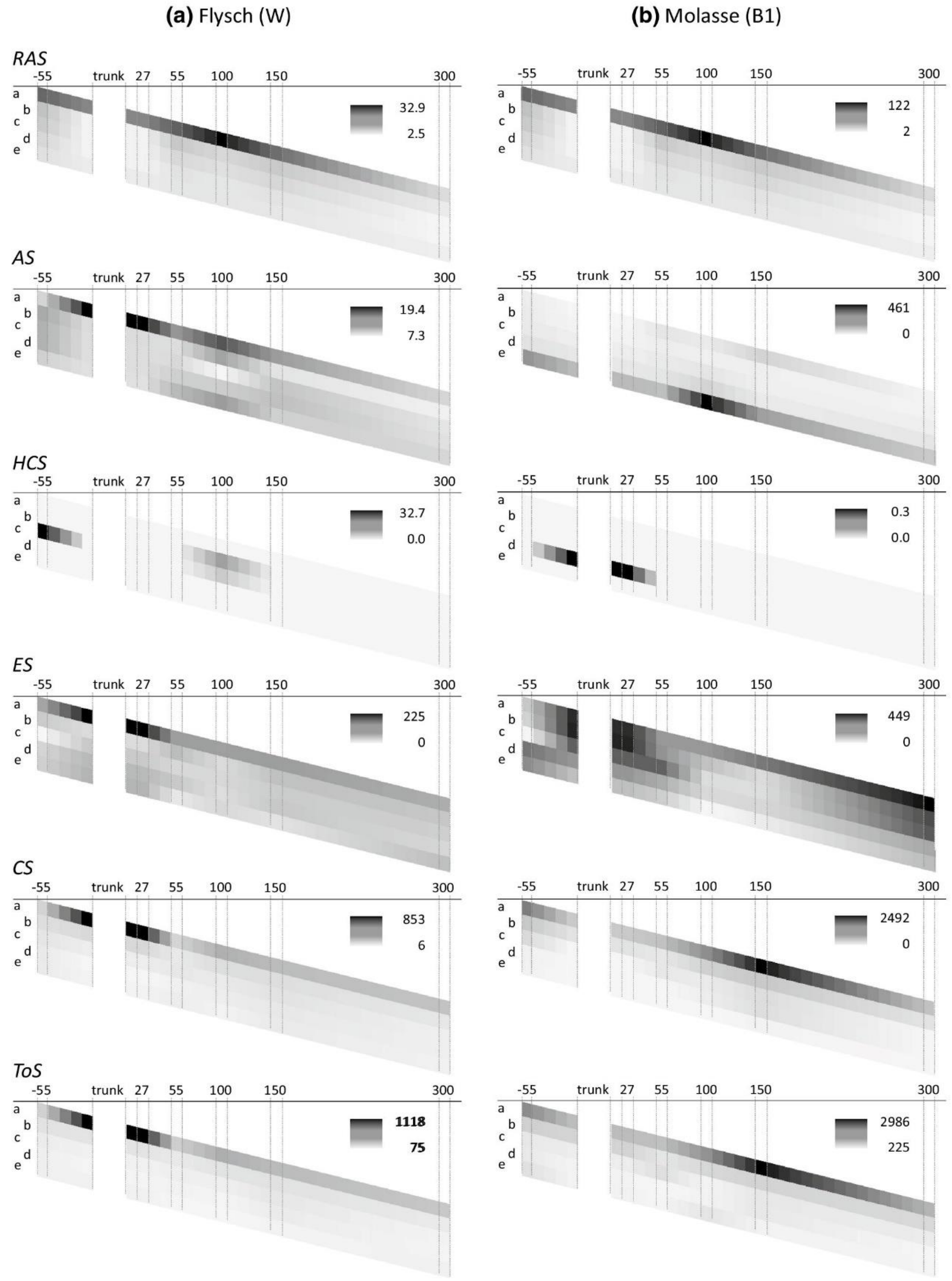
Sulfur contained in soil organic matter (*OS*) represented large pools of up to 95% of total S (*ToS*) on both bedrocks which is in accordance with data reported by Vannier et al. (1993) and Erkenberg et al. (1996). The *ES* fraction amounted 38 and 54% of *OS* at W and B1 (mean of average soil profiles), respectively. This is in contradiction to Havlin et al. (2005), suggesting that *ES* represents the majority of the organic S. However, data of *ES* (33 and 39% of *ToS* on W and B1, respectively) are higher than reported contributions of 16% by David et al. (1982) in forest soils, but close to the 38% contribution as found at the Hubbard Brook Experimental Forest (Likens et al. 2002). The total S pool of a haplic podzol in the control watershed Schluchsee consisted of >90% *OS*, 64% of *ToS* was *CS*, and 28% *ES*. However, in a dystric cambisol in the control watershed Villingen, inorganic S bound in the mineral soil accounted for 67% of the *ToS* pool (Prietzel et al. 2001).

Bivariate Pearson correlation coefficients (*R*) are given in Table 4. Caution must be exercised when interpreting correlations because they do not give information about "controlling factors or processes". Conclusions may be drawn from cases where the coefficients for W and B1 were very contrary. e.g., high positive correlation between *OS* and *AS* at W (0.75; $p < 0.001$) may be explained by high mineralization rates on Flysch, while the lack of this relation (even negative sign) at B1 may indicate that immobilization (microbial uptake) is an important process on Molasse. Ester sulfate-S, that is considered to be easily mineralized after hydrolysis (Morche 2008), was positively correlated with *AS* at W (indicating mineralization on Flysch) but vice versa at B1 (indicating immobilization on Molasse).

Bivariate relations among S fractions, which were similar for both sites, can be summarized as follows (Table 4): Organic sulfur increased with decreasing pH since acidic conditions promote the accumulation of organic matter and vice versa (i.e., organic matter accumulation produces organic acids). Organic sulfur was positively correlated with all other S fractions at both sites, except with *AS* at B1 (see assumption on preferential S immobilization on Molasse above), *HCS* (probably due to the fact that most data were zero) and with *ES* at B1 (which was distributed more patchy than at W; see Fig. 1). Extremely high coefficients were recorded for both sites between *OS* and *ToS* (W: 1.00; B1: 0.98), which is not surprising as *OS* represents in total the major S fraction in the investigated soils.

Organic C was positively related to *RAS, OS* and *ToS* ($p < 0.001$) stating the general link between C and S in terrestrial ecosystems (Table 4). The facts that C_{org} was (a) positively related to *AS* at W but negatively at B1, and (b) positively to *ES* at W but not significant at B1 is striking. Possible interpretations are suggested below.

Plant debris (C_{org}) provided *CS* at both substrates (*R*, C_{org}/*CS*: 0.94–0.96). Carbon bonded S seemed to be rather stable at B1 (*R, CS/ES*: ns; suggesting immobilization) but contributed to labile *ES* at W1 (*R, CS/OS*: 0.86; suggesting mineralization). According to Ghani et al. (1991), carbon-bonded S is converted, probably oxidatively, to *ES* and is then mineralized to SO_4^{2-}. Labile *ES* probably contributed to *RAS* at W (0.62; note: R, *ES/RAS* at B1: ns) which, finally, showed up as adsorbed sulfate-S at W (*R, RAS/AS*: 0.67 vs ns at B1). This underlines the suggestion that *AS* originates mainly from decomposition (mineralization) of organic material at Flysch (see high accumulation of *AS* in the top soil of Fig. 1) while *AS* is accumulated in the deep soil at Molasse (Bs horizons with high contents of Al and Fe oxides) as visible in Fig. 1. The fact that a considerable proportion of atmospherically deposited SO_4^{2-} may be cycled through the organic S pool before being released to soil solution, as suggested for the Flysch sites, was indicated by $^{34}S/^{32}S$ ratios (Alewell et al. 1999; Zhang et al. 1998). Selected water samples of rainwater (precipitation and throughfall) and soil solution at the study site W were measured for $\delta^{34}S_{VCDT}$ by Hanousek et al. (2016) and ranged between 4 and 6‰, the ratio in soil solution being slightly lower. The lower ratio indicated that a considerable portion of the atmospherically deposited sulfate was cycled through



(a) Flysch (W)

(b) Molasse (B1)

◂**Fig. 1** Distribution of readily available sulfate-S (*RAS*), adsorbed sulfate-S (*AS*), HCl-soluble sulfate-S (*HCS*), ester sulfate-S (*ES*), carbon bonded sulfur (*CS*) and total sulfur (*ToS*) on **a** Flysch (study site W) and **b** Molasse (study site B1). Values were measured in the soil horizons 0–3 (*a*), 3–10 (*b*), 10–20 (*c*), 20–30 (*d*) and 30–50 (*e*) cm in −55, 27, 55, 100, 150 and 300 cm distance from a tree trunk. Values between these points were calculated as moving average. Maximum and minimum values are given in the legend of each sub-plot. All data are given in $\mu g\ S\ g^{-1}$

the organic S pool before being released to the soil solution and supports our conclusions for the site W above, deduced from relations between the different S-fractions.

Interpreting bi-variate correlation coefficients of Table 4 is difficult due to many inter-correlated variables. e.g., we would expect a positive relation between Al_d and C_{org} at B1 (which was negative: $R = -0.82$; $p < 0.001$) as was recorded at W (R: 0.47; $p < 0.01$), since C_{org} was coupled with negative soil pH (R: −0.42 and −0.83 at W and B1, respectively) promoting the formation of Al oxides via silicate weathering (Rampazzo et al. 1999). Because Fe and Al oxides increased significantly with increasing depth at B1 (Bs horizons of podzols on Molasse) but C_{org} contents decreased (similar to depth profiles of *OS*, Table 3) this relation Al_d (Al_o) to C_{org} was negative. As a consequence, the relations of Al_d (Al_o) to *RS* (readily available S is mainly provided from the humus layer), *CS*, *OS* and *ToS* were all negative as well. However, Al oxides played a major role for adsorption of *AS*, as indicated by positive correlations at B1 (R, Al_d/*AS* 0.48, $p < 0.05$; Al_o/*AS* 0.55, $p < 0.01$). At W (Flysch), there was a declining trend of Al_d with increasing depth, causing significant positive relations to C_{org} and all other S-fractions (except *HCS*). This is why, *AS* was correlated negatively with soil pH ($R = -0.33$) and positively with C_{org} ($R = 0.73$) at W (due to increasing positive charges of humus particles and Al oxides for adsorbing sulfate) but positively with soil pH ($R = 0.65$) and negatively with C_{org} ($R = -0.40$) at B1 (due to increasing Al oxides with increasing depth at increasing soil pH but decreasing C_{org} contents).

Dithionite-soluble Fe_d at B1 showed similar coefficients as Al_d, though relations with C_{org} and *AS* were not significant (Table 4). At high pH-soils on Flysch (W), the formation of Fe oxides was lower and its role to adsorb sulfate was negligible.

Are stemflow induced soil changes reflected in spatial patterns of S fractions?

The average pH values of the six total soil profiles per site indicated clearly soil acidification at near downhill distances on Flysch but not on Molasse (Table 3). As soil pHs were lower on Molasse, stemflow induced acidification was not significant as a consequence of the logarithmic pH-scale, of lower base contents (compare Table 2), which do not allow redistribution of large amounts of base cations, and of buffering by the aluminum system at soil pH below 4.2.

Wide soil pH ranges (3.2–7.0) on Flysch (pH: $W < E < J$; Table 3) are the reason that pH did neither differ with depth within individual soil profiles nor with distance from the stem within one soil depth. On Molasse, at relatively narrow soil pH ranges (2.8–4.4), top soil acidification was recorded for the average soil profiles at all six distances as related to podzolization (Table 3).

Changes within an individual horizon over distance and vice versa (Table 3) were compared to disentangle the soil depth effect on S fractions from stemflow induced spatial patterns. There was no significant effect of stemflow (distance) on distribution of *ToS* and *OS* within the mineral soil, however, total S and *OS* (both for *ES* and *CS* at W) tended to be higher at 27 cm distance in the top soil of Flysch (Table 3a; Fig. 1a). Total S and *OS* showed a general decline with increasing depth. This decline was steeper on Molasse (Table 3b) due to surface accumulation of organic matter on podzolic soils, characterized by slower decomposition and mineralization rates than on Flysch.

Mean contents of total S of 97 beech sites in the Vienna Woods decreased markedly in the stemflow area (0–5 cm soil depth; 20 cm downhill from the base of the stem) but slightly increased in the between trees area (0–5 cm soil depth; at least 3 m away from beech stems) from 1984 to 2012 (Berger et al. 2016), corresponding to a S loss (0–5 cm horizon) of 18.2 $g\ m^{-2}$ and a S gain of 8.2 $g\ m^{-2}$, respectively (unpublished data by the same authors). Therefore, high amounts of atmospherically deposited SO_4^{2-}, partly cycled through the organic S pool, must have been mineralized on Flysch since the 1980s. Berger et al. (2009a, b) concluded for comparable beech sites on Flysch that net mineralization of S in the top soil is the major reason for negative SO_4^{2-} input - output budgets. Preferential mineralization over immobilization on Flysch was suggested from the correlation analysis (Table 4). Narrow mineral soil C_{tot}/S_{tot} ratios on Flysch (Table 2) support this conclusion.

Net immobilization of S on Molasse may be another reason why no acidifying effects of stemflow were visible (Table 3b). In fact, SO_4^{2-} stemflow fluxes caused an accumulation of *ES* along the tree stem (Fig. 1b). Wide mineral soil C_{tot}/S_{tot} ratios on Molasse (Table 2) support the theory of high microbial S immobilization rates. High amounts of precipitated Al^{3+} and SO_4^{2-} as Al hydroxy sulfates in the stemflow area (27 cm) were likely reflected via HCl extractions (as indicated as *HCS* in Table 3b and Fig. 1b), since, according to Prietzel and Hirsch (1998, 2000), phosphate extraction (*AS*) may underestimate inorganic S in acidic soils. A high rate of precipitation/dissolution of Al hydroxy sulfates in the stemflow area would be another reason for buffering acidic input via stemflow.

Table 4 Coefficients of correlation (*R*, Pearson; $n=6$ profiles × 5 horizons = 30) among pH, C_{org}, readily available sulfate-S (*RAS*), adsorbed sulfate-S (*AS*), HCl-soluble sulfate-S (*HCS*), organic sulfur (*OS*), total sulfur (*ToS*), ester sulfate-S (*ES*), carbon bonded sulfur (*CS*), dithionite-soluble Fe (Fe_d) and Al (Al_d), and oxalate-soluble Fe (Fe_o) and Al (Al_o) at the study sites W (Flysch) and B1 (Molasse)

Parameter	Site	pH	C_{org}	*RAS*	*AS*	*HCS*	*OS*	*ToS*	*ES*	*CS*	Fe_d	Fe_o	Al_d
C_{org}	W	-0.42	X										
	B1	**-0.83**	X										
RAS	W	-0.45	**0.72**	X									
	B1	**-0.74**	**0.96**	X									
AS	W	*-0.33*	**0.73**	**0.67**	X								
	B1	**0.65**	-0.40	ns	X								
HCS	W	ns	ns	ns	ns	X							
	B1	ns	ns	ns	ns	X							
OS	W	-0.36	**0.97**	**0.62**	**0.75**	ns	X						
	B1	**-0.68**	**0.95**	**0.94**	ns	ns	X						
ToS	W	-0.37	**0.97**	**0.65**	**0.77**	ns	**1.00**	X					
	B1	**-0.65**	**0.94**	**0.96**	ns	ns	**0.98**	X					
ES	W	-0.42	**0.89**	**0.62**	**0.60**	*-0.31*	**0.91**	**0.90**	X				
	B1	ns	ns	ns	*-0.36*	ns	ns	ns	X				
CS	W	*-0.34*	**0.96**	**0.60**	**0.77**	ns	**0.99**	**0.99**	**0.86**	X			
	B1	**-0.67**	**0.94**	**0.96**	ns	ns	**0.97**	**0.98**	ns	X			
Fe_d	W	ns	ns	ns	ns	ns	ns	ns	ns	ns	X		
	B1	ns	ns	**-0.52**	ns	ns	-0.41	-0.47	ns	**-0.51**	X		
Fe_o	W	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.59**	X	
	B1	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.92**	X	
Al_d	W	ns	**0.47**	*0.36*	0.45	ns	**0.52**	**0.52**	0.45	**0.53**	**0.74**	**0.53**	X
	B1	**0.92**	**-0.82**	**-0.74**	0.48	ns	**-0.63**	**-0.65**	ns	**-0.66**	ns	ns	X
Al_o	W	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.59**	**0.49**	**0.67**
	B1	**0.95**	**-0.85**	**-0.76**	**0.55**	ns	**-0.68**	**-0.67**	ns	**-0.69**	ns	ns	**0.97**

p
ns
0.1
0.05
0.01
0.001

The level of significance is given as: ns (not significant), italic ($p < 0.1$), normal ($p < 0.05$) bold ($p < 0.01$) and bold plus underlined ($p < 0.001$)

Desorption of SO_4^{2-} in response to input of high amounts of water with low SO_4^{2-} concentrations at the stem after the end of the 1980s is put forward to explain significantly reduced *AS* at the stem base (27 cm) on Flysch (Table 3a). A similar (not significant) trend was visible on Molasse (Table 3b). This trend can be explained by "natural water extractions" (i.e., desorption) over historic time-periods.

Do these patterns match the reported negative S-budgets or recovery from acid rain?

Contents of *AS* were much lower on the high pH soils on Flysch than on the low pH soils on Molasse, since the density of net positive surface charges decreases with increasing soil pH. Hence, the conclusion by Berger et al. (2009a, b) that top soil mineralization of organic S is the major source of net SO_4^{2-} output on Flysch agrees with our data. The mean 2-year (2005–2007) net SO_4-S balance on comparable beech stands (between trees area) on Flysch was estimated −2.0 kg S ha^{-1} $year^{-1}$ (Berger et al. 2009a).

The fact that *AS* increased with depth on Molasse can be explained by the vertical wash-out direction (desorption starts at the top soil) and increasing amounts of Fe- and Al oxides or hydroxides in deeper soil layers (adsorption in the deeper soil; Table 3), as stated in the results section. Reuss and Johnson (1986) pointed out that a long time may be required for passage of the SO_4^{2-} front to any particular soil depth. It is striking that the soil profile next to the stem (27 cm) is the only one, which did not show a significant vertical increase of adsorbed SO_4^{2-} (*AS*). It can be suggested that this passage from top to deeper soil horizons has ceased due to accelerated recovery after the reduction of S deposition. Solute SO_4^{2-} flux profiles (between trees area) for the same beech sites on Molasse by Berger et al. (2009a) showed a steady increase with depth. Hence, desorption of historically deposited S below 10 cm can be assumed as the major source of net S loss for these acidic soils. The mean 2-year (2005–2007) net SO_4-S balance on the same beech stands on Molasse (between trees area) of this study was estimated −1.4 kg S ha^{-1} $year^{-1}$ (Berger et al. 2009a).

In accordance to Reuss and Johnson (1986), it can be concluded that increasing soil solution fluxes with decreasing distance from the stem cause a quicker steady state of soil SO_4^{2-} pools in response to currently decreased inputs. The presented data on *AS* support this hypothesis. Comparison of old and recent top soil pH values indicates a higher increase in the infiltration zone of beech stemflow than in the "between trees area" (Berger et al. 2016). The data (Table 3) did not show any horizontal pH depression close to the stem, as reported for Flysch sites in the Vienna Woods in the 1980s (Berger and Muras 2016; Kazda 1983; Sonderegger 1981).

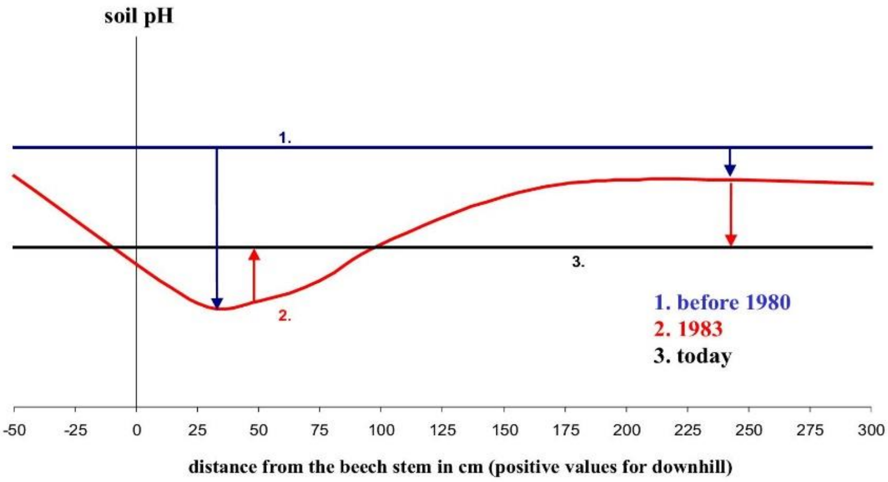


Fig. 2 Generalizing spatial soil recovery of the top soil, expressed as pH change (redrawn from Berger and Muras 2016)

In contrast to the reported negative S budgets for whole forested watersheds, the stem area of beech seems to have recovered from acid rain - if recovery is defined as the state where input–output S budgets turn from negative to zero. It is concluded that reduced atmospheric sulfate inputs affected soil conditions. Our results match nicely with predictions by Berger and Muras (2016) that the top soil will recover from acid deposition, as already recorded in the infiltration zone of stemflow near the base of the stem. However, in the between trees areas and especially in deeper soil horizons recovery may be highly delayed (Fig. 2).

Conclusions

Fractionation of S compounds in forest soils is a powerful tool for interpreting S dynamics and S biogeochemistry in forest ecosystems. Stemflow resulted in spatial soil patterns of S fractions (overall hypothesis). On Flysch, *RAS* represented 3–6%, *AS* 2–12%, *HCS* 0–8% and *OS* 81–95% of *ToS*. On Molasse, *RAS* amounted 1–6%, *AS* 1–60%, *HCS* 0–8% and *OS* 37–95% of *ToS*. It was possible to discuss relations between S fractions in regard to important soil processes, e.g., mineralization/immobilization and adsorption/desorption (question 1). Stemflow clearly caused spatial soil patterns of the inorganic S fractions (question 2). Desorption of SO_4^{2-} in response to input of high amounts of water with low SO_4^{2-} concentrations at the stem after the end of the 1980s is put forward to explain reduced *AS* at the stem base. Our data contribute to current reports on negative input–output S budgets of forest watersheds, suggesting that mineralization of *OS* on nutrient rich soils and desorption of historic *AS* on nutrient-poor soils are the dominant S sources (question 3), which have to be considered in future modeling of sulfur. Finally, we conclude that the impact of heavy S deposition loads via stemflow and recovery from these inputs can be fingerprinted from the S fraction distribution in the soil. We suggest that combining the soil S fractionation method with analyses of stable isotopes of S and in situ soil solution studies will complete our knowledge on S biogeochemistry.

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15) Potential of three microbial bio-effectors to promote maize growth and nutrient acquisition from alternative phosphorus fertilizers in contrasting soils

Potenciál tří mikrobiálních biostimulantů k podpoře růstu a odběru živin z alternativních fosforečných hnojiv rostlinami kukuřice

Tato publikace vznikla v rámci evropského projektu BioFector a jsou zde shrnuty výsledky z nádobových pokusů 5-ti evropských univerzit (ČR, Německo, Itálie, Švýcarsko a Dánsko) v kontrastních půdních podmínkách. Cílem bylo vyhodnotit působení tří mikrobiálních biostimulantů (*Trichoderma harzianum, Bacillus amyloliquefaciens* a *Pseudomonas sp.*) na růst a odběr živin rostlinami kukuřice hnojenými různými fosforečnými (mletý fosfát, trojitý superfosfát) a organickými (digestát, kompostovaný hnůj, čistírenské kaly) hnojivy. Z dosažených výsledků je zřejmé, že efektivita jednotlivých biostimulantů je silně závislá jednak na půdních vlastnostech, jednak na druhu aplikovaného hnojiva. Dobré perspektivy vykázaly většinou varianty hnojené kompostovaným chlévským hnojem, digestátem a čistírenskými kaly. Naproti tomu byl vliv biostimulantů na uvolňování živin z hůře rozpustných forem v minerálních hnojivech prokázán pouze v jednom z pokusů (Buus, Švýcarsko). Z hlediska půdních vlastností je zřejmé, že biostimulanty vykazovaly největší potenciál na půdách s vysokým pH a zároveň chudých na živiny. Dalším cílem realizovaných pokusů bylo hledání nejvhodnější kombinace organického hnojiva a biostimulantu v interakci s půdními vlastnostmi (pH, mikrobiální aktivita, obsah organické hmoty, a celkový obsah fosforu a dusíku). Z tohoto sledování vyplývá, že se všechny tři biostimulanty podílely na získání živin (zejména P) dvěma hlavními cestami, tj. podporou růstu kořenů a mobilizací fosforu prostřednictvím urychlení mineralizace dodaných organických hnojiv. I přesto, že na alkalických půdách bylo zjištěno vyšší procento kolonizace kořenů houbami, nepodařilo se jednoznačně potvrdit, zda tento jev hrál klíčovou roli ve zlepšení příjmu fosforu. Byly rovněž potvrzeny výsledky některých studií (Lekfeldt et al. 2016, Kundel 2016, Neumann 2015), že v půdách chudých na fosfor biostimulanty nevykazují zpravidla žádný vliv na růst rostlin, nebo dokonce působí negativně. Příčinou je konkurence o limitující živinu.

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RESEARCH **Open Access**

Potential of three microbial bio-effectors to promote maize growth and nutrient acquisition from alternative phosphorous fertilizers in contrasting soils

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Abstract

Background: Agricultural production is challenged by the limitation of non-renewable resources. Alternative fertilizers are promoted but they often have a lower availability of key macronutrients, especially phosphorus (P). Biological inoculants, the so-called bio-effectors (BEs), may be combined with these fertilizers to improve the nutrient use efficiency.

Methods: The goal of this study was to assess the potential of three BEs in combination with alternative fertilizers (e.g., composted manure, biogas digestate, green compost) to promote plant growth and nutrient uptake in soils typical for various European regions. Pot experiments were conducted in Czech Republic, Denmark, Germany, Italy, and Switzerland where the same variety of maize was grown in local soils deficient in P in combination with alternative fertilizers and the same set of BEs (*Trichoderma*, *Pseudomonas,* and *Bacillus* strains). Common guidelines for pot experiment implementation and performance were developed to allow data comparison, and soils were analyzed by the same laboratory.

Results: Efficiency of BEs to improve maize growth and nutrient uptake differed strongly according to soil properties and fertilizer combined. Promising results were mostly obtained with BEs in combination with organic fertilizers such as composted animal manures, fresh digestate of organic wastes, and sewage sludge. In only one experiment, the nutrient use efficiency of mineral recycling fertilizers was improved by BE inoculation.

Conclusions: These BE effects are to a large extent due to improved root growth and P mobilization via accelerated mineralization.

Keywords: Biofector, Bio-effector, Bio-inoculants, *Bacillus*, *Pseudomonas*, *Trichoderma*, Maize, Organic fertilizer, PGPR, Phosphorus, Recycling fertilizer

Background

To meet global demands for agricultural crops, farming systems in industrialized countries have undergone profound transformations. On the one hand, high application rates of synthetic fertilizers and manure together with the use of pesticides, irrigation, and short crop rotations have increased yields and have helped to reduce hunger in these countries [1]. On the other hand, modern high-input agriculture has created a multitude of environmental problems. In this context, groundwater pollution and the eutrophication of aquatic systems due to soil erosion and nutrient leaching and run-off from agricultural fields are of great concern [1, 2]. Additionally, the extensive use of synthetic

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fertilizers is contributing to climate change as fertilizer production and use cause significant greenhouse gas emissions [3]. Moreover, for some plant nutrients such as phosphorous (P), natural fixation processes in soil leads to a decrease in the efficiency of the fertilizers as only a fraction of the applied P fertilizer is taken up by plants. Depending on the soil pH and mineralogy, a considerable amount of P fertilizers gets either adsorbed to iron, aluminum oxides, or calcium and is no longer directly available to the plant [4]. Consequently, surplus fertilizer inputs are often maintained to meet the crop's demands. Finally, commonly used P fertilizers in modern agriculture are regarded as non-renewable resources with a limited supply, and the reserves are concentrated in a few countries around the globe [4, 5]. Hence, judicious use of the resource by effective recycling is required for long-term sustainability.

Today, increasing attention is being paid to the downside of high-input agricultural systems and much research is aimed at developing alternative ways to produce sufficient food in a sustainable and environmentally sound way. A number of different approaches have been investigated with the intention to reduce fertilizer inputs into agroecosystems, including breeding plant varieties with better P acquisition efficiency [6], fertilizer placement strategies [7], or application of soil organisms and natural extracts with plant growth-promoting potential [8–10]. These so-called bio-effectors (BEs) include plant growth-promoting rhizobacteria (PGPR) for instance *Pseudomonas* and *Bacillus* spp., as well plant-associated fungi, such as arbuscular mycorrhizal fungi (AMF) or *Trichoderma spp.* [11]. Moreover, BEs comprise non-living agricultural bio-stimulants (ABs) such as extraction products from macroalgae, compost, and plants [12]. The plant growth-promoting effect of BEs is based on different mechanisms, for example, the control of plant pathogens either by stimulation of the plant's defense mechanisms or by microbial antagonism [11, 13]. Some BEs induce plant growth by stimulating the plant's own hormone production such as auxins or cytokinins [13, 14], or by producing directly hormone-like compounds [15]. Regarding P acquisition, some BEs are associated with increased phosphatase activities in the soil [16–18] and have also shown the ability to liberate P from soil particles [9, 16, 19, 20] and from P-recycling fertilizers [21, 22]. Another important mechanism is based on the so-called "mycorrhiza helper" effect [23] which has been observed for many bacterial BEs [24]. This term refers to a stimulation of AMF which fulfill important functions regarding P uptake by plants.

Although BEs have shown the potential to promote plant growth, their effectiveness is influenced by numerous biotic and abiotic factors. Consequently, depending on the properties of the soil and the structure of the naturally occurring rhizosphere community, their plant growth-promoting effect has often shown limited reproducibility. As reported in Mosimann [25], soil properties such as pH can have impacts on the BEs' ability to exert plant growth-promoting effects. Moreover, the amount of total and plant available P seems to be a decisive factor for the performance of bacterial BEs [26, 27]. Also, competition with naturally occurring rhizosphere microorganisms can limit the survival of introduced BEs [28]. This indicates that more work is needed to unravel the conditions under which BEs can exert plant growth-promoting effects, in order to achieve more homogenous and predictable effects. With this new knowledge, specifically adapted BEs could be developed which might improve fertilizer use efficiency and decrease the dependence of agriculture on large external inputs of synthetic fertilizers.

Maize (*Zea mays*), a major crop globally, is particularly sensitive to low phosphorus availability [29] and has shown responsiveness to BE applications [30–32]. For this reason, maize was selected as the study crop to evaluate the potential of three BEs: the bacterial BEs *Pseudomonas* sp. DSMZ 13134 and *Bacillus amyloliquefaciens* FZB42 and the fungal BE *Trichoderma harzianum* T-22. We applied all BEs individually in combination with different organic and inorganic P fertilizers in order to study the impact of the different P sources on the BEs' ability to promote P acquisition and growth of maize. In order to shed more light on the impact of soil properties on the effectiveness of the applied BEs, we used six soils originating from different sites in Europe. We hypothesized that BE application improves plant P acquisition from soil and from recycling fertilizers, and that soil properties, e.g., pH and P levels (total P and plant available P), influence the effectiveness of the three BEs.

Methods

Overall experimental design

Maize growth (variety Colisée, KWS Saat, Germany) was investigated in pots using soils collected from six different sites (Table 1) and by applying a factorial design including the two following factors: P fertilizer (minimum 3 levels) and microbial BE application (2–4 levels). Table 2 gives an overview of the treatments applied to each soil.

Collection of soils and soil characterization

Topsoil was collected from the six different locations. The soils were characterized by one analytical lab (LA Chemie, University of Hohenheim, Germany) for pH (0.01% $CaCl_2$), texture, organic carbon content [33], and plant available soil P (CAL method for neutral and alkaline soils [33] and DL method for acidic soils [33]). Additional information regarding geographic origin of the

Table 1 Soil characteristics

Soil-ID	Geographic origin	Management	Soil type	Texture			Soil pH	C org	Phosphorus
				Clay (%)	Sand (%)	Silt (%)	($CaCl_2$)	(%)	CAL/DL[d] (mg P kg^{-1})
1-Buus[a]	Buus (Switzerland)	Rotation: Lay rotation Fertilization: organic, low input Site type: farmer field site	Loamy soil	29.9	3.90	66.2	6.6	2.64	6.5
2-Castel[a]	Castel Volturno (Italy)	Rotation: Maize-clover Fertilization: mineral, low input Site type: farmer field site	Vertic Xerofluvent	36.5	19.0	44.5	7.3	1.34	19.2
3-Humpolec[a]	Humpolec (Czech Republic)	Rotation: P-WW-SB[c] Fertilization: mineral, low input Site type: experimental station	Cambisol Loamy sand	15.9	52.9	31.2	5.2	1.63	45.0/59.8
4-Lukavec[a]	Lukavec (Czech Republic)	Rotation: long-term protection area around field experiment Fertilization: none Site type: experimental station	Cambisol Loamy sand	14.0	56.0	30.0	5.4	1.5	48.0/49.8
5-Vörden[b]	Vörden (Germany)	Rotation: before 2009 grazing area for wildlife – since 2009 field forage cultivation Fertilization: low input (organic and mineral) Site type: farmer field site	Cambisol	12.0	41.1	46.9	5.0	0.72	11.4/9.4
6-Taastrup[a]	Taastrup (Denmark)	Rotation: Barley-barley Fertilization: no P fertilizer since 1964 Site type: long-term field trial	Sandy loam	13.4	55.4	31.2	5.8	1.09	15.3/17.9

[a] Data were recorded on the pure soil

[b] Data were recorded on the 2:1 soil: sand mixture used in the pot experiments

[c] *P* potato, *WW* winter wheat, *SB* spring barley

[d] P measured after double lactate (DL) extraction is also given for soil with pH < 6 (exception for soil 3)

soils, management of the sites, and soil type is given in Table 1. Most soils had low to very low levels of available P, which makes them ideal for testing the efficiency of BEs in improving P availability and/or P uptake of added fertilizers by maize. The pH was ranging from slightly acidic to medium alkaline.

BE treatments

The three BE strains tested (Table 3) were formulated within three commercial products, namely Trianum-P (Koppert, The Netherlands) containing *Trichoderma harzianum* strain T-22 (BE1), Proradix WP (Sourcon Padena, Germany) containing *Pseudomonas* sp. strain DSMZ 13134 (BE2), and Rhizo Vital 42 fl. (Abitep, Germany) containing *Bacillus amyloliquefaciens* strain FZB42 (BE3). The choice of these three BEs is based on their published ability to act as potential bio-stimulants and biofertilizers. The strain *Trichoderma harzianum* Rifai 1295-22 (T22) has demonstrated abilities to increase the solubilization of sparingly soluble calcium phosphates [34] and to promote plant growth in various plant species [35, 36] including maize [37]. The strain *Pseudomonas* DSMZ 13134 is an efficient root colonizer [38] and has been shown to solubilize inorganic phosphate under in vitro conditions [39]. Moreover, it has recently shown the potential to improve plant growth and phosphorus acquisition when inoculated to maize [10, 25]. The gram-positive strain *Bacillus amyloliquefaciens* FZB42 is mainly studied for its biocontrol potential but other studies also indicate its ability to act as a biofertilizer [40, 41]. The *Trichoderma* strain was inoculated at a concentration of 2.5×10^4 spores per g of substrate and the two bacterial strains at a concentration of 2×10^6 cfu per g of substrate. A non-inoculated control (BE0) was included for every soil and in combination with every P treatment. The exact amount of inoculated cells or spores in the different products was checked in preliminary work where inoculation suspensions were plated on specific media and counted afterward. This has led to a standardized inoculation protocol that was later applied for all pot experiments.

P fertilization treatments

The three common P treatments were negative control without the addition of P fertilizer (P0), Triple Super Phosphate (TSP), and Rock Phosphate (RP). In most

Table 2 Overview of the experimental design and setup at the different locations (1: Switzerland, 2: Italy, 3 and 4: Czech Republic, 5: Germany, 6: Denmark)

	1-Buus	2-Castel	3-Humpolec	4-Lukavec	5-Vörden	6-Taastrup
Factors tested (experimental design)						
Crop						
Maize (variety: Colisée)	x	x	x	x	x	x
Bio-effectors						
No bio-effectors (BE0)	x	x	x	x	x	x
BE1: Trianum-P	x	x	x	x		
BE2: Proradix	x	x	x	x	x	x
BE3: Rhizovital	x	x	x	x	x	
Fertilizers						
No P fertilizer (P0)	x	x	x	x	x	x
Triple Super phosphate	x	x	x	x	x	
Rock phosphate	x	x	x	x	x	
Local sewage sludge ashes					x	x
P-enriched BOF slag					x	
Fresh Digestate	x					
Local composts	x				x	
Composted cattle manure		x				
Composted horse manure		x				
Local sewage sludges					x	x
Experimental setup						
Amount of substrate per pot (kg-DW equivalent)	2.5	2.5	5	5	5	2.5
Number of plants per pot at harvest	1	1	3	3	1	1
Growth period (weeks)	8	8	15	15	8	6.5
Number of replicates	5	5	5	5	4	3
Soil to sand ratio	2:1	2:1	2:1	2:1	2:1	1:1
Growing facilities[a]	GC	GC	GH	GH	GH	GH

[a] GC means growth chamber and GH greenhouse

Table 3 Bio-effector strains applied

Product	Producer	Abbr.	Species	Type of organism	Formulation	Application rate (cfu g^{-1} soil)
Control	n.a.	BE0	n.a.	n.a.	n.a.	n.a.
Trianum-P	Koppert, The Netherlands	BE1	*Trichoderma harzianum*, strain T-22	Fungus	Powder	2.5×10^4
Proradix WP	Sourcon Padena, Germany	BE2	*Pseudomonas sp.* strain DSMZ 13134	Bacteria	Powder	2×10^6
Rhizovital 42 fl.	ABiTEP, Germany	BE3	*Bacillus amyloliquefaciens*, strain FZB42	Bacteria	Liquid	2×10^6

n.a. means not applicable

cases, a number of additional P treatments (Table 4) were investigated and consisted of organic fertilizers and/or recycled inorganic fertilizers. These fertilizers were locally available and previously studied for their properties by the individual groups. As a result, some soils were tested with organic fertilizers and others only with recycled inorganic fertilizers (in addition to TSP and RP). Except for the P0 treatment, all pots received P at a dose of 50 mg P per kg of dry substrate. The TSP (20% P) was provided by Landor in Switzerland and RP (12% P) by Herbert Molitor in Germany. The additional P treatments consisted of recycled inorganic fertilizers (sewage sludge ashes and P-enriched BOF (basic oxygen furnace) slags [42]) and organic fertilizers (fresh biogas digestate, mature compost from green

Table 4 Main characteristics of the fertilizers used

Fertilizers	Abbr.	Inorg/org	Dry matter content (%)	Total P (mg P g^{-1})	Water extractable P (mg P g^{-1})	Total N (mg N g^{-1})	Ammonium ($NH_4^+ - N$) (mg N g^{-1})
Triple super phosphate	TSP	Inorg	100	201.0	201.0	0	0
Rock phosphate	RP	Inorg	100	115.4	0.22	0	0
Sewage sludge ashes (Germany)	SSA-DE	Inorg	100	103.5	0.79	n.d.	n.d.
Sewage sludge ashes (Denmark)	SSA-DK	Inorg	100	89	n.d.	n.d.	n.d.
P-enriched BOF slag (Germany)	LSD/SSA	Inorg	100	18.0	0.0	n.d.	n.d.
Fresh digestate (Switzerland)	FrDi	Org	48.0	4.36	0.130	14.6	0.61
Compost (Switzerland)	Comp-CH	Org	59.6	2.45	0.043	10.4	0.24
Compost (Germany)	Comp-DE	Org	57.2	1.42	0.67	n.d.	n.d.
Composted cattle manure (Italy)	C-MComp	Org	60.0	7.75	n.d.	22.7	n.d.
Composted horse manure (Italy)	H-MComp	Org	67.0	10.0	n.d.	14.2	n.d.
Sewage sludge (Germany)	SS-DE	Org	99	38.1	0.52	n.d.	n.d.
Sewage sludge (Denmark)	SS-DK	Org	23.0	30.4	1.5	n.d.	n.d.

n.d. means not determined

wastes, composted cow and horse manures, and sewage sludge). They were characterized by the local laboratories (Table 4) for their dry matter content, total P content, water extractable P, total potassium (K) and N content, and their ammonium N content.

Pot experiment setup, growing conditions, and harvest

After sieving, the soil was mixed with quartz sand (0.6–1.2 mm) in the ratio 2:1 or 1:1 (soil dry weight (DW)/sand DW; W/W). Each pot contained the equivalent of 2.5 kg or 5 kg DW of the experimental substrate (Table 2). All pots received N (100 mg N kg^{-1} substrate) and K (166 mg K kg^{-1} substrate) in form of calcium nitrate and Kalimagnesia, respectively. Where alternative P treatments were applied, N and K were measured and reduced from the basal dose of N and K (except for the German and the Danish trials where the addition of N and K was not reduced).

The N, K, and P fertilizers were added individually to every pot, and water addition was adjusted to reach 60–70% of the substrates water holding capacity (WHC). Three seeds were sown per pot, and the BE suspension was added in the seeding hole (5 ml per seeding hole = 15 ml per pot). BE suspensions were prepared under sterile conditions by diluting the products with 2.5 mM $CaSO_4$. The surface of the pots was then covered by a fine layer of quartz sand to avoid formation of surface crusts after watering.

The pots were transferred to growth chamber or greenhouse (see Table 2) and watered according to plant's need in order to keep the initial WHC (increased to 70–80% after two weeks). Thinning (including the root systems) was performed one week after sowing, leaving one plant in each pot. During the growth period, plant height was measured every second week and final harvest took place 8 weeks after sowing. Any deviation from this standard protocol (size pot, growing period, number of plant per pot, standard P fertilizers) is described in Table 2. Every treatment was replicated five times (or at minimum three times, see Table 2), and pots were randomly distributed in the growth chambers or greenhouses. The guidelines for establishing the pot experiments were common to all soils, and deviations to experimental setup are due to particular constraints of the different involved groups.

Plant analyses

At harvest, shoots were collected, dried (65 °C), and stored for further analyses. Root systems were washed from the substrate, and a subsample was kept (only in Swiss and Italian soils) to assess percentage root length colonization (RLC %) by arbuscular mycorrhizal fungi (AMF) following the protocol of Phillips and Hayman [43] and Brundrett et al. [44]. The remaining root was weighed and dried for total root DW assessment. Dried shoot material was milled for later determination of total N and P concentration. Shoot nitrogen concentration was measured using CHN analyzers, while shoot P concentration was determined spectrophotometrically or using flow injection analysis (Danish experiment) after acid extraction.

Data analyses and statistics

Residuals of the data (shoot DW, N and P content in shoot and RLC %) were tested for normality using the Shapiro–Wilk test. For every soil, a two-way ANOVA was performed to test the significance of each factor

(P fertilizer and BE strain) and their interaction. Since this interaction was always significant and for the sake of the graphical representation (in Figs. 2, 3, 4, and 5), a one-way ANOVA was performed within each P treatment and for each soil to determine which BE treatment (BE1, BE2, or BE3) was significantly different from the un-inoculated controls (Tukey's test, $P < 0.05$). Due to differences in growing conditions (Table 2, lower part) leading to substantial differences in the aboveground biomass response among single experiments, we normalized the aboveground biomass, P, and N content relative to the negative control treatment (P0, BE0) in the following way (exemplified for biomass):

$$normalized\ biomass_{sample} = \frac{biomass_{sample}}{\overline{biomass_{control(P0,BE0)}}}$$

Furthermore, in order to quantify the relative effect of BE additions across soils and P fertilizer types, absolute values for shoot DW (g plant^{-1}) were converted to shoot biomass improvement (in %) for every BE (BE1, BE2, and BE3) calculated relative to the BE0 control. For this, we applied the following equation:

$$improvement_{\%} = 100 \cdot \left(\frac{biomass_{sample(P_x,BE_y)} - \overline{biomass_{sample(P_x,BE_0)}}}{\overline{biomass_{sample(P_x,BE_0)}}}\right)$$

A value of 0% means that the shoot biomass of maize inoculated with the BE strain in question did not improve shoot biomass as compared to the un-inoculated control (using the same P fertilizer), whereas a value below or above 0% indicates that the specific BE treatment led to a decrease or an increase in aboveground biomass, respectively. In order to investigate the relative effect of BEs across different experiments, we categorized the added P fertilizers as either organic or inorganic. Data from experiments conducted in the Czech Republic were not considered here as they only included inorganic fertilizers (TSP and RP). For each BE, we performed a separate two-way ANOVA on these data with "soil" and "fertilizer type" and their interaction as factors, in order to analyze if the effect of BE was dependent on soil and/or fertilizer type (Table 6).

For each fertilizer, the fertilizer efficiency (FE %) relative to TSP (positive control) was calculated as the mean efficiency measured in n replicated pots. The efficiency in individual pots was calculated as follows:

$$FE_{\%} = 100 \cdot \left(\frac{biomass_{sample} - \overline{biomass_{control(P0,BE0)}}}{\overline{biomass_{TSP}} - \overline{biomass_{control(P0,BE0)}}}\right)$$

Finally, the P uptake efficiency (PUE %) from the different fertilizers was calculated as follows:

$$PUE_{\%} = 100 \cdot \left(\frac{P\ content_{sample} - \overline{P\ content_{control(P0,BE0)}}}{\overline{P\ content_{TSP}} - \overline{P\ content_{control(P0,BE0)}}}\right)$$

Results

The aboveground biomass was highly variable across the experiments (Fig. 1). A two-way ANOVA on raw biomass data (Fig. 1) for the negative (P0) and positive controls (TSP) without addition of BE (BE0) yielded highly significant effects of soil (P < 0.001) and P fertilizer ($P < 0.001$), as well as a highly significant interaction between the two factors (P < 0.001). In five out of six soils, there was a significant positive effect of TSP addition on the aboveground biomass (Fig. 1, Tukey's test, $P < 0.05$). The Humpolec soil is the only soil not affected by TSP addition with regards to aboveground biomass production.

In absence of BE inoculation (BE0), plants responded differently to the addition of the various P fertilizers. This was true for normalized biomass (Fig. 2), normalized P content (Fig. 3), and normalized N content (Fig. 4). The most effective fertilizers were the P-enriched BOF slag (Vörden trial) and in general the composted material from green wastes (FrDi, Comp-CH, Comp-D (see Table 4 for abbr.)) and sewage sludges from Denmark (incinerated or not).

The following combinations of BE and P fertilizers resulted in a higher normalized shoot biomass (Fig. 2), when compared to the negative control treatment (BE0) receiving the same P fertilizer (treatments

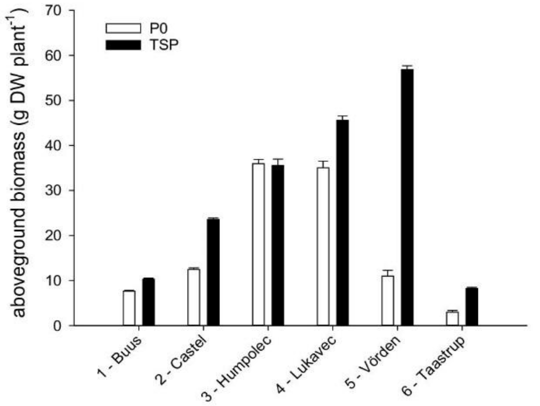

Fig. 1 Aboveground biomass of maize plant at harvest in the different trials: 1: Switzerland, 2: Italy, 3 and 4: Czech Republic, 5: Germany, 6: Denmark [without BE inoculation and either supplemented with Triple Super Phosphate (TSP) or not (P0)]. *Error bars* represent Standard Error of the Mean (SEM)

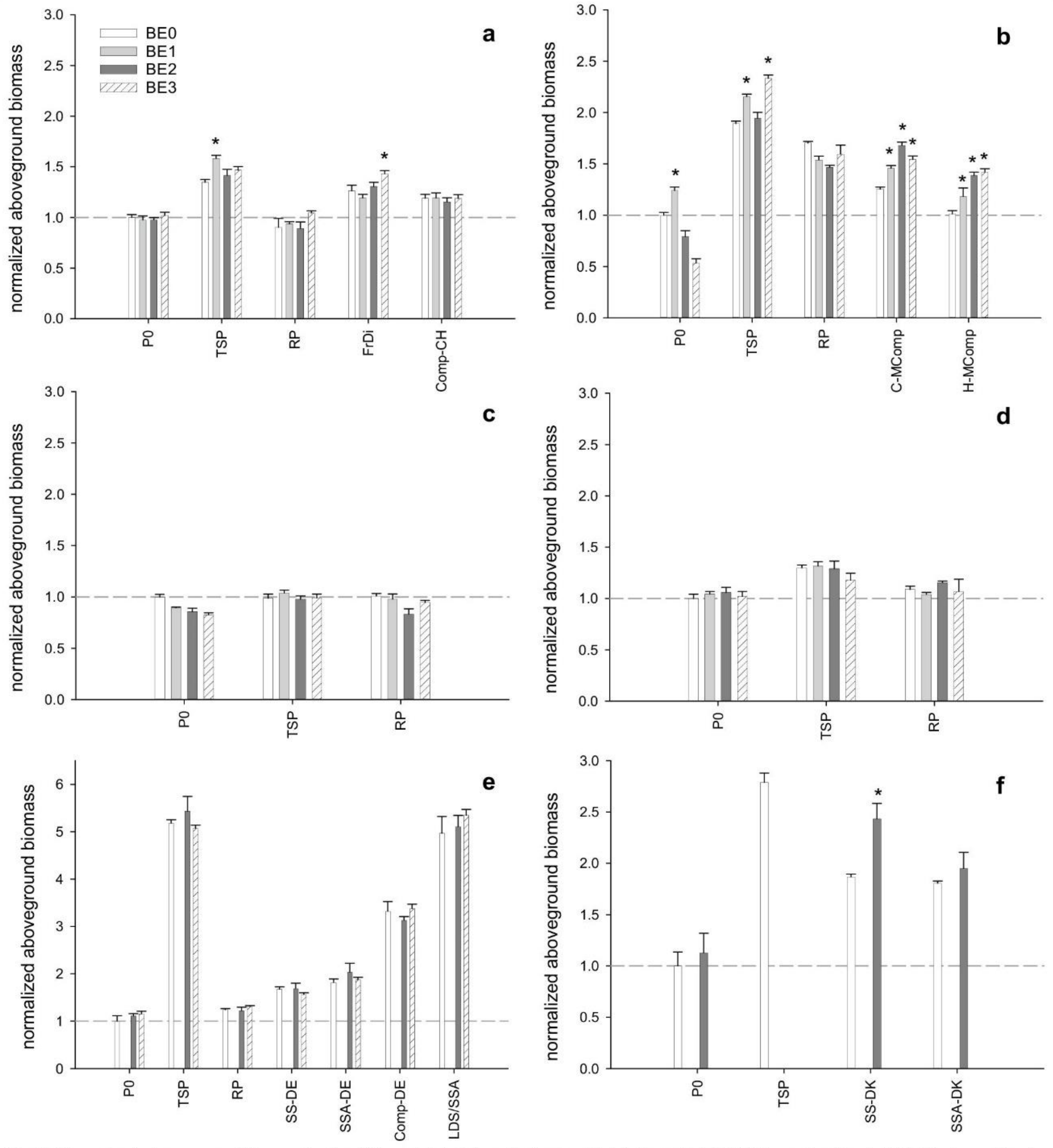

Fig. 2 Normalized aboveground biomass for the different trials: 1-Buus Switzerland (**a**), 2-Castel Italy (**b**), 3-Humpolec Czech R. (**c**), 4-Lukavec Czech R. (**d**), 5-Vörden Germany (**e**), 6-Taastrup Denmark (**f**). *Bars* represent the mean of five replicates ($n = 4$ for **e**, and $n = 3$ for **f**). *Error bars* represent SEM. BE treatments (BE1-3) resulting in a significantly higher normalized biomass compared to the BE0 control within each P fertilizer treatment and within each trial are indicated with an *asterisk* (*Tukey's test with $P < 0.05$). See Tables 3 and 4 for explanation of BE and P treatment abbreviations

highlighted with an asterisk): BE1 combined with TSP in the Buus (Fig. 2a) and Castel soils (Fig. 2b); BE2 combined with sewage sludge in the Taastrup soil (Fig. 2f); BE3 combined with fresh digestate and TSP in the Buus (Fig. 2a) and Castel (Fig. 2b) soils, respectively; and all BEs combined with composted animal manures

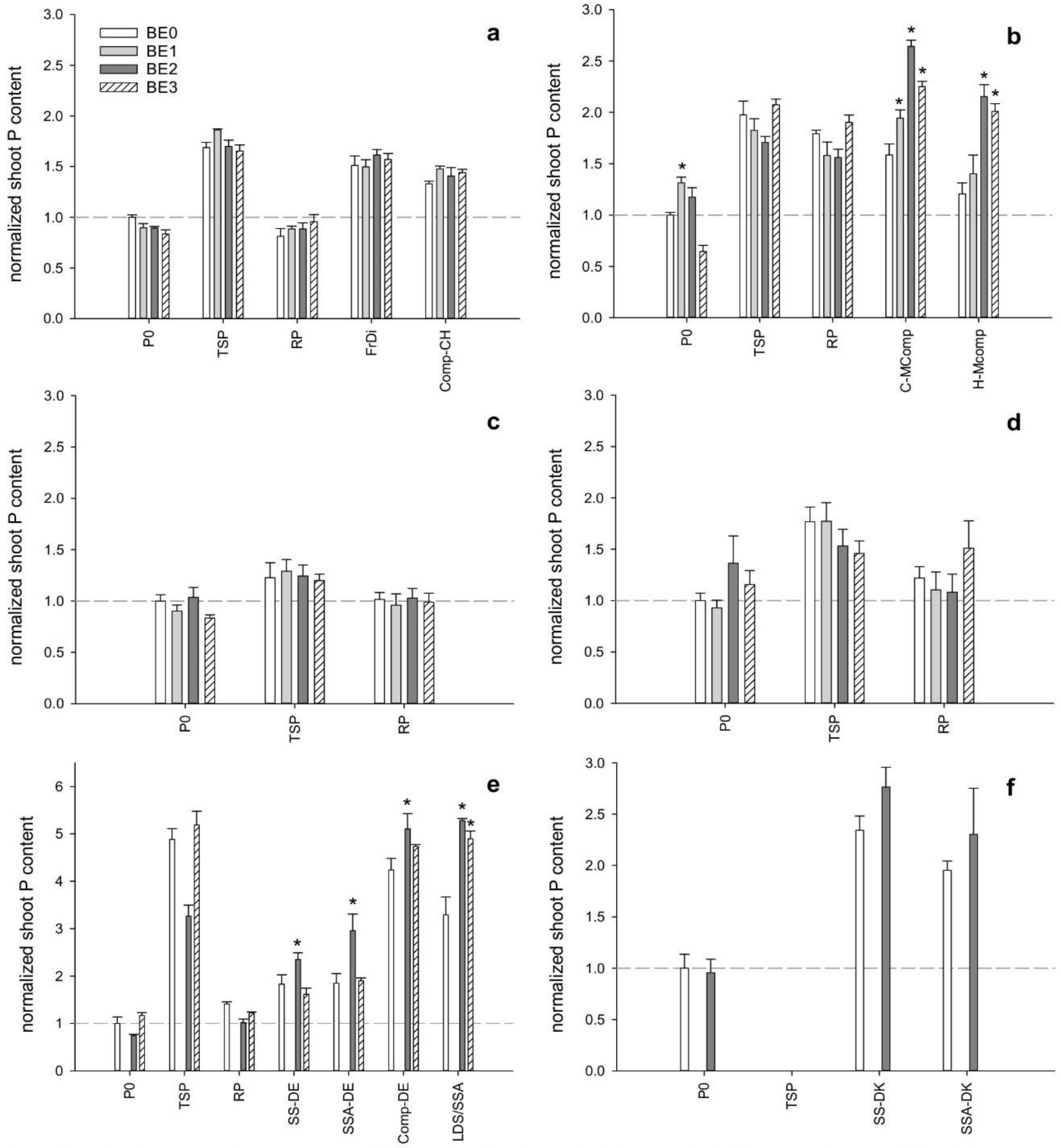

Fig. 3 Normalized aboveground P content for the different trials: 1-Buus Switzerland (**a**), 2-Castel Italy (**b**), 3-Humpolec Czech R. (**c**), 4-Lukavec Czech R. (**d**), 5-Vörden Germany (**e**), 6-Taastrup Denmark (**f**). *Bars* represent the mean of five replicates ($n = 4$ for **e**, and $n = 3$ for **f**). *Error bars* represent SEM. BE treatments (BE1-3) resulting in a significantly higher normalized P content compared to the BE0 control within each P fertilizer treatment and within each trial are indicated with an *asterisk* (*Tukey's test with $P < 0.05$). See Tables 3 and 4 for explanation of BE and P treatment abbreviations

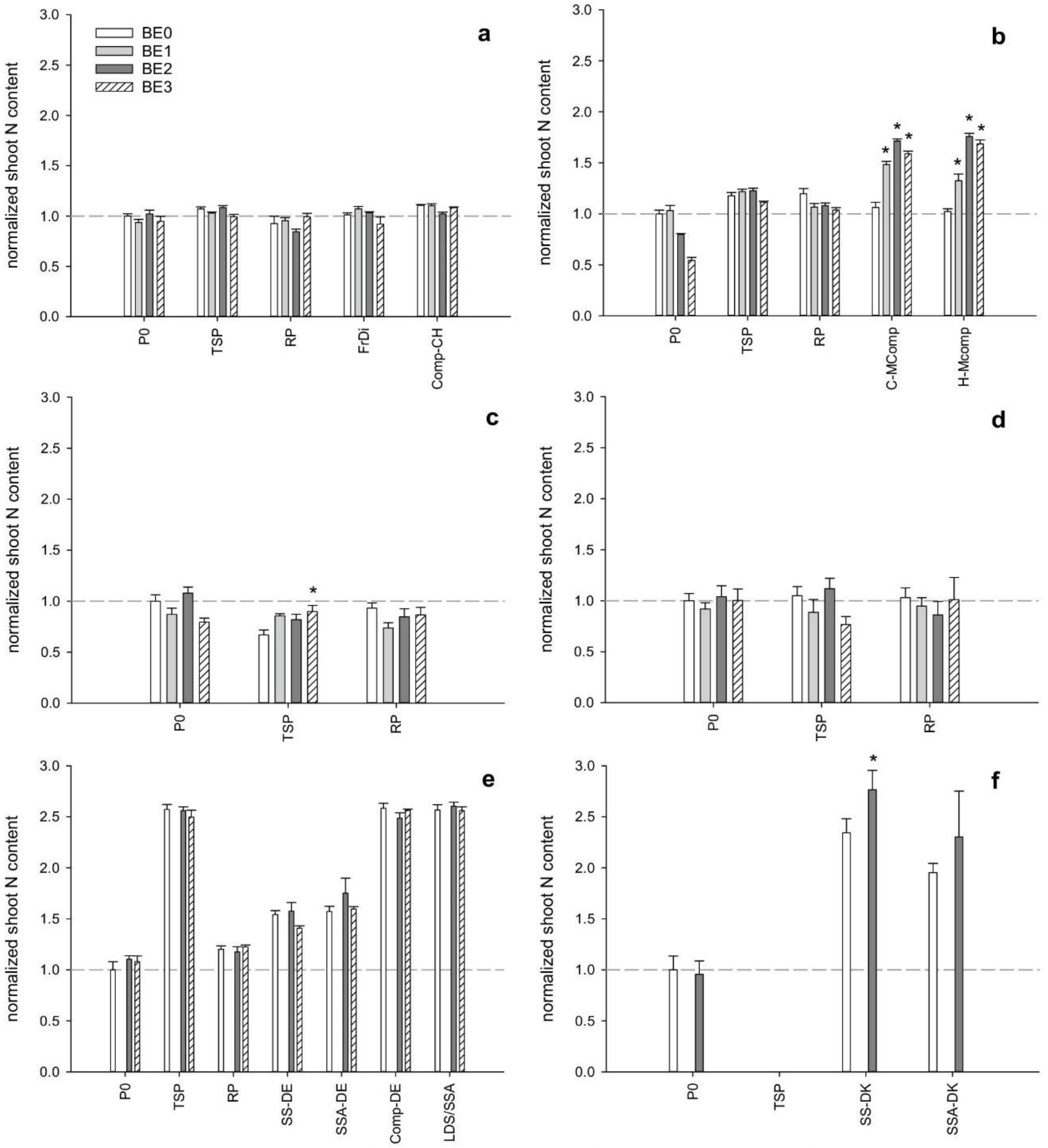

Fig. 4 Normalized aboveground N content for the different trials: 1-Buus Switzerland (**a**), 2-Castel Italy (**b**), 3-Humpolec Czech R. (**c**), 4-Lukavec Czech R. (**d**), 5-Vörden Germany (**e**), 6-Taastrup Denmark (**f**). *Bars* represent the mean of five replicates ($n = 4$ for **e**, and $n = 3$ for (**f**). *Error bars* represent SEM. BE treatments (BE1-3) resulting in a significantly higher normalized N content compared to the BE0 control within each P fertilizer treatment and within each trial are indicated with an *asterisk* (*Tukey's test with $P < 0.05$). See Tables 3 and 4 for explanation of BE and P treatment abbreviations

in the Castel soil (Fig. 2b). In the Humpolec, Lukavec, and Vörden soils, BE inoculation did not result in any increased normalized shoot biomass (Fig. 2 c–e).

Across the four experiments included in Table 6 (all except the two Czech experiments), BE inoculations induced an increase in shoot DW of 5–7.5%, as compared to un-inoculated controls (Table 6). For all three tested BEs, soil influenced the BE-derived increase in shoot biomass ($P < 0.01$). When looking across fertilizer types, the largest BE effects were observed in the Castel soil for BE1 (+12%), in the Taastrup soil for BE2 (+17%), and in the Buus soil for BE3 (8%). The factor "fertilizer type" significantly influenced the improvement in biomass when plants were inoculated with BE2 and BE3 (Table 6, $P < 0.0001$), but no such effect of "fertilizer type" was observed with BE1 (Table 6, $P > 0.05$). Furthermore, there was a significant interaction between "soil" and "fertilizer type" for all BE treatments (Table 6, $P < 0.0001$).

Across all soils, there was a significant positive effect of adding BE2 (+14%) and BE3 (+13%) when organic fertilizers were applied (Table 6, $P < 0.05$). For inorganic fertilizers, there was only a significant effect of BE3 (+7%, $P < 0.05$). When no fertilizer was added, none of the three BEs resulted in an improvement of the shoot biomass (Table 6, $P > 0.05$).

The normalized aboveground P content was significantly different between P fertilizers in all experiments (Fig. 3). In the Castel soil, inoculation with BE1 led to a significantly larger P content in the P0 and cow manure compost treatments, while inoculation with BE2 and BE3 resulted in an increased P content in both animal manure treatments (Fig. 3b). In the Vörden soil, inoculation with BE2 resulted in an increased P content when combined with any of the recycling fertilizers (Fig. 3e). In the remaining soils (Buus, Humpolec, Lukavec, and Taastrup), BE inoculation did not result in increased aboveground P contents. In the Castel soil, the higher P content due to BE2 addition (combined with cow and horse manures) is reflecting the significant increase in P concentration in the aboveground biomass (Table 7). BE3 increased P concentration only in the P0 treatment, while BE1 did not induce any P concentration increase. In the Vörden soil, where plants also accumulated more P in the aboveground biomass (Fig. 3e), P concentration was also higher (as compared to the corresponding BE0 control), but only when sewage sludge ashes were combined with BE2 and when P-enriched BOF slags were combined with BE2 or BE3.

The normalized aboveground N content (Fig. 4) showed less difference between P fertilization treatments than the normalized P content did (Fig. 3). Only in Vörden (Fig. 4e) and Taastrup (Fig. 4f) soils, a clear effect of P fertilizers on aboveground N content was observed.

In the Castel soil, the effect of P fertilization depended on BE addition since we only observed a significantly larger normalized aboveground N content when pots had been inoculated with BEs 1–3 (Fig. 4b). In the Taastrup soil, a significantly higher aboveground N content was observed in plants inoculated with BE2 and when sewage sludge was used as fertilizer. In the Humpolec soil, we found a significant increase in aboveground N content due to inoculation with BE3, when TSP was used as P fertilizer (Fig. 4c). However, the recorded N content (Fig. 4c) in this treatment (TSP/BE3) was numerically smaller than in the negative control (P0/BE0). In the remaining soils (Buus, Lukavec, and Vörden), BE inoculation did not result in increased aboveground N contents.

Intensity of RLC % by AMF was only measured in roots of plants growing in the Buus and Castel soils (Fig. 5). For un-inoculated treatments which were not amended with P fertilizer (BE0, P0), the two soils induced different

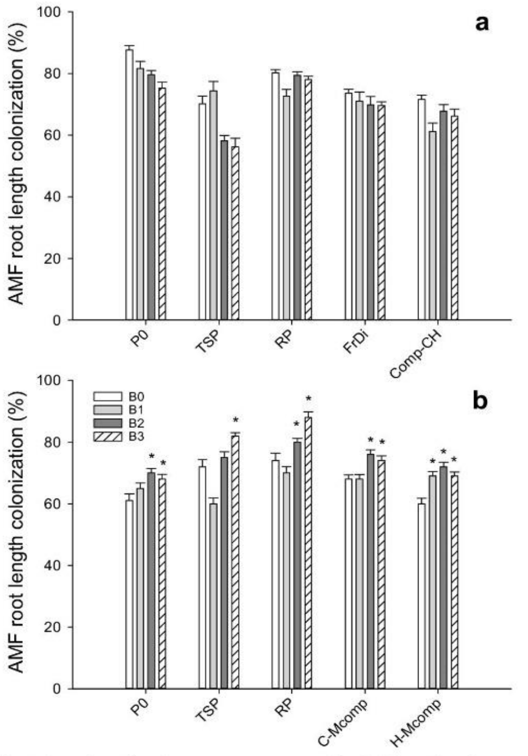

Fig. 5 Root length colonization percentage by AMF (in %) in Buus (**a**) and Castel soil (**b**). *Bars* represent the mean of five replicates. *Error bars* represent SEM. BE treatments (BE1-3) resulting in a significantly higher RLC % compared to the BE0 control within each P fertilizer treatment and within each trial is indicated with an *asterisk* (*Tukey's test with $P < 0.05$). See Tables 3 and 4 for explanation of BE and P treatment abbreviations

levels of RLC % (87% for the Buus soil and 60% for the Castel soil). In the Buus soil, BE inoculation leads to either no change in RLC % or a decrease, especially for plants amended with TSP (Fig. 5a). In contrast, the roots from the Castel soil had significantly higher RLC % in BE-inoculated pots, especially for BE3 (independent of P fertilizer applied) and for the combination of BE2 and animal manures (Fig. 5b).

Discussion

Variable levels of soil fertility and harvested aboveground biomass (P0, BE0 and TSP, BE0)

Due to variation in the experimental setup (e.g., pot size and growth duration), different aboveground biomasses were harvested ranging from 4 to 36 g per plant in the negative control treatment (P0, BE0). The different increases due to TSP addition (TSP, BE0) indicated that soils had varying initial fertility levels (Figs. 1, 2) with the Czech soils (Humpolec and Lukavec) being the less depleted in P. Plants growing in the Vörden (DE), the Taastrup (DK), and, to a lesser extent, in the Castel (IT) soil, were obviously P limited (increase between 2- to fivefold due to TSP addition), while the plants in the Buus (CH) soil showed a moderate P increase (1.6-fold). The same response to TSP addition was observed with spring wheat with the Vörden and Taastrup soils [45], where a similar experimental setup was used. The Taastrup soil originates from a long-term field trial where no nutrients have been added for approximately 50 years [46], thereby explaining the response to application of easily available P sources. The Castel soil was taken from an alkaline site where native P is largely bound in calcium complexes. The Buus soil is from an organic field site where the plant available P fraction is indeed low (P CAL = 6.5 mg P/kg), but with a large organic P fraction (data not shown) which likely contributed to P supply during the experiment causing a moderate response to TSP addition.

Variable P fertilizer effects (P, BE0)

We observed a range of responses due to the application of different P fertilizers. The calculated fertilizer efficiencies (Table 5) indicate that, in the absence of inoculated BEs, recycling fertilizers (organic or inorganic) were superior to the rock phosphate fertilizer (the Castel experiment was an exception). The fertilizer and P use efficiencies for sewage sludge, and sewage sludge ashes were quite similar within the same experiment but quite different between the two experiments where these products were included (16–22% for the Vörden experiment and 45–49% for the Taastrup experiment). This is in line with the data on water extractable P in the sludges where the level in the Danish sludge was three times higher than in the German sludge (Table 4). Furthermore, the availability of P in sewage sludge ashes can be quite different depending on the processing of the sludge in the water treatment plant [47].The very high fertilizer efficiency recorded for the P-enriched BOF slag has been observed earlier in wheat [45] and maize [42].

Moderate effects in the absence of added P (P0, BE1-3)

When no P was added, the three BEs neither improved aboveground biomass nor P uptake. In one case (Castel-IT), there was even a reduced aboveground biomass due to the application of BE2 and BE3. The absence of response after BE application was observed in many other experiments [45, 48, 49] and in particular in soils characterized by extraordinary low levels of available P (20 mg kg^{-1} or lower) or that were limiting in other soil nutrients [10, 50, 51]. In contrast, other studies have shown that for maize and other crops, the application of BEs was more efficient in the absence of added P fertilizers [36–38]. However, in these cases, soil P availability was distinctly higher and P CAL ranged between 35 and 45 mg/kg.

The observed absence of plant responses to BE applications when soils are extremely low in available P could be explained by direct growth limitation of the host plants, which finally limits sufficient production of roots and root exudates to initiate beneficial plant–microbial interactions with indigenous or inoculated soil microbes in the rhizosphere. This phenomenon is well documented, e.g., for mycorrhizal associations frequently absent on extremely nutrient-impoverished soils [52, 53] or for the establishment of the Rhizobium symbiosis in leguminous plants on low P soils which is promoted by starter applications with soluble P [54]. Moreover, in the absence of significant soil P pools that can be mobilized by BEs, their presence will have no beneficial effect on plant P supply, or may even be in competition with plants by immobilizing P in microbial tissue.

BE effects depend on soil and fertilizer type

In the present study, it was observed that for the three tested BEs, the soil strongly modulated the observed BE effect (Table 6). Various studies have shown that soil pH, soil organic carbon content, soil microbial activity, and soil nutrient status are all influencing the success of BE application [16, 25, 55–57]. Our findings indicate that the most responsive soils to BE application (in combination with P fertilizers) were either alkaline (Castel-IT) or depleted in soil nutrients (Taastrup-DK and Vörden-DE for P acquisition), confirming the influence of soil pH and soil nutrient statues on the effectiveness of BEs. The type of fertilizer used along with BEs was also significantly influencing the magnitude of BE effects (Table 6). Since all tested fertilizers were different (with the exception of

Table 5 Relative fertilizer efficiencies (FE) and P use efficiencies (PUE)

P fertilizer	Abbr.	1-Buus (Switzerland)		2-Castel Volturno (Italy)		3-Humpolec (Czech R.)		4-Lukavec (Czech R.)		5-Vörden (Germany)		6-Taastrup (Denmark)	
		FE (% of TSP)	PUE (% of TSP)	FE (% of TSP)	PUE (% of TSP)	FE (% of TSP)	PUE (% of TSP)	FE (% of TSP)	PUE (% of TSP)	FE (% of TSP)	PUE (% of TSP)	FE (% of TSP)	PUE (% of TSP)
Negative control	P0	0bc	0c	0d	0d	0a	0a	0b	0b	0d	0c	0c	n.a.
Triple super phosphate	TSP	100a	100a	100a	100a	100a	100a	100a	100a	100a	100ab	100a	n.a.
Rock phosphate	RP	−28c	−27c	79b	81ab	−70.1a	7a	30b	28b	6cd	11 c	n.a.	n.a.
Fresh digestate	FrDi	76a	74ab	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Composts	Comp-CH, Comp-DE	54ab	48b	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	55b	84 ab	n.a.	n.a.
Composted cow manure	C-MComp	n.a.	n.a.	28c	60bc	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Composted horse manure	H-MComp	n.a.	n.a.	2d	21cd	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Sewage sludges	SS-DE, SS-DK	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	16cd	21c	49b	n.a.
Sewage sludge ashes	SSA-DE, SSA-DK	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	20cd	22c	45b	n.a.
P-enriched BOF slag	LSD/SSA	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	95 a	59b	n.a.	n.a.

The efficiencies are calculated for the BE0 treatments only. Efficiencies are calculated relative to TSP as the positive control (see "Methods" section for details). For each column, different letters after mean values represent significantly different means (Tukey's test, $P < 0.05$)

n.a. means not applicable

Table 6 Shoot biomass improvement (%) of the different BE treatments relative to the un-inoculated control (BE0)

	Trianum-P (BE1)	Proradix (BE2)	Rhizovital (BE3)
Overall BE effect (across all treatments)	7.5%	5.2%	6.0%
Number of observations	50	87	78
BE effect in the different soils (across all fertilizers types)			
Buus (Switzerland)	2.7%b^{a}	0.4%b^{a}	8%a^{a}
Castel (Italy)	12.3%a^{a}	7.8%b^{a}	6.7%a^{a}
Vörden (Germany)	Not measured	3.4%ab^{a}	3.6%b^{a}
Taastrup (Denmark)	Not measured	17.0%a^{a}	Not measured
BE effect with the different fertilizer types (across all soils)^{b}			
No fertilizer added	10.8%a^{a}	−1.2%b^{a}	−11.5% b^{a}
Organic fertilizer	6.9%a^{a}	*14.0%a*^{a}	*13.0% a*^{a}
Inorganic fertilizer	6.4%a^{a}	1.5%b^{a}	7.3% a^{a}
ANOVA on the effect of soil, fertilizer type and their interaction			
Soil (*p* value)	0.0002 (*df* = 1)	0.0070 (*df* = 3)	0.0054 (*df* = 2)
Fertilizer type (*p*-value)	ns (*df* = 2)	<0.0001 (*df* = 2)	<0.0001 (*df* = 2)
Soil x fertilizer type (*p*-value)	<0.0001 (*df* = 2)	<0.0001 (*df* = 6)	<0.0001 (*df* = 4)

df means degree of freedom

See "Methods" section for description of the calculations

[a] Levels not connected by the same letter are significantly different (Tukey's HSD test $P < 0.05$)

[b] Underlined combinations of BE and P fertilizer type indicate when BE addition significantly improved shoot biomass as compared to the un-inoculated control with the same fertilizer type (*t*-test with $P < 0.05$)

Table 7 Aboveground P concentration (mg/g)

	BE0	BE1	BE2	BE3
1-Buus				
Control P0	1.57a	1.45ab	1.44ab	1.29b
TSP	1.97a	1.85a	1.89a	1.77a
RP	1.42a	1.49a	1.63a	1.44a
FrDi	1.87ab	1.97a	1.94a	1.72b
Comp-CH	1.76a	1.95a	1.91a	1.91a
2-Castel Volturno				
Control P0	0.96c	1.01bc	1.40a	1.15b
TSP	1.00a	0.81b	0.84ab	0.85ab
RP	1.00a	0.98a	1.01a	1.15a
C-MComp	1.20b	1.27b	1.50a	1.39ab
H-MComp	1.13b	1.12b	1.48a	1.35ab
3-Humpolec				
Control P0	1.03a	1.03a	1.26a	1.04a
TSP	1.28a	1.27a	1.29a	1.25a
RP	1.03a	1.01a	1.26a	1.07a
4-Lukavec				
Control P0	0.88a	0.78a	1.10a	0.98a
TSP	1.19a	1.19a	1.04a	1.11a
RP	0.99a	0.92a	0.82a	1.21a
5-Vörden				
Control P0	1.12a	n.a.	0.76b	1.14a
TSP	1.06a	n.a.	0.68b	1.15a
RP	1.28a	n.a.	0.94b	1.05b
SS-DE	1.23ab	n.a.	1.58a	1.16b
SSA-DE	1.15b	n.a.	1.62a	1.14b
Comp-DE	1.45a	n.a.	1.84a	1.58a
LSD/SSA	0.75b	n.a.	1.17a	1.03a
6-Taastrup				
Control P0	1.99a	n.a.	1.71b	n.a.
SS-DK	2.49a	n.a.	2.28a	n.a.
SSA-DK	2.15a	n.a.	2.31a	n.a.

For each row, means which are followed by different letters are significantly different (Tukey's HSD test $P < 0.05$, performed within each P treatment and for each soil)

n.a. means not applicable

TSP and RP that were tested in almost all experiments), we categorized them in two types (organic or inorganic) in order to investigate if there were some more general trends in the interaction between P fertilizer applied and the effect of the three BEs across the experiments. Clearly, BE2 and BE3 (both bacterial) applied with organic fertilizers were the best combinations and in particular when these BEs were combined with composted animal manures (for BE2 and BE3) or with the Danish sewage sludge (for BE2). In the Vörden soil, sewage sludge was also combined with BE2. But here, BE2 failed to exert any effect on the obtained biomass, showing again the complexity of these processes and why the interaction between soil and fertilizer type was significant in the performed two-way ANOVA (Table 6). In two cases (Buus and Castel soils), BE1 (fungal) was able to increase the aboveground biomass when combined with TSP, indicating that the three BEs have likely different modes of action (see below). The study of Lekfeldt et al. [45] indicates that, to some extent, these effects and interactions are also crop specific since they did not observe any positive effects of BE1-3 on plant growth or P uptake when wheat was fertilized with recycling fertilizers.

Possible modes of action

Several studies [27, 39, 58] have shown that, in in vitro conditions, many BEs are able to dissolve insoluble tricalcium phosphates by releasing protons and/or organic acids (e.g., gluconic acids), decreasing the pH of the growth medium and/or act as cation chelators (Al, Fe, and Ca) [59]. Nevertheless, in pot experiments where soil low in available P is used as the growth substrate, this solubilization ability was limited, as almost none of the BEs could increase plant P content in P0 and RP treatments, with the exception of BE1 in the alkaline Castel soil (P0 treatment,). This limited solubilization ability under in situ soil conditions was also observed in other studies [27, 45, 49] and may be explained by the soil buffering capacity that inhibits the BE-induced soil acidification [33] that otherwise would have favored solubilization of calcium phosphates from soil and/or RP. However, we observed that in the Vörden soil, the amendment of sewage sludge ashes or P-enriched BOF slags (both inorganic fertilizers containing P in calcium phosphates complexes) increased plant P uptake when they were inoculated with BE2 or BE3 (Fig. 3e). Since this was not associated with increased N content in shoot (Fig. 4e), we can exclude that the better acquisition of P is the result of improved root growth. Rather, it is possible that the higher P content and concentration (Fig. 3e and Table 7) measured in the shoots are indeed the result of P solubilization (from these fertilizers), induced by BEs and in particular BE2. Yet, it remains to be understood why the biomass of these plants was not larger than the BE0 control and why the same BE could not solubilize P when combined with rock phosphate in the same soil.

Inoculated plants with higher biomass, P, and N contents (in the Castel soil, BE1-3 with composted animal manures; or in the Taastrup soil, BE2 with sewage sludge) could result from BE-induced mineralization of organic P and N contained in the tested organic fertilizers. For P, several studies have shown that phosphatase activities (acid and alkaline) measured in soil can be increased upon inoculation with BEs [16–18, 60]. Although it was not measured in our experiments, we argue that increased mobilization of P from organic fertilizers via higher phosphatase activities is likely one of the major mechanisms behind the successful BE-organic fertilizer combinations observed in this study. Nevertheless, plants can also produce phosphatases, and therefore BEs may also have induced growth of more roots and these may in return have produced more of these enzymes. Indeed, the fact that BEs combined with TSP lead in some cases to higher aboveground biomass suggests that BEs can increase root growth and therefore the volume of soil explored for nutrient uptake. This mechanism has been described in several studies [61–64] and results from the direct or indirect production of root growth regulators such as auxin.

The volume of soil explored for nutrient uptake can also be increased via higher root length colonization percentage (RLC %) by AMF. In this study, the only roots analyzed for RLC % were from the experiments with the Buus and Castel soils. In the latter case, an increase in RLC % after BE inoculation was recorded with or without application of P fertilizers (Fig. 5b). This mycorrhizal boosting effect is known for several microorganisms [65–67] and in particular for BE2 [68]. In the Castel soil, the increased RLC % due to BE inoculation in combination with the P fertilization treatments P0, RP, and TSP did not necessarily translate into higher biomass or acquired P. This indicates that the mycorrhizal boosting effect of BE inoculation in that soil was of minor importance for plant P uptake and growth, although we do not know to which degree it contributed to the improvement in P uptake and growth for the plants inoculated with the three BEs and amended with the animal manures. In the Buus soil (Fig. 5a), RLC % was not increased after BE inoculation. This can be explained in part by the fact that roots were already highly colonized (almost 90%). But it also seems that in some combinations, BE inoculation had a negative impact on RLC % under these conditions, and this could be due to competition. Yet, the clear mechanisms remain to be explained.

Conclusion

We have observed that in our experiments, the most responsive soils for the three tested BEs were either alkaline or highly depleted in nutrients, and that a positive effect of the BEs was dependent on an input of P fertilizer under these conditions. Of all tested fertilizers, the combination of BE with organic fertilizers (in particular composted animal manures) was the most promising. Nevertheless, the combination of BE and organic fertilizers was not always improving biomass production or P uptake (e.g., Buus soil). The next challenge will be to understand what properties of organic fertilizers make them more compatible with a given BE and how this interacts with soil parameters, such as soil pH, soil microbial activities, organic matter content, and soil P and N status. Regarding the mechanisms, the results from this study suggest that the three BEs likely improved root growth (volume of soil explored for nutrients uptake) and P mobilization via accelerated mineralization, whereas chemical P solubilization likely took place in very few cases. Although higher mycorrhizal RLC% were observed upon inoculation with the three BEs in the alkaline soil of Castel, it remains to be determined if this was the principal mechanism contributing to improved P acquisition and aboveground biomass in that experiment.

Clarification of mechanisms together with new experiments with more diverse soils and characterization of BE-compatible fertilizers will give further insights about the window of conditions leading to successful use of BEs in agriculture. Nevertheless, our results show which kind of soils and fertilizers type is more responsive to BE inoculation.

Authors' contributions

The study was elaborated by PM, GN, AP, AdN, MK, MR, VC, MW, and CT. The experiments were performed by SS, CT, CM, FW, MR, MK, JSDL, and VC. The manuscript was written by CT, JSDL, and DK. All authors read and approved the final manuscript.

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Competing interests

All authors confirm that they have no competing interests in any of the products tested in this work, or any results presented in the manuscript.

Availability of data and materials

Raw data may be available on request to the authors, please contact corresponding author.

Consent for publication

All the authors consented for publication.

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16) Evaluating of soil sulfur changes under different fertilizing systems during long-term field experiments

Vyhodnocení změn různých forem síry v půdě během dlouhodobých pokusů s různými systémy hnojení

Síra se v posledních desetiletích stává limitující živinou ve výživě rostlin. To je způsobeno poklesem vstupu síry do půdy prostřednictvím atmosférických depozic, menší měrou využití minerálních hnojiv obsahujících S jako balastní látku a v neposlední řadě pěstováním výkonnějších odrůd kulturních plodin náročnějších na živiny včetně síry (Eriksen 2005, Lehmann et al. 2008, Scherer 2009). Proto byla vypracována tato publikace zaměřená na změny obsahů síry v dlouhodobých pokusech s různými systémy hnojení. Dva základní cíle byly následující: 1) v průběhu 18 let pokusu (1996-2014) sledovat na kontrolních nehnojených variantách vývoj obsahu různých frakcí síry v půdě, 2) ve stejném období posoudit vliv různých systémů hnojení (hnůj, čistírenské kaly, minerální NPK) z hlediska změn obsahu S v půdě. K dosažení objektivních výsledků byla zvolena dvě kontrastní stanoviště (kambizem a černozem) a zároveň byly sledovány 3 různé frakce minerální síry. Na obou stanovištích je patrný postupný pokles obsahu síry v půdě na kontrolní nehnojené variantě, který je nejvýraznější v období 1996-1999, kdy ještě dozníval vliv zvýšených atmosférických depozic z předchozích let. Dále je rovněž patrný výrazný vliv různých systémů hnojení. Zatímco čistírenské kaly se ukázaly být dobrým zdrojem přístupné síry v půdě, hnojení minerálním NPK působilo pozitivně na hladinu síry stanové 1 mol/l HCl. Příčinou této situace byla pravděpodobně síra dodaná v trojitém superfosfátu (1 % S), sloužícím u varianty NPK jako zdroj fosforu. Aplikace chlévského hnoje naopak nevedla k výrazným změnám oproti kontrolní variantě, ačkoliv dosažené hodnoty zde byly téměř ve všech případech ve srovnání s kontrolou vyšší. Obecně lze na základě výsledků tvrdit, že sledované formy síry v půdě korespondovaly s dodanými obsahy S pouze částečně.

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Evaluating of soil sulfur forms changes under different fertilizing systems during long-term field experiments

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ABSTRACT

The aim of this work was to evaluate the changes of different soil sulfur forms during long-term field experiments supplied with sewage sludge (SS), farmyard manure (FYM) or mineral fertilizers. The experiment was established at three different sites with the crop rotation of potatoes, wheat and barley. Fertilizing system was based on the same nitrogen dose per the whole crop rotation. Soil samples from the beginning of the experiment (1996) and at the end of each crop rotation were analysed after all three crops harvest. Similar tendencies of changes in water extractable (S_w), adsorbed (S_{ads}), 1 mol/L HCl estimated (S_{HCL}) sulfur and their sum of (ΣS) related to the fertilizing systems were observed among all studied sites. A decreasing tendency of ΣS in soil was observed during the experiment (except for the year 2005). The biggest decrease was always visible compared to control treatment. Mineral fertilizers (S added with superphosphate) appeared to be the best source of S_{HCL}. Application of SS increased the S_w content in soil samples after potatoes harvest, whereas a decreasing tendency among the following crops was visible. Contents of other S forms (S_w at control, FYM and mineral fertilizers included) were usually balanced during the crop rotation.

Keywords: plant nutrient; fertilization; sulfur fractions; sulfur deficiency; essential element

For the future, it is important to further understand the influence of fertilization on the dynamics of plant nutrients in the soil as well as its influence on the plant growth. The content of bioavailable nutrients in soils varies not only depending on the fertilizer used, but also on the seasonal influence, soil properties and many other factors. Therefore, the data of the long-term field experiments provide valuable information for optimizing fertilization applications (Johnston 1997, Scherer 2001, Hejcman et al. 2012, Neugschwandtner et al. 2014).

Mineral sulfur (S) deficiency is an actual problem in many sites (Eriksen 2005, Lehmann et al. 2008, Balík et al. 2009, Scherer 2009 and others). The content of mineral sulfur forms in soil rapidly decreased in last 25 years (Balík et al. 2009, Scherer 2009). Although sulfur has been known as an essential element for plant growth for over two centuries, intentionally S fertilizing was not realized before 1980's. At that time the most of the cultivated plants did not show evidence of S deficiency, because of air pollution from industry, intensive application of mineral fertilizers containing S as the ballast substance, application of manure and cropping of plant cultivars with lower S uptake in crop rotation (Zhao et al. 1999, Scherer 2001, Blair 2002).

From the mentioned reasons it may be expected that sulfur becomes a limiting factor for yield formation and product quality (Eriksen et al. 2004). Therefore, it is necessary to pay attention to the sulfur fertilization possibilities under different soil-climatic conditions.

The total soil sulfur appears in two basic forms: (i) organic and (ii) inorganic. Inorganic sulfur, on which is mainly focused this manuscript, represents usually less than 10% from total S (Tisdale et al.

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1993, Wang et al. 2006, Förster et al. 2012) and appears in different forms. The most important form for S uptake by plants is bioavailable sulfate (SO_4^{2-}) in the soil solution. This form represents usually only about 1% of total S (Knauff 2000). Another form is sulfate adsorbed to weak binding sites on the surface of soil particles. It directly influences the perseveration of the sulfate balance with the soil solution (McLaren and Cameron 1996). The most stable mineral S form represents sulfur co-precipitated with calcium and magnesium carbonates (Tisdale et al. 1993, Chen et al. 1997). Mentioned forms are possible to estimate using different sequential extraction methods, for example after Shan et al. (1992).

Therefore, the aim of this work is to evaluate the changes of water extractable (S_w), adsorbed (S_{ads}) and 1 mol/L HCl estimated (S_{HCL}) sulfur forms under different fertilizing systems (sewage sludge (SS), farmyard manure (FYM) and mineral fertilizer) compared to the zero control during the long-term field experiments.

MATERIAL AND METHODS

The long-term field experiment was established at experimental fields of the Czech University of Life Sciences in Prague and Crop Research Institute in Prague-Ruzyně (Czech Republic). The basic characteristics of the sites are given in Table 1.

Potatoes, winter wheat and spring barley were cropped in crop rotation on small plots (60 m^2). All three crops were cropped parallel each year of the experiment. Organic fertilizers were applied only to the potatoes in crop rotation. The total amounts of N, P, K, Ca, Mg and S added per one crop rotation in different fertilizing treatments are summarized in Table 2.

For this experiment, SS from a wastewater treatment plant in Prague-Troja was used. The whole system of fertilizing is based on the uniform amount of 330 kg N/ha per 3 years (expect of unfertilized control).

Archive air-dried soil samples (< 2 mm) from the beginning (year 1996) of the experiment and from the end of each crop rotation (years 1999, 2002, 2005, 2008, 2011 and 2014) were chosen for analysis. All samples were taken after harvest of each single crop in crop rotation and before fertilizers application. Each sample was analysed in two replications. The samples were extracted to obtain water extractable, 0.032 mol/L NaH_2PO_4 extractable adsorbed and 1 mol/L HCl extractable sulfur fractions (Morche 2008, Kulhánek et al. 2011). All extracts were measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Varian VistaPro, Mulgrave, Australia). The content of total estimated sulfur (ΣS) was calculated like a sum of S_w, S_{ads} and S_{HCL}.

For the basic statistical evaluation and excluding the outlaying observations, Statistica software was used (StatSoft Inc. 2015). Thereafter, the tendencies in sulfur fractions changes were evaluated in Microsoft Office Excel (Excel 2007).

RESULTS AND DISCUSSION

The contents of different sulfur forms at the beginning of the experiment (year 1996) are mentioned in Table 3. The values of S_w were very similar among the studied sites (about 11 mg S_w/kg). The amounts of S_{ads} ranged between 5.97 and 9.44 mg

Table 1. Characteristics of experimental fields

	Humpolec	Hněvčeves	Suchdol
Latitude	49°33'15''N	50°18'46''N	50°07'40''N
Longitude	15°21'02''E	15°43'3''E	14°22'33''E
Altitude (m a.s.l.)	525	265	286
Mean yearly temperature (°C)	7.0	8.2	9.1
Mean yearly rainfall (mm)	665	573	495
Soil type	Cambisol	Luvisol	Chernozem
Soil texture	sandy loam	clayey loam	sandy loam
pH[1]	5.1	5.9	7.5

[1]Estimated in 0.01 mol/L $CaCl_2$, 1:10 w/v

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Table 2. Application rates of macronutrients (kg/ha) – 3-year cycle

Treatment		Potatoes	Wheat	Barley
Control	N, P, K, Ca, Mg, S	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Sewage sludge	N	330[1]	0	0
	P	201[1]	0	0
	K	55[1]	0	0
	Ca	268[1]	0	0
	Mg	70[1]	0	0
	S	81[1]	0	0
Farmyard manure	N	330[1]	0	0
	P	70[1]	0	0
	K	352[1]	0	0
	Ca	250[1]	0	0
	Mg	30[1]	0	0
	S	73[1]	0	0
Mineral fertilizers[2]	N	120	140	70
	P	30	30	30
	K	100	100	100
	Ca, Mg	36, 0	42, 0	21, 0
	S	6.5	6.5	6.5

[1] P, K, Ca, Mg and S dose was calculated based on the average nutrient content in organic fertilizers. N application rate was based on the fertilizers analyses. [2] Mineral fertilizers used: N – calcium ammonium nitrate (27% N); P – triple superphosphate (21% P, 3% S); K – potassium chloride (50% K)

S_{ads}/kg. The biggest differences were found in the S_{HCL} contents, where the values reached from 5.35–22.8 mg S_{HCL}/kg. The highest S_{HCL} contents (Suchdol and Hněvčeves sites) are also reflected in the differences among the ΣS contents. Due to the used ICP-OES method to estimate different sulfur forms, it has to be taken into account that not only inorganic sulfur forms, but also some organic S compounds were estimated (Kowalenko and Grimmett 2007). For this reason, the better way is to use ion chromatography like e.g. in Förster et al. (2012). Anyway, the ICP-OES method was used, because for our purposes it can be successfully used as well (Kowalenko and Grimmett 2007, Morche 2008, Kulhánek et al. 2011).

In the further evaluation of the experiment (in order to eliminate the plots variability) the data from the year 1996 (before fertilizing) were converted to 100%. The data from the following years were then compared with the year 1996. For the better transparency, the average contents of studied sulfur forms were calculated for each evaluated year. The changes of estimated soil sulfur forms related to different crops in crop rotation and terms of the fertilizers application are mentioned further.

Figure 1 shows the changes in estimated sulfur fractions at Suchdol site. The S_w content was always the highest in the SS treatment. This treatment is followed by fertilizing with FYM. It was probably

Table 3. The contents of estimated sulfur forms in the year 1996 (mg S/kg soil)

	S_w	S_{ads}	S_{HCL}	ΣS
Suchdol	10.40 ± 0.98	7.46 ± 0.72	22.8 ± 4.64	40.7 ± 5.00
Humpolec	12.3 ± 0.77	9.44 ± 0.72	15.6 ± 1.03	37.3 ± 1.75
Hněvčeves	10.5 ± 0.76	5.97 ± 0.38	5.35 ± 0.54	21.8 ± 1.19

S_w – water extractable; S_{ads} – adsorbed; S_{HCL} – 1 mol/L HCl estimated; ΣS – total estimated sulfur

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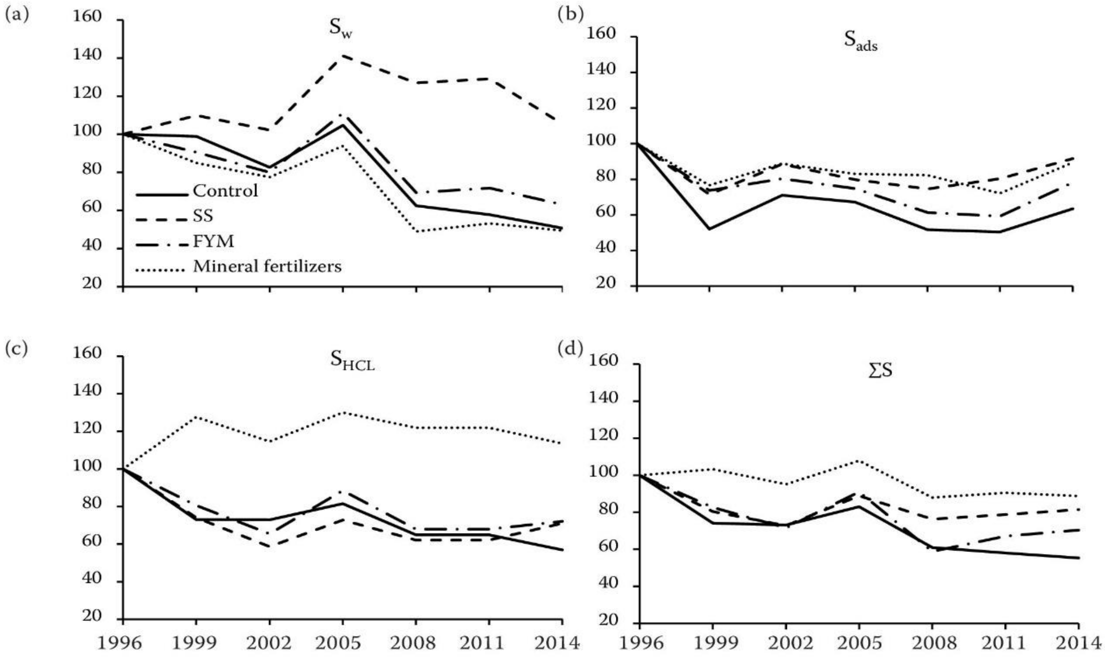

Figure 1. Changes of sulfur contents (%) at Suchdol site between 1996 and 2014. S_w – water extractable; S_{ads} – adsorbed; S_{HCL} – 1 mol/L HCl estimated; ΣS – total estimated sulfur

due to the slightly higher S dose applied using SS and better SS mineralization described further.

The lowest contents of S_w were always found in the treatment fertilized with mineral fertilizers. It is the treatment where the highest yields of all studied crops were reported (Černý et al. 2010). Therefore, the higher yields probably resulted in the higher sulfur uptake by plants and lower contents of S_w in comparison to the control treatment. Furthermore it is possible to assume that sulfate applied with mineral fertilizers is more prone to leaching and that S sequestration depends on both the fertilizer type and S application rate (Förster et al. 2012). This was confirmed at Hněvčeves site as well, but not at Humpolec site. The S_w and S_{ads} contents estimated at Humpolec site were almost always higher compared to Suchdol and Hněvčeves. Therefore the bioavailable sulfur content at Humpolec site was probably more sufficient to cover the plant uptake and unused sulfur added with mineral fertilizers caused the higher values of S_w compared to control.

The contents of S_{ads} were relatively low and ranged between 3.80 and 10.6 mg S_{ads}/kg. There were no big differences between the studied fertilizing treatments. The lowest S_{ads} contents were always found in the control treatment without fertilizing. The rest of the studied treatments showed almost the same tendencies. It can be partially explained due to the fact, that SO_4^{2-} adsorption is strongly pH-dependent and increases with decreasing pH (Förster et al. 2012). In our case, the lowest pH after 14 years of experiment was measured in the control treatment at Suchdol site. Similar results were observed at Lukavec site (soil-climatic conditions are comparable with Humpolec site) where the lowest pH values were measured at mineral fertilizers and control treatment. Only at Hněvčeves site, the highest pH decrease was observed at mineral fertilizers treatment followed by SS and control (Vašák et al. 2015). The second reason for the lowest S_{ads} contents in the control could be the lowest S input at this treatment. Location and climatic conditions of different sites play important role as well (Förster et al. 2012).

The S_{HCL} form in soil showed interesting tendencies. The amounts of S_{HCL} among the FYM, SS and control treatment were very similar during the studied period, but the highest content was always found in the treatment fertilized with mineral fertilizers. This is probably due to the fact that almost whole sulfur added in the form of superphosphate (6.5 kg S/ha/year) was in the calcium sulfate form. This tendency was confirmed at all studied sites. Suchdol site has about 2.5% of carbonates; sulfur estimated using 1 mol/L HCl was probably mostly in Ca and Mg coprecipitated form. However, the results of Morche (2008) show that soils with less than 1% $CaCO_3$ provide evidence that most of the S extracted with 1 mol/L HCl is organically bound sulfur. Moreover, some other HCl soluble minerals (e.g. Fe and Al oxides and sulfates), mainly in non-calcareous, acidic soils can contribute to this S fraction as indicated by Chen et al. (1997).

The sum of estimated sulfur forms was influenced by the high amount of S_{HCL} in the mineral fertilizers treatment. There is a visible decreasing tendency of ΣS content in soil during the experiment, especially in the control treatment. It can be explained by the fact that the sulfur inputs to the soil strongly decreased at the end of 20th century (Balík et al. 2009, Scherer 2009).

Figure 2 shows the tendencies in mineral soil sulfur changes at Humpolec site during the experiment. The highest S_w contents were found in the SS treatment. This treatment was again followed by FYM. Compared to Suchdol site, the lowest S_w content was always obtained in the control treatment; probable reasons are discussed above.

SS also appeared as a good source of S_{ads}. Only in this treatment, the level of S_{ads} was higher in the end of the experiment compared to its beginning. The differences among following fertilizing treatments (control, FYM and mineral fertilizers) were not clearly visible during the whole experiment.

The average content of S_{HCL} (calculated for control) during the whole experiment was 10.5 mg S_{HCL}/kg. It is the value about 7.5 mg S_{HCL}/kg lower compared to Suchdol site. The soil from Humpolec site contains less than 0.35% of carbonates. As discussed before, estimated sulfur was probably mainly in organic form (Morche 2008). Nevertheless, the S_{HCL} contents were always the highest in mineral fertilizers treatment in each studied site and year. Therefore, it is assumed that the differences between this treatment and others were mainly caused

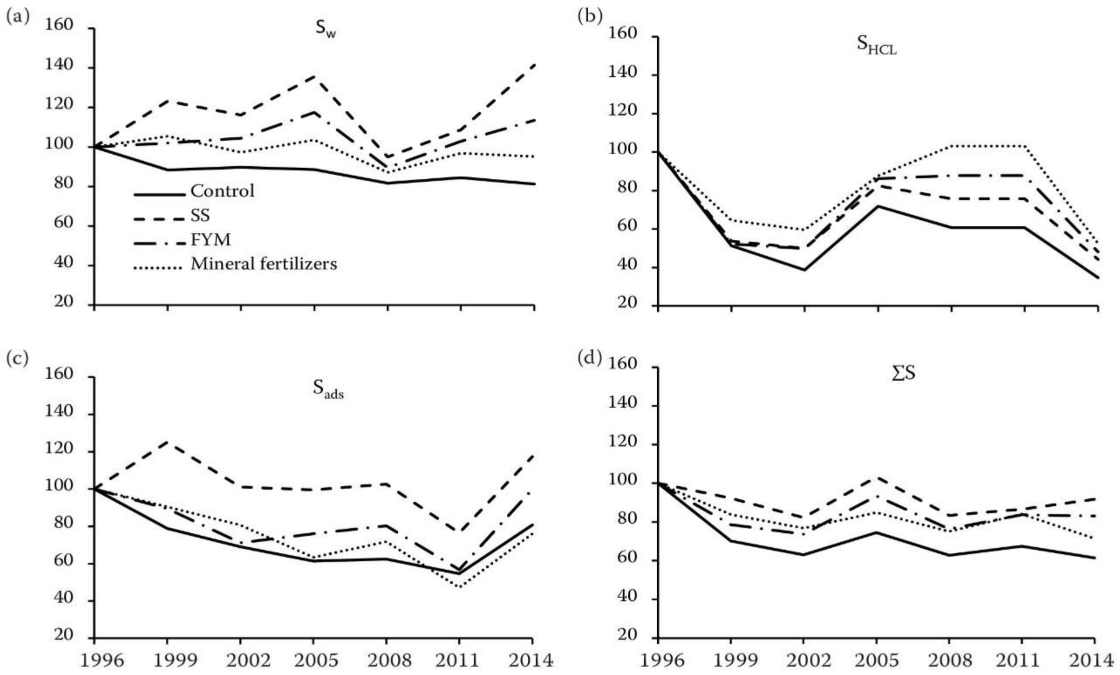

Figure 2. Changes of sulfur contents (%) at Humpolec site between 1996 and 2014. S_w – water extractable; S_{ads} – adsorbed; S_{HCL} – 1 mol/L HCl estimated; ΣS – total estimated sulfur

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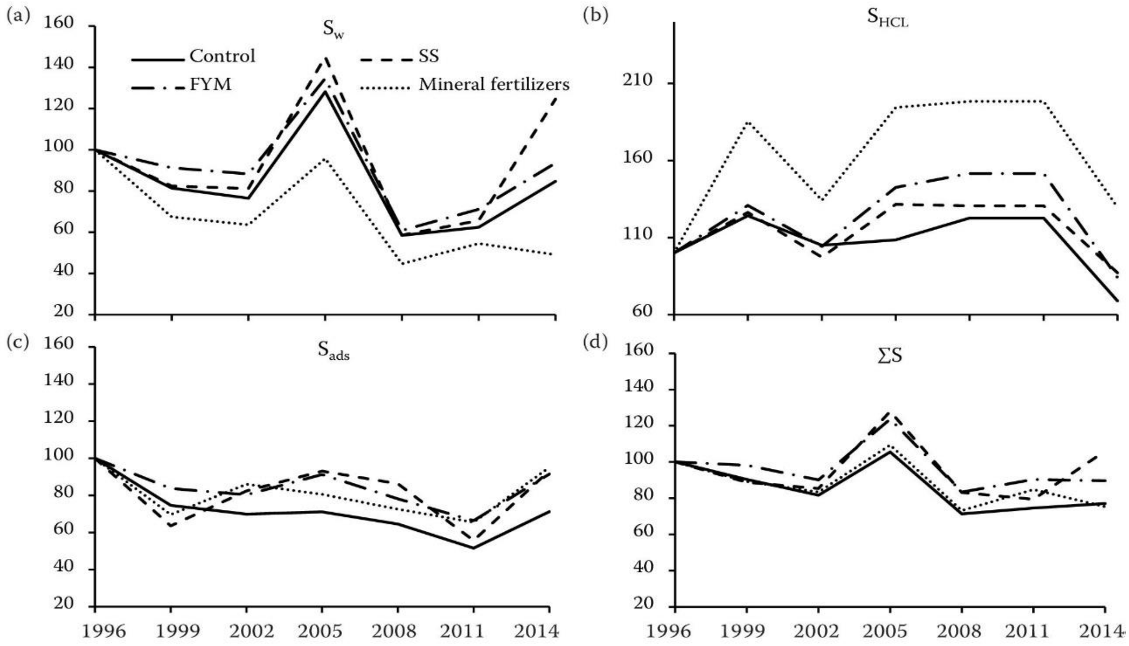

Figure 3. Changes of sulfur contents (%) at Hněvčeves site between 1996 and 2014. S_w – water extractable; S_{ads} – adsorbed; S_{HCL} – 1 mol/L HCl estimated; ΣS – total estimated sulfur

due to the calcium-bound sulfates contained in superphosphate estimated with 1 mol/L HCl as well. This was also confirmed at Hněvčeves site, which has about 0.7% of carbonates. This fact partially prove the statement of Kulhánek et al. (2011) who estimated the increasing content of occluded sulfur in soil after calcium sulfate S application at three different sites in three year experiment.

Figure 3 shows the results from Hněvčeves site. The differences between studied years are the most visible here. Especially the contents of S_w sulfur in the year 2005 are higher compared to other years. In spite of this fact, the differences among the fertilizing treatments are clearly visible as well as on the other sites. The lowest contents of S_w, like at Suchdol site, were always found in the mineral fertilizers treatment. Thereafter follows the control treatment. Treatments fertilized with SS and FYM showed quite similar results, which were higher than mineral fertilizers and control.

Control treatment showed usually the lowest values of S_{ads}. Differences between other fertilizing systems were not clearly visible. Curtin et al. (2007) studied the influence of long-term application of S in the form of single superphosphate on the content of S_{ads} in long-term experiment. They found the significantly increasing tendency with both superphosphate doses (7 and 14 kg S/ha/year) against zero control. In our experiments, only 6.5 kg S/ha/year was added as superphosphate. Therefore, the differences between control and mineral fertilizers were not so clearly visible, but the S_{ads} contents in the mineral fertilizers treatment were almost always higher in comparison to control among all three studied sites and seasons.

The studied S forms content increase is typical for all three sites in the year 2005. It was probably caused due to the weather conditions in this year, which were optimal for the mineralization of the organic sulfur sources (higher rainfall in July and August together with higher temperatures in September) (CHMI 2016). It confirms the role of climatic conditions on the soil S fractions contents mentioned in Förster et al. (2012).

The differences between soil sulfur contents related to the crops in crop rotation were also evaluated. Almost in all cases, the highest contents of S_w were clearly visible in samples taken

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up in the SS treatment after potatoes harvest. At the next crops in crop rotation (wheat and barley) a decreasing tendency follows. This was caused because SS was always applied only before the potatoes for the whole crop rotation. This tendency appeared in a lesser extent among the S_{ads} and ΣS as well, but not among the S_{HCL}. A similar result was expected during FYM fertilizing, but the contents of three measured S forms did not change during the crop rotation. It is probably due to the fact that in the FYM sulfur is in more stable organic forms compared to SS. That confirms the study of Lima et al. (2009), who estimated the long-term influence of SS or FYM application and found significantly higher organic matter (especially lignin and lignin-like products) accumulation in the FYM treatment. The second reason of lower S_w content among the FYM treatment can be the fact that its long-term application led to the S accumulation in the dry soil aggregates (Yang et al. 2007) whose size increased with the increasing organic fertilizers rate (Scherer et al. 2012). After Scherer et al. (2012) the soil particle size plays the key role in the S availability, where the content of plant available S decreases with the increasing soil particle size, while the influence of the kind and amount of used organic fertilizers (FYM, SS and composts) is negligible. The above-mentioned fact also partially confirms the results of Odlare et al. (2008) who compared the microbial activity in soil after SS or cattle manure application. They found slightly higher microbial activity in soil after SS application. Based on the results of Sastre et al. (1996), SS had a positive influence on microbial activity in soil and due to this process on the organic matter decomposition as well. According to Tabatabai and Chae (1991) SS has also more optimal C:S ratio for organic compounds mineralization compared to FYM. This confirmed also Förster et al. (2012) who stated that SS has a large pool of potentially mineralizable S. It was probably the reason for S_w content increase in the SS treatment after potatoes harvest. As expected, the content of studied S forms did not change among control and mineral fertilizers treatments during the crop rotations.

Based on the results of our three long-term field experiments it can be concluded that a decreasing tendency of ΣS content is visible in the period from 1996 to 2014 (except for the year 2005). The biggest decrease is always visible in the control treatment. SS application led to the highest S_w contents compared to the other fertilization treatments. Mineral fertilizers treatments appeared to be the best source of S_{HCL}. The contents of studied sulfur forms in soil were (especially in the year 2005) influenced by the local climatic conditions as well.

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17) Effect of bioeffectors and recycled P-fertilizer products on the growth of spring wheat

Vliv biostimulantů a recyklovaných fosforečných hnojiv na růst jarní pšenice

Přiložená studie je zaměřena na kombinaci dvou moderních cest představujících potenciál pro lepší hospodaření s fosforem. První z nich je recyklace odpadních materiálů a druhá využití biostimulantů. Jedná se o meta-analýzu pokusů, které probíhaly v rámci evropského programu BioFector. Recyklace odpadních materiálů představuje perspektivní cestu k částečnému uzavření koloběhu některých živin, zejména fosforu (Hilton et al. 2010, Karunanithi et al. 2015). Ve většině recyklovaných hnojiv se však fosfor vyskytuje jen v obtížně dostupných formách. Proto jsou intenzivně hledány možnosti, jak uvolnit takto vázaný fosfor. Je obecně známo, že některé mikroorganismy disponují metabolickým aparátem umožňujícím uvolnit i velmi silně vázaný P (např. Richardson 2001, Harman et al. 2004). Proto izolace těchto mikroorganismů a jejich řízená aplikace představuje jednu z možností, jak zefektivnit působení recyklovaných hnojiv. Možnosti využití těchto tzv. biostimulantů jsou v současné době předmětem řady studií. Přiložená publikace vychází z výsledků nádobových pokusů s jarní pšenicí. Ačkoliv byla testována široká škála biostimulantů i odpadních materiálů, pozitivní výsledky z pohledu výnosu *nadzemní hmoty* pšenice a odběru P jsou pouze sporadické. Konkrétně pouze v jednom experimentu byl na pozitivní kontrole hnojené trojitým superfosfátem zaznamenán zvýšený výnos biomasy po aplikaci *Pseudomonas sp.* a *Bacillus Amyloliquefaciens*. Ve stejných pokusech však bylo působení *Penicillium sp.* a *Bacillus Amyloliquefaciens* v kombinaci s Thomasovou moučkou negativní. Rovněž odběr fosforu nebyl zmíněnými biostimulanty zásadně ovlivněn. Pouze bakterie *Pseudomonas sp.* vykazovala neprůkazné tendence ke zvýšenému odběru P rostlinami pšenice. Naopak signifikantní rozdíly z hlediska výnosů nadzemní biomasy vykazovaly různé odpadní materiály. Zatímco popel z čistírenských kalů vykazoval pouze 20-40% účinnost ve srovnání s trojitým superfosfátem (TSP, 100 %), sušené čistírenské kaly, Thomasova moučka, struska obohacená o fosfor a vláknitá frakce kejdy dosahovaly účinnosti vyšší než 74 % ve vztahu k TSP. Komposty (převážně ze zahradního odpadu a čistírenských kalů) vykazovaly střední efektivitu, tj. výnosy představující 40-70% ve srovnání s TSP. Závěrem lze konstatovat, že některé odpadní materiály mohou být perspektivním zdrojem fosforu. Efekt biostimulantů na výnos nadzemní hmoty pšenice a využití fosforu byl zpravidla neprůkazný.

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RESEARCH **Open Access**

Effect of bioeffectors and recycled P-fertiliser products on the growth of spring wheat

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Abstract

Background: The recycling of waste products into P fertilisers in agriculture is advisable from the perspective of sustainability. Bioeffectors (BEs), which have the ability to increase the plant uptake of P from recycled fertiliser products, may increase the fertiliser value of these products. This paper investigated the effect of a range of different recycled fertilisers on the growth and P uptake of wheat in pot experiments conducted at three different locations in Europe. Furthermore, investigations were undertaken as to whether the addition of a range of bioeffectors could significantly enhance P availability, P uptake and plant growth.

Results: BE additions were found not to significantly increase the aboveground biomass of wheat plants or the uptake of P when plants were fertilised with recycled fertiliser products. This was shown across a range of pot experiments with soils of different P status. Only in the case of the positive control P fertiliser (TSP) was a positive effect of Proradix and RhizoVital on plant growth observed in one of the experiments, while in the same experiment RhizoVital and Biological fertiliser DC had a negative impact on plant biomass when the P fertiliser was Thomas phosphate. With regard to P uptake, there was only a slight positive effect of Proradix in plants not supplied with P fertiliser in this experiment. Clear differences were seen in the efficiency of P fertilisers. Generally, sewage sludge ash performed quite poorly (20–40 % of TSP), while sewage sludge, Thomas phosphate, P-enriched slag and the fibre fraction of pig manure all had a high availability of P (>74 % relative to TSP). Compost composed mainly of garden/park waste and sewage sludge was intermediate in availability (40–70 %). The elemental composition of the harvested wheat plants was significantly affected in all cases by the different P fertilisers added. The BE treatments significantly affected the elemental composition of the aboveground biomass in one of the experiments where the product Proradix had the greatest effect on elemental composition.

Conclusions: In conclusion, the experiments revealed a wide difference in the bioavailability of P in the different waste products, but the added microorganisms demonstrated a limited capacity to influence plant P uptake across a range of soils and waste products.

Background

Phosphorus (P) is a non-renewable resource [1], and currently the majority of P added as fertiliser in agriculture is in the form of inorganic fertilisers. From a sustainability point of view, it is sensible to make better use of P resources that are discarded as waste from urban areas; hence, there is a need to improve the recycling of P from agricultural and urban wastes [2]. Recycled fertiliser products are by no means homogenous and the availability of P for plant uptake from recycled fertiliser products may vary considerably, depending on the feedstock of the fertiliser and the subsequent type of processing [3]. Sewage sludge as a fertiliser may contain a range of different P forms depending

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on the specific process used to recover P from the sewage water, but a precipitation reaction using Al, Fe, Mg or Ca to precipitate P is often employed [3]. Sewage sludge may contain different types of organic contaminants [4]. Although it is still used as a fertiliser in many countries across Europe [5], the use of sewage sludge has declined in a number of European countries, while sewage sludge application on agricultural land was banned in Switzerland in 2008 [6]. A common practice that eliminates the organic contaminants in sludge is to incinerate the sewage sludge, thus producing sewage sludge ash. However, the availability of P in sewage sludge ash is quite low, but is also observed to be variable depending on the type of treatment in the sewage treatment plant [7]. Techniques such as acid leaching and thermal treatment have been investigated for their potential to recover P from sewage sludge ash while separating it from the detrimental heavy metals with varying success [5, 7], but there is also a possibility of combining the upgrading of sewage sludge ash with the recycling of metallurgical slags. Slags from the metallurgical industry had been recycled as fertiliser in Germany in the form of Thomas phosphate for more than 100 years, but this is no longer produced [8]. BOF (basic oxygen furnace) or LD (Linz–Donawitz) slag from steel production may be used as a liming agent in agriculture [8]. It is also possible, however, to use sewage sludge ash as a means to enrich the hot liquid BOF slag with P, resulting in a fertiliser with a markedly higher P availability compared to the feedstock sewage sludge ash, due to a conversion of $Ca_3(PO_4)_2$ (whitlockite) of low neutral ammonium citrate (nac) solubility into Ca-silico-phosphates with a higher nac solubility [9]. P contained in animal manures can be used more sustainably if it is up-concentrated, and thereby more easily transported from areas with a P surplus to areas where soils have a P deficit [3]. The majority of P (60–90 %) found in pig manure is inorganic [10] and only very little is in the form of phytate [11, 12]. Biomass ash (e.g. wood and straw ash) from bioenergy-plants could also potentially serve as a P fertiliser [13].

Different types of biostimulants or bioeffectors (BEs) have been investigated for their ability to increase plant productivity in agricultural systems [14]. The concept of BEs covers quite a diverse group of natural products [15]. In the present paper, the scope was limited to plant growth-promoting microorganisms (PGPM) focusing on plant growth-promoting rhizobacteria (PGPR) [16] and free-living fungi, such as species of the genus *Trichoderma* [17]. PGPM may have the potential to enhance plant uptake of P from soil [14, 18, 19]. Improved growth under P-limiting soil conditions as a result of microbial inoculations has been observed in many different plant species, such as mung bean [17], bean [20], maize [21] and wheat [22]. In soil, a large proportion of the total P pool is not directly available for plant uptake [23], and the ability to solubilise phosphates in the rhizosphere has been viewed as an important function of PGPM [24]. The plant growth-promoting effect may, however, be overestimated due to a publication bias, and an observed positive plant growth response may be due to mechanisms other than an increased availability of P in the rhizosphere brought about by PGPM solubilisation of P, e.g. changes in root architecture and total root length [24].

Further research is therefore required into the potential of BEs to facilitate the plant uptake of nutrients from soil. Fungi of the ascomycete genera *Trichoderma* and *Penicillium* have been extensively studied for their potential as PGPM [25], and fungi of the genus *Trichoderma* may have an ability to increase plant nutrient uptake from soil [26, 27]. The specific *T. harzianum* strain Rifai 1295-22 (T22) has been observed to increase the solubilisation of sparingly soluble calcium phosphates [28] and to have a plant growth-promoting effect in willow [29], chickpea [17] and maize [30]. Fungi of the genus *Penicillium* have been shown to have P-solubilising capabilities [31, 32], and members of this genus have been observed to have a positive effect on biomass and P uptake of wheat and bean [20]. Wakelin et al. [33] found that a strain of *P. bilaii* is both capable of increasing the yield of medic and lentil in the field and of significantly increasing the level of HCO_3-extractable P in soil microcosms, and more recently *P. bilaii* has been found to increase yield in maize in field trials [21]. On the other hand, the positive effect of *P. bilaii* under P-limited conditions has also been linked in pea to an increase in the root adsorptive capacity under P-limited conditions rather than through increased P solubilisation [34]. However, an investigation across a number of field studies involving wheat showed that *P. bilaii* does not significantly affect P uptake and yield [35]. Gram-negative gammaproteobacteria of the genus *Pseudomonas* are ubiquitous bacteria in soil, are known to proliferate greatly in the rhizosphere [36] and have been studied for their plant growth-promoting activities for many years [37]. Bacteria from this genus have been observed to increase plant productivity under P-limiting conditions [38, 39]. As a representative of *Pseudomonas* PGPR, the product *Proradix* was selected. This product has primarily been developed and investigated for its effects on plant resistance to pathogens [40–42], but there is also evidence that this product may improve plant growth under nutrient-limiting conditions [43]. Low G + C Gram-positive bacteria of the genus *Bacillus* have been shown to solubilise calcium phosphates and increase the dry matter yield of wheat in a pot trial in which no P fertiliser or calcium phosphates were applied [22]. A number of *B. amyloliquefaciens* strains have been investigated for their biocontrol capabilities [44] and the type strain (FZB42) for the subspecies *plantarum* of *B. amyloliquefaciens* [45] is reported to work as a biofertiliser and provide protection against various soil-borne diseases [46, 47].

The aim of the present paper was to investigate whether a variety of BE organisms could significantly enhance the availability and uptake of P from a range of recycled P-fertiliser products with very different P availability. The paper encompasses a number of pot experiments carried out in Denmark, Germany and the Czech Republic. The experiments included a negative control as well as a positive control (in two out of three studies) in which highly available triple superphosphate was added.

Sewage sludge ash in particular is an example of a product with quite low P solubility, offering considerable potential for improvement by BEs. The microorganisms were expected to have a positive effect on the solubilisation of fertiliser-derived P in the soil, as well as a direct effect on the plants through hormonal effects. The latter effects might occur in all P-addition treatments, whereas the positive effects on soil P availability are expected to be of greater significance in treatments with lower P availability, such as sewage sludge ash. It was therefore hypothesised that: (i) inoculation with the selected BE strains would increase the availability of P from the recycled fertilisers and (ii) inoculation with the selected BE strains would increase the uptake of P by wheat from soil, leading to a larger production of aboveground biomass.

Due to differences in the conditions between the individual experiments, it is not possible to compare the concentrations of elements or biomass produced per kg soil across experiments. We therefore only analyse the relative changes in, for instance, biomass compared to the negative and positive controls (where included) across experiments. We analyse the effect of BEs in the individual experiments. The fact that some of the BEs are tested using different soils and slightly different growing conditions serves as a stronger test of their performance than a single pot experiment would have.

Methods

The paper deals with the results of three separate pot trials. The pot trials were carried out at Arbeitsgemeinschaft Hüttenkalk e.V., Germany (HK Kalke experiment), the University of Copenhagen, Denmark (UCPH experiment), and the Czech University of Life Sciences Prague, Czech Republic (CULS experiment).

Sampling of soil and soil characterisation

The three pot experiments included in this publication were performed with four different soils (Table 1). Soil for the HK Kalke experiment was sampled from the plough layer of a field with arable feed production that had not had any P fertilisation for over 30 years, located in Marienmünster-Vörden in East Westphalia, Germany. Soil for the UCPH experiment was sampled from the plough layer of the long-term nutrient depletion trial at the University of Copenhagen's experimental farm in Taastrup, Denmark, where cereals have been grown continuously for more than 50 years without the addition of P fertilisers. Finally, the soils used in the CULS pot experiment were sampled from the plough layer of either the long-term experimental farm in Humpolec, Czech Republic, where wheat, potato and barley were grown in rotation continuously without fertilisation for 20 years (Humpolec soil), or a field managed by a conventional farming system with low P inputs (Poděbrady soil). The air-dried soil was analysed in the laboratory of the *Landesanstalt für Landwirtschaftliche Chemie* at the University of Hohenheim, Germany, for the HK Kalke and UCPH experiments. Selected results of these analyses are presented in Table 1. Texture was analysed using a combination of wet sieving and pipetting according to the VDLUFA standard method C 2.2.1 [48]. Organic carbon content was measured according to the VDLUFA standard method A 4.1.3.1 [48]. pH was measured in 0.01 M $CaCl_2$ according to the VDLUFA standard method A 5.1.1 [48]. Finally, calcium-acetate lactate-extractable P (P_{CAL}) was measured according to the VDLUFA standard method A 6.2.1.1 [48]. The soils for the CULS experiments were analysed at the Czech University of Life Sciences following the same protocols.

Table 1 Soil data

Soil	Management	Texture (%)			OC (%)	pH[a]	P_{CAL} (mg kg^{-1})	P_{TOT} (mg kg^{-1})
		Sand	Silt	Clay				
Vörden[b]	Conventional farming system. Arable land food production. No P addition for more than 30 years	41.1	46.9	12.0	0.8	5.0	26	310
NDT-A[c]	Continuous cropping. No addition of P fertiliser for more than 30 years	55.4	31.2	13.4	1.1	5.8	35	397
Humpolec[c]	Continuous cropping (potato, wheat, barley). No addition of P fertilisers	30	49	21	1.6	4.5	59	587
Poděbrady[c]	Conventional farming system. Low P input	57	24	19	1.9	6	30	384

[a] pH measured in $CaCl_2$

[b] Data were recorded on the 2:1 soil:sand mixture used in the pot experiments

[c] Data were recorded on the pure soil

Bioeffector (BE) treatments

A range of different bioeffectors (BEs) was added to the growth medium at sowing. A control treatment without the addition of BEs was included (BE0). The BEs investigated were a *Trichoderma harzianum* isolate marketed as Trianum-P by Koppert (TrP), Proradix (Pro) from Sourcon Padena containing *Pseudomonas* sp., RhizoVital 42 (RhVi) produced by ABiTEP containing *Bacillus amyloliquefaciens* ssp. *plantarum*, strain FZB42, biological fertiliser DC (Bio-DC) produced by Bayer Crop Science Biologics GmbH containing *Penicillium* sp. and BactoProf (BaPr) produced by Terra Bioscience, Germany, which contains isolates of *T. harzianum* and five species of *Bacillus*. BE suspensions were prepared in 0.25 mM $CaSO_4$. The concentrations used for the inoculation are given in Table 2.

P fertilisers and P-fertilisation treatments

A number of recycled P fertilisers were applied in the experiment (Table 3). Thomas phosphate was obtained from the Luxengrais steel plant in Luxembourg. Sewage sludge and sewage sludge ash for the HK Kalke experiment were obtained from a municipal treatment plant in Bonn, processing wastewater mainly from households and from an attached sewage sludge incineration plant. The sewage sludge and sewage sludge ash used for the UCPH experiment originated from a public treatment plant receiving wastewater from households and industries (Spildevandscenter Avedøre). A P-enriched steelmaking slag (LDS/SSA in the HK Kalke experiment) was produced by blowing sewage sludge ash into a liquid 1500 °C basic oxygen furnace slag (prepared by the Linz–Donawitz process) from a steelwork in Salzgitter [9]. A fibre fraction of pig manure (FFPM) was obtained using the decanter centrifuge method. A compost (Comp) consisting of a mixture of mainly garden park waste and sewage sludge (42 % garden park waste, 36 % sewage sludge, 14 % straw and horse manure, 8 % wood mass) was obtained from the private company KomTek, Denmark. Straw ash (StA) was a mixture of fly and bottom ash from a grate-fired boiler (15 MWt) and originated from cereal straw combustion. Finally, wood ash (WoA) was obtained from a fluidised bed reactor (15 MWt) in which wood chips were combusted.

For the majority of the fertilisers, the equivalent of 50 mg P kg^{-1} soil was added. There were, however, some deviations from this in the CULS experiment (Table 3). For both the HK Kalke experiment and the UCPH experiment, sewage sludge (SS) and sewage sludge ashes (SSA) were included. Furthermore, both TSP and a low-grade type of TSP, termed superphosphate (SP) here, were included in both these experiments as positive controls. In all three experiments, a negative control without the addition of P fertiliser (P0) was included. An overview of the BE and P-fertilisation treatments included in the three pot experiments is presented in Table 4.

Pot trial setup, growing conditions and harvest

Soil preparation, growing conditions, harvest days and nutrient application are presented in Table 5.

HK Kalke experiment

The air-dried and sieved soil (mesh size 5 mm) was mixed with water-washed quartz sand in a proportion of 2:1. This substrate was mixed with 0.843 g kg^{-1} $Ca(NO_3)_2 \cdot 4H_2O$ and 0.719 g kg^{-1} Patentkali (27.8 % K_2O, 9.49 % MgO, 15.8 % S). Each pot was filled with 6 kg of the fertilised soil/sand mixture and watered to 70 % of WHC. Before watering, Bio-DC was mixed into the substrate of the Bio-DC treatment. Spring wheat (cultivar Aranka) was sown in 32 separate sowing holes (approximately, 2 cm deep). To each of the sowing holes, 2 ml of the Pro or RhVi suspensions were added in the corresponding BE treatments. After germination, plants were reduced to 24 wheat plants per pot. The pots were placed in a randomised design, with four replicates per treatment, in an outdoor roofed vegetation hall. Pots were irrigated with demineralised water to 60–70 % WHC (controlled gravimetrically once a week) during the whole vegetation

Table 2 Bioeffector (BE) products applied in the experiments

Product	Producer	Abbr.	Name of organism(s)	Type of organism	Application rate (cfu g^{-1} soil)
Control	n.a.	BE0	n.a.	n.a.	n.a.
Trianum-P, T22	Koppert, The Netherlands	TrP	*Trichoderma harzianum*, strain T-22	Fungi	$2.5 \cdot 10^4$
Proradix	Sourcon Padena, Germany	Pro	*Pseudomonas* sp., strain DSMZ 13134	Bacteria	$2 \cdot 10^6$
RhizoVital 42	ABiTEP, Germany	RhVi	*Bacillus amyloliquefaciens*	Bacteria	$2 \cdot 10^6$
Biological fertiliser DC	Beyer/Prophyta, Germany	Bio-DC	*Penicillium* sp.	Fungi	$1 \cdot 10^5$
Bacto prof	Terra Bioscience, Germany	BaPr	*T. harzianum* and five species of *Bacillus*	Bacteria + fungi	$2 \cdot 10^6$

Table 3 P-fertilisation treatments applied in the experiments

P fertiliser	Treatment abbreviation	Total P content in product (g kg^{-1})	Water-extractable P (% of total P)	App. rate (g dry product kg^{-1} soil)	P app. rate (mg P kg^{-1} soil)
Negative control	P0	n.a.	n.a.	n.a.	n.a.
Triple superphosphate	TSP	200	43.3	0.25	50
Superphosphate	SP	81	11.4	0.62	50
Thomas phosphate	Thph	68	0	0.73	50
Sewage sludge, HK Kalke	SS	36	n.d.	1.40	50
Sewage sludge, UCPH	SS	37	n.d.	1.36	50
Sewage sludge ash, HK Kalke	SSA	103	0.18	0.48	50
Sewage sludge ash, UCPH	SSA	89	n.d.	0.56	50
Fibre fraction of pig manure	FFPM	2.4	n.d.		50
SSA-enriched LD slag	LDS/SSA	17	0	2.92	50
Compost mainly consisting of sewage sludge and garden/park waste	Comp	3.6[a,b]	n.d.	13.8[b]	50
Ashes from cereal straw	StA	13.6	6.5	10	136
Ashes from wood chips	WoA	10.2	0.05	10	102
Dipotassium phosphate	DKP	178	100	0.18	32

[a] According to information from the producer

[b] These measurements are in g kg^{-1} fresh matter

period. The plants were supplemented with an additional 50 mg N kg^{-1} soil on day 40 in the form of $Ca(NO_3)_2$. The plants were harvested 8 weeks after sowing. The plants were at stage 59 (without P fertilisation) or stage 63 (with P fertilisation).

UCPH experiment

Soil was partially air dried and sieved (mesh size 5 mm). The soil was mixed with quartz sand in the proportion of 1:1. The water-holding capacity of the soil/sand mixture was determined. For each pot, 2.5 kg of the soil/sand mixture was mixed with 0.645 g kg^{-1} $Ca(NO_3)_2$ and 0.667 g kg^{-1} Patentkali (30 % K_2O, 10 % MgO, 42.5 % SO_3). Subsequently, this substrate was mixed with either 50 g sand (P0 treatment) or 50 g sand mixed with one of the P fertilisers being investigated (Table 5). The fertilisers were mixed with sand prior to being added to the soil to ensure thorough mixing throughout the whole soil volume. The soil was watered to 40 % of WHC. Fifteen wheat seeds (cultivar Scirocco, KWS) were sown in separate sowing holes (approximately, 2 cm deep). After the seeds were sown, 1 mL of BE suspension (or 0.25 mM $CaSO_4$ in the BE0 controls) was added to each of the sowing holes before these were closed. For each treatment, five replicate pots were set up, resulting in a total of 140 pots in the experiment. The pots were placed in a greenhouse in a randomised block design. After germination, the wheat plants were thinned out, leaving ten plants in each pot. During the experiment, the pots were watered to weight (initially, 60 % and subsequently 70 % of WHC) at regular intervals (every 1–3 days). The blocks were rotated and reshuffled once or twice a week during the experiment. At 25 days after sowing, the youngest fully developed leaf was removed from one plant in three replicates of the BE0 treatments (all P treatments), giving a total of 21 samples. After 32 days, five plants from each pot were harvested. After 42 days, extra N was added to each pot (33 mg N kg^{-1} soil). After 54 days, the remaining five plants were harvested from each pot. At harvest, the plants were at stage 55. A follow-up experiment partially replicating the UCPH experiment was carried out as well (see Additional file 1 for details).

CULS experiment

Soil was air dried and sieved (mesh size 10 mm). No sand was added to the soil. For each pot, 5 kg (d.w.) of soil was used, which was mixed with 1.67 g of NH_4NO_3. 50 g of WoA or StA was thoroughly mixed with the soil prior to filling the pots (final dose 10 g of ash per kg soil). K_2HPO_4 was applied as a water solution and was also thoroughly mixed into the whole soil volume. The soil was watered to 40 % of WHC, 25 wheat seeds (cultivar Aranka) were sown in separate sowing holes (approximately, 2 cm deep) and 2 ml of BE suspension (or 0.25 mM $CaSO_4$ in the BE0 controls) was applied to each hole prior to closing. After germination, the number of plants was reduced to 20 wheat plants per pot, and these were inoculated again by irrigation with 100 ml of BE suspension per pot. The pots were placed in an outdoor roofed vegetation hall. Pots were irrigated with demineralised water to 60–70 % WHC (controlled gravimetrically once a week)

Table 4 Overview of the treatments applied in the different experiments (soils)

Soil	1. HK Kalke (Germany)	2. UCPH (Denmark)	3. CULS (Czech Republic)	
	Vörden	NDT	Humpolec	Poděbrady
Bio-effectors				
Negative control (BE0)	X	X	X	X
TrP		X		
Pro	X	X		
RhVi	X	X	X[a]	X[a]
Bio-DC	X			
BaPr			X[a]	X[a]
P fertilisers				
Negative control (P0)	X	X	X[b]	X[b]
DKP			X[b]	X[b]
TSP	X	X		
SP	X	X		
Thph	X			
SS	X	X		
FFPM		X		
Comp		X		
SSa	X	X		
LDS/SSA	X			
StA			X	X
WoA			X	X

[a] Only in combination with StA and WoA

[b] Only in combination with BE0

during the entire vegetation period. The experiment was undertaken in a randomised design with four randomisation procedures during the experiment. The plants were harvested after 16 weeks. At harvest, the plants were at full maturity.

Soil data recorded during the HK Kalke experiment

Soil (40 g) was sampled 27 days after sowing. The soil was air dried and completely passed through a 2 mm mesh sieve. For pH measurement, 10 g of soil was suspended in 25 ml of a 0.01 molar $CaCl_2$ solution for 1 h, stirred twice and pH determined using a pH electrode (VDLUFA standard method A 5.1.1). For water extraction of soil phosphate according to Van der Paauw [49] and Murphy and Riley [50], 4.25 ml of soil was suspended in demineralised water for approximately 22 h. Thereafter 250 ml water was added; the mixture was mechanically shaken for 1 h and filtered. P determination was undertaken using a spectrophotometer and molybdenum blue method.

Plant analyses

HK Kalke experiment

After harvest, the aboveground wheat plant material was dried at 60 °C. 400 mg of plant material was digested with 8 ml 69 % HNO_3 supra and 1 ml 15 % H_2O_2 in high-pressure MARS express vessels in a MARS microwave digestion system. The element analyses of P, K, Mg, Ca, Mn and Na were performed by ICP-OES.

UCPH and CULS experiment

The plant material was dried at 65 °C and weighed to measure the dry aboveground biomass. For elemental analysis, the dry plant material was finely ground. Subsequently, 100 mg of dry plant material was mixed with 2.5 ml 70 % HNO_3 and 1 ml 15 % H_2O_2, followed by digestion in a pressurised single-chamber microwave oven (UltraWAVE, Milestone Srl, BG, Italy). Samples were then diluted to 50 ml using Milli-Q water and analysed for their elemental content (B, Ca, Cu, Fe, K, Mg, Mn, P, S, Zn in UCPH and Ca, K, Mg, Mn, Na, P in CULS) by ICP-OES. For the samples from the final harvest in the UCPH experiment, only P was measured using flow injection analysis.

Data analyses and statistics

The measurements of aboveground biomass were normalised relative to the control treatment (P0, BE0):

$$\text{Normalized biomass}_{\text{sample}} = \frac{\text{biomass}_{\text{sample}}}{\text{biomass}_{\text{control(P0, BE0)}}}. \quad (1)$$

In the CULS experiment, the normalisation was undertaken separately for the two soils. Significance testing of differences between treatment means was performed using one- and two-way ANOVAs and Dunnett's test (for comparisons versus the control only) or Tukey's test (for all possible comparisons) for post hoc multiple comparisons. These were performed using the statistics module in Sigma Plot 13.0. In the UCPH experiment in which two separate samplings had been performed, the difference in normalised biomass between sampling days was tested using a paired *t* test. For the CULS experiment, all the P-fertiliser treatments were combined with the BE0 treatment only. The effect of different P substrates was therefore analysed by a two-way ANOVA, excluding data for the RhVi and BaPr BE treatments. The effect of BE treatments in the CULS experiment was tested in two separate two-way ANOVAs for the two soils, where only data from the straw and wood ash treatments were included.

The efficiency of the fertilisers relative to TSP (positive control) was calculated as the mean efficiency measured

Table 5 Growing conditions in pot experiments

Exp.	Wheat cultivar	Soil:sand ratio (mass)	Size of pots (L)	Mass of substrate (kg)	No of plants	No. of harvests	Final harvest (weeks)	Rep	App. of macronutrients at setup (mg kg^{-1} substrate)				
									Ca[a]	K[b]	Mg[b]	N	S[b]
HK Kalke	*Aranka*	2:1	6	6	24	1	8	4	158	166	40	100[a]	114
UCPH	*Scirocco*	1:1	3	2.5	10	2	8	5	158	166	40	100[a]	114
CULS	*Aranka*	1:0	6	5	20	1	16	3	0	0	0	100[c]	0

[a] Supplied as $Ca(NO_3)_2$

[b] Supplied as Patentkali (30 % K_2O, 10 % MgO, 42.5 % SO_3)

[c] Supplied as NH_4NO_3

in n replicate pots. The efficiency in the individual pots was calculated as follows where data were available:

$$\mathrm{FE}(\%) = 100 \times \left(\frac{\mathrm{biomass}_{\mathrm{sample}} - \overline{\mathrm{biomass}_{\mathrm{P0,BE0}}}}{\overline{\mathrm{biomass}_{\mathrm{TSP}}} - \overline{\mathrm{biomass}_{\mathrm{P0,BE0}}}} \right). \quad (2)$$

Similarly, the P-uptake efficiency from the different fertilisers was calculated as follows where the necessary data were available:

$$\mathrm{PUE}(\%) = 100 \times \left(\frac{\mathrm{P\ content}_{\mathrm{sample}} - \overline{\mathrm{P\ content}_{\mathrm{P0,BE0}}}}{\overline{\mathrm{P\ content}_{\mathrm{TSP}}} - \overline{\mathrm{P\ content}_{\mathrm{P0,BE0}}}} \right). \quad (3)$$

The data for biomass in the HK Kalke experiment were expressed as a function of the available P level (P_{H2O}) in the pot experiment and the three-parameter exponential rise to maximum (Mitscherlich) curve was fitted to the data [51]:

$$y = y_0 + a(1 - e^{-\mathrm{bx}}). \quad (4)$$

The same model was used to express biomass as a function of the P concentration in the youngest fully developed leaf at day 25 in the UCPH experiment. These regressions and simple linear regressions were performed using the regression wizard in SigmaPlot 13.0 (Systat).

Principal component analysis (PCA) was performed on data for elemental concentrations. Beforehand, PCA data were standardised by subtracting the mean for each element and then dividing this by the standard deviation. PCA was performed in R version 3.1.1 [52] using the ade4 package [53] with a chosen number of principal components of 10.

Results

Aboveground biomass and P content

HK Kalke experiment

In the KALKE experiment (Fig. 1a), the normalised aboveground biomass was significantly different between the P-fertilisation treatments (two-way ANOVA, $P < 0.001$) and BE treatments (two-way ANOVA, $P < 0.05$), and there was a significant interaction between the two factors (two-way ANOVA, $P < 0.001$). For the TSP treatment, the inoculation with Proradix and RhizoVital resulted in a significantly higher normalised aboveground biomass (Dunnett's test, $P < 0.05$). When Thomas phosphate (Thph) was applied as a P fertiliser, inoculating with Proradix and biological fertiliser DC resulted in a significantly lower normalised aboveground biomass compared to the BE0 control (Dunnett's test, $P < 0.05$). For the remaining P fertilisers, the applied BEs (Pro, RhVi and Bio-DC) did not have a significant effect when compared with the control. The post hoc analysis of P-content data (Table 6a) showed that although there was a highly significant effect of both the P fertiliser, the BE application and the interaction between the two (two-way ANOVA, $P < 0.001$), there was not a significant positive effect of any of the BE treatments on P uptake from any of the P fertilisers compared to the BE0 control (Dunnett's test, $P > 0.05$). Only in the P0 treatment, there was a significant positive effect of Pro and RhVi on total aboveground P content (Table 6a).

UCPH experiment

In the UCPH experiment, plants were harvested after 32 (Fig. 1b) and 54 days (Fig. 1c). The normalised biomass across all treatments was significantly different between harvests (paired t test, $P < 0.001$); therefore, the normalised aboveground biomass data from the two harvests were analysed individually. The normalised biomass was significantly different between P-fertilisation treatments at both harvests (two-way ANOVA, $P < 0.001$). In contrast to this, the different BE inoculations did not affect the biomass obtained (two-way ANOVA, $P > 0.05$) and no interaction was observed between the two factors (two-way ANOVA, $P > 0.05$). At both harvests, all treatments in which a P fertiliser was applied resulted in a significantly higher aboveground biomass than the control without added P fertiliser (Dunnett's test, $P < 0.05$). The absence of a significantly higher aboveground biomass when inoculating with the three BEs (TrP, Pro, RhVi) compared to the uninoculated control (BE0) was confirmed for sewage sludge, sewage sludge ash and compost as the P fertilisers in a follow-up experiment at the University of Copenhagen in which only five wheat plants were grown in each pot (Additional file 1: Fig. S1). P uptake was only evaluated for the P fertilisers SSA and FFPM at the second harvest (Table 6b). No effect was produced by either of the two main factors (P fertiliser and BE addition) and there was no interaction between the two factors in relation to the aboveground P content (two-way ANOVA, $P > 0.05$).

CULS experiment

In the CULS experiment (Humpolec and Poděbrady soils), there was no significant effect of the soil type on the aboveground biomass in the control (P0/BE0) treatment (one-way ANOVA, $P > 0.05$, data not shown). For the Humpolec soil, the DKP treatment yielded a significantly lower normalised biomass compared to the control (two-way ANOVA on BE0 data with soil and P fertiliser as factors, Dunnett's test $P < 0.05$), while in the Poděbrady soil the normalised aboveground biomass was not significantly different from the control when adding DKP (Dunnett's test, $P > 0.05$). The addition of straw

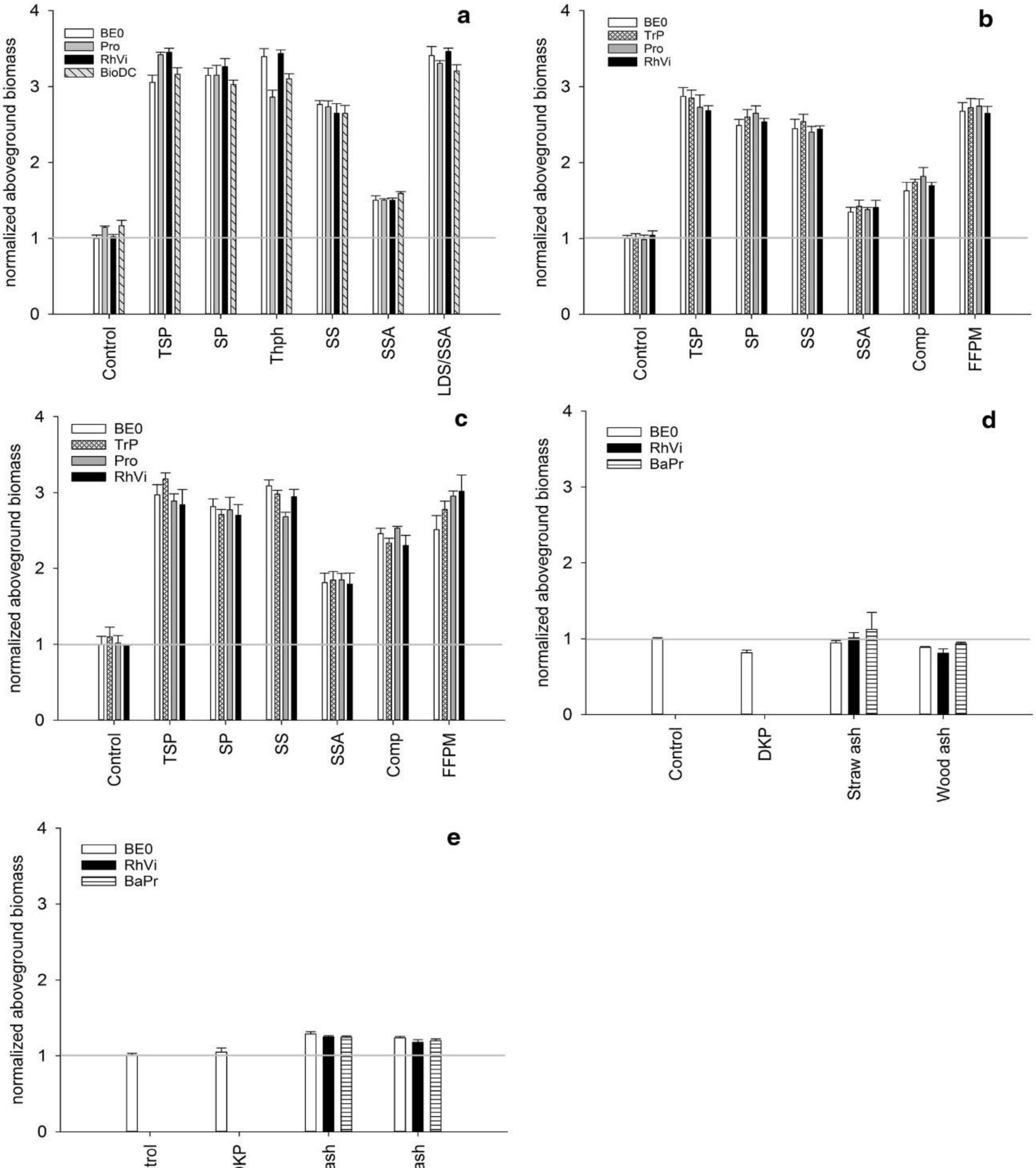

Fig. 1 Normalised aboveground biomass of wheat plants in the HK Kalke (**a**), UCPH (**b**, **c**) and CULS (**d**, **e**) experiments. The data are normalised by dividing by the mean of the control treatment (no P fertiliser and BE0) in each experiment and in each of the two soils in **d** and **e**. The following P fertilisers were added in the experiments: no P fertiliser (Control), triple superphosphate (TSP), superphosphate (SP), Thomas phosphate (Thph), sewage sludge (SS), sewage sludge ash (SSA), compost of sewage sludge and garden/park waste (Comp), P-enriched steelmaking slag (LDS/SSA), fibre fraction of pig manure (FFPM), K_2HPO_4 (DKP), straw ash and wood ash. The soil was either not inoculated (BE0) or inoculated with Proradix (Pro), RhizoVital (RhVi), biological fertiliser DC (Bio-DC), Trianum P (TrP) or BactoProf (BaPr). Data in **b** are for four plants harvested from each pot after 32 days, while data in **c** are for five plants harvested after 54 days. The Humpolec soil was used in **d** while the Poděbrady soil was used in **e**

Table 6 P content in aboveground biomass (mg P kg^{-1} soil) in the HK Kalke (a), UCPH (b) and CULS (c) experiments

a

P fertilizer	Tukey's test (BE0 results)[a]	Control (BE0)	Pro	RhVi	Bio-DC
Control (P0)	a	3.8	***5.4***	***4.7***	4.3
TSP	c	8.9	10.5	10.2	9.5
Superphosphate	c	9.4	9.6	9.4	8.6
Sewage sludge	c	8.7	9.8	9.8	7.8
Sewage sludge ashes	b	5.3	6.1	6.1	*4.2*
Thomas phosphate	c	10.3	*7.5*	10.7	8.5
LDS/SSA	c	9.3	9.0	9.1	8.0

b

	Tukey's test (BE0 results)	Control (BE0)	TrP	Pro	RhVi
Sewage sludge ashes	n.s.	5.9	5.8	6.1	5.9
FFPM	n.s.	4.1	6.2	5.6	6.1

c

P fertilizer	Tukey's test (BE0 results)[a]	Control (BE0)	RhVi	BaPr
Humpolec soil				
Control (P0)	a	11.2	n.a.	n.a.
DKP	a	10.4	n.a.	n.a.
Straw ash	b	13.5	14.4	16.5
Wood ash	a	11.3	10.2	12.4
Poděbrady soil				
Control (P0)	A	12.4	n.a.	n.a.
DKP	A	14.4	n.a.	n.a.
Straw ash	B	19.8	20.2	19.1
Wood ash	AB	15.6	14.2	14.9

a Data were analysed by two-way ANOVA. Data were log-transformed prior to statistical analysis due to unequal variances (Brown–Forsythe test, $P < 0.05$). Tukey's post hoc test was used to test whether there was a significant difference in P uptake between the different P fertilisers within the BE0 treatment. For each P fertiliser, values in bold italics are significantly higher than the BE0 treatment and values in italics are significantly lower than the BE0 control according to Dunnett's test

b Data were log-transformed prior to statistical analysis due to unequal variances (Brown–Forsythe test, $P < 0.05$)

c For each of the two soils independently, Tukey's post hoc test was used to test if there was a significant difference in P uptake between the different P fertilisers within the BE0 treatment. Small letters indicate differences for the Humpolec soil, while capital letters indicate differences within the Poděbrady soil. For each row, values in bold italics are significantly higher than the BE0 treatment and values in italics are significantly lower than the BE0 control according to Dunnett's test (one-way ANOVA with data for straw ash and wood ash)

[a] Different letters indicate means that are significantly different

ash resulted in a significantly higher biomass at harvest in the Poděbrady soil (Dunnett's test $P < 0.05$), while the biomass after adding straw ash was not significantly different from the control without the addition of P fertiliser in the Humpolec soil (Dunnett's test $P > 0.05$). Finally, the addition of wood ash led to a significantly lower biomass compared to the control in the Humpolec soil (Dunnett's test $P < 0.05$), while in contrast the addition of wood ash led to an increase in biomass compared to the control for the Poděbrady soil (Dunnett's test $P < 0.05$). Overall, there was only a fairly limited difference in the harvested biomass in a comparison across P-fertilisation treatments (Fig. 1d, e). The maximum increase observed when looking across the BE0 treatments was 22 %. This increase was observed for both straw and wood ash in the Poděbrady soil. The effect of the addition of the two different ash types (straw and wood ash) in combination with the different BE inoculation treatments included here (BE0, RhVi, BaPr) was analysed in two separate two-way ANOVAs for the two soils (Humpolec & Poděbrady) included in this experiment. No significant effect was observed of ash type or BE addition or an interaction between the two factors for any of the two soils investigated ($P > 0.05$, two-way ANOVA). There was a significant effect of soil on P uptake in the P0/BE0 treatment (Table 6c, one-way ANOVA, $P < 0.001$). The data for P content (Table 6c) were subsequently analysed for the two soils independently, and a significantly higher P content was observed in the straw ash treatment compared to the control in both soils (Tukey's test, $P < 0.05$), but the

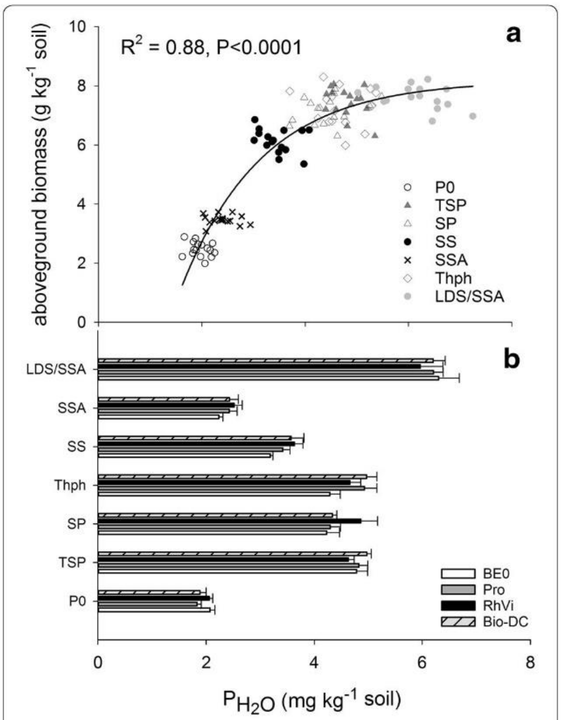

Fig. 2 **a** Total aboveground biomass of wheat plants in the KALKE experiment as a function of P_{H_2O} measured after 27 days and **b** the measured values of P_{H_2O} across P fertilisers and BE treatments. In **a,** data are fitted to three-parameter functions of the form $y = y_0 + a \cdot \left[1 - e^{(-b \cdot x)}\right]$. In **b,** each *bar* represents the mean of data from four replicate samples. *Error bars* represent SEM. The following P fertilisers were added in the experiment: no P fertiliser (P0), TSP, superphosphate (SP), sewage sludge (SS), sewage sludge ash (SSA), Thomas phosphate (Thph), P-enriched LD slag (LDS/SSA)

BE treatments did not result in a total P content that was significantly different from the control (Dunnett's test, $P > 0.05$).

Soil-available P in the KALKE experiment and relationship with biomass

Water-extractable P (P_{H2O}) was able to explain a large part of the variation in the aboveground biomass in the HK Kalke experiment across P-fertilisation treatments and BE treatments (Fig. 2a). Using the Mitscherlich equation, a model with P_{H2O} measured after 27 days explained 88 % of the variation in the aboveground biomass (Fig. 2a, $R^2 = 0.88$, $P < 0.0001$). The water-extractable P in soil was significantly different between the P-fertiliser treatments, and all P-fertiliser treatments were significantly different from the control (Fig. 2b, two-way ANOVA, $P < 0.001$). The sequence was as follows: P0 < SSA < SS < TSP = SP = Thph < LDS/SSA. There was no significant effect of BE inoculation on the level of water-extractable P in the pots (two-way ANOVA, $P > 0.05$) and there was no significant interaction between the two factors (two-way ANOVA, $P > 0.05$).

Correlation between plant P data and aboveground biomass

The variation in the aboveground biomass in the UCPH experiment in the BE0 treatments at both harvests (after 32 and 54 days) was explained by the P concentration in the youngest fully developed leaf harvested from one plant during early growth after 25 days. Using the three-parameter exponential rise to maximum (Mitscherlich) curve equation, highly significant relationships between the two variables were found (Fig. 3: first harvest, $R^2 = 0.73$, $P < 0.0001$; second harvest, $R^2 = 0.78$, $P < 0.0001$).

Fertiliser use efficiencies

Thomas phosphate and sewage sludge ash-enriched BOF slag both had a relative fertiliser efficiency and relative P-use efficiency comparable to or higher than TSP (Table 7). Sewage sludge tended towards slightly lower relative efficiencies compared to TSP (76–106 %), but these differences were only significant in the follow-up experiment at UCPH (Table 7). Sewage sludge ash, on the other hand, gave quite low efficiencies (24–31 %) and

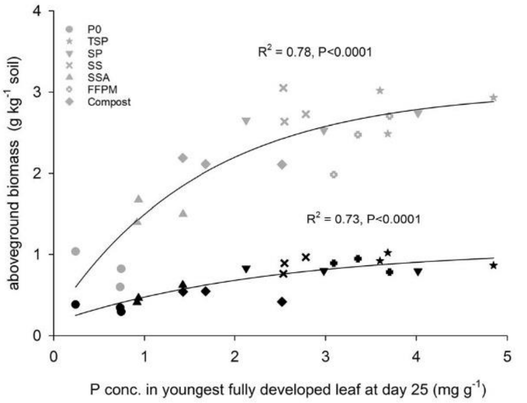

Fig. 3 Total aboveground biomass of wheat plants in the UCPH experiment from the first harvest (32 days after sowing, *black symbols*) or the second harvest (54 days after sowing, *grey symbols*) as a function of the P concentration in the youngest fully developed leaf from one plant measured at 25 days after sowing. Only data from three replicate pots were included for each treatment and only BE0 treatments were included. P treatments are: negative control (*P0*), *TSP*, low-grade TSP (*SP*), sewage sludge (*SS*), sewage sludge ashes (*SSA*), fibre fraction of pig manure (*FFPM*), compost of sewage sludge and garden/park waste (*Compost*). The data are fitted to three-parameter functions of the form $y = y_0 + a \cdot \left[1 - e^{(-b \cdot x)}\right]$

Table 7 Relative fertiliser efficiencies

P fertiliser	HK Kalke ($n = 4$)		UCPH ($n = 5$)		UPCH_2[a] ($n = 4$)
	FE (% of TSP)	PE (% of TSP)	FE (% of TSP), first harvest	FE (% of TSP), second harvest	FE (% of TSP)
Negative control	0 a	0 a	0 a	0 a	0
Triple superphosphate	100 cd	100 b	100 c	100 cd	100 c
Superphosphate	104 cd	110 b	79 c	92 cd	103 c
Thomas phosphate	117 d	127 b	n.a.	n.a.	n.a.
Sewage sludge	86 c	96 b	76 c	106 d	80 b
Sewage sludge ash	24 b	31 a	18 ab	41 b	36 a
Fibre fraction of pig manure	n.a	n.a.	93 c	77 c	n.a.
SSA-enriched LD slag	117 d	108 b	n.a.	n.a.	n.a.
Compost of sewage sludge and garden/park waste	n.a.	n.a.	33 b	74 c	54 a

The efficiencies are calculated for the BE0 treatment only. Efficiencies are calculated relative to TSP as the positive control (see "Methods"). For each column, different letters after the mean values represent significantly different means (Tukey's test, $P < 0.05$)

[a] This experiment was partly a replication of the UCPH experiment limited to five plants pot^{-1} (see Additional file 1 for details)

the relative P-use efficiency recorded in the HK Kalke experiment and the relative fertiliser efficiency recorded at the first harvest in the UCPH experiment were not significantly different from the negative control without the addition of a P fertiliser (Table 7). The compost included in the UCPH experiment had a relative fertiliser efficiency (33–74 %) between those of sewage sludge ash and sewage sludge (Table 7).

PCAs on plant compositional data

UCPH experiment

For the UCPH experiment, the elemental composition (B, Ca, Cu, Fe, K, Mg, Mn, P, S, Zn) was analysed in the youngest fully developed leaf sampled after 25 days from the different P-fertilisation treatments (all BE0). The composition was found to be very similar in treatments P1 (TSP), P4 (sewage sludge) and P6 (fibre fraction of pig manure), whereas the treatments P5 (sewage sludge ash) and particularly P0 (no P fertiliser added) were clearly separated in a PCA plot showing the first two principal components (Fig. 4a). The two groups of treatments were partly separated along the first principal component, but were more clearly separated along the second principal component. The loadings of the second principal component (Fig. 4b) showed that the most negative loading seen was for the element P followed by Zn and K.

HK Kalke experiment

The samples were grouped according to the P fertiliser applied along the first principal component in a PCA on the elemental composition (Ca, K, Mg, Mn, Na, P) of the aboveground biomass from the final harvest in the HK Kalke experiment (Fig. 5a). There was also a tendency towards a grouping along the second principal component due to the different BE inoculations across P-fertiliser treatments (Fig. 5b). Thus Proradix treatment was separated from the uninoculated control (BE0) in this plot. When looking at the loadings of the second principal component (Fig. 5c), higher concentrations of Mn in the BE0 plants were observed to be important for the separation in elemental composition between BE0 and Proradix plants.

CULS experiment

There was a clear grouping of pots according to the P fertiliser applied when concentrations of Ca, K, Mg, Mn, Na and K in leaves, stems and grain were used in a PCA (Fig. 6a). The clearest separation was between plants that had received no P fertiliser (P0) and plants that had received straw ash (StA). The treatments were primarily separated along the second principal component, which explained 30.8 % of the variation in the dataset. The loading plot of PC2 (Fig. 6d) shows that higher concentrations of P, K and Mn were especially important for the grouping of samples along the second principal component and that plants that had received straw ash as a fertiliser generally contained higher concentrations of P and K in the three tissues investigated compared to the remaining treatments, while higher concentrations of Mn in plant tissues pulled the samples that had received P0, DKP and WoA in the opposite direction in the PCA plot (Fig. 6a, d). The samples were grouped along the first principal component according to soil (Humpolec or Poděbrady), where higher concentrations of P and Mn were generally observed in plants grown in the Humpolec soil, while higher concentrations of Ca and Mg were recorded in plants grown in the Poděbrady soil (Fig. 6a, c).

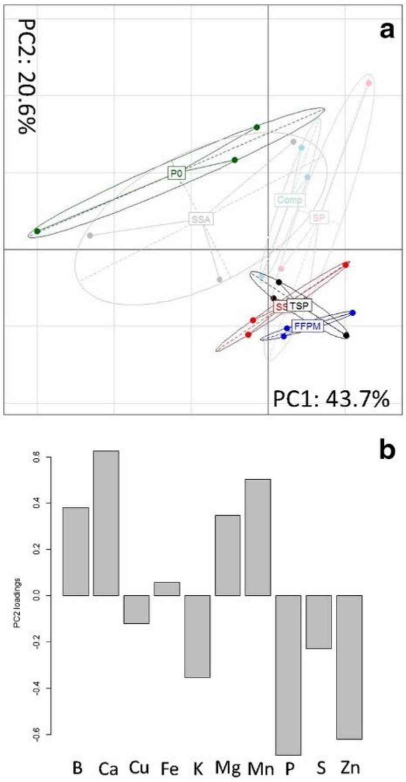

Fig. 4 Score plot (**a**) showing the result of a PCA on the elemental (B, Ca, Cu, Fe, K, Mg, Mn, P, S, Zn) composition of the youngest fully developed leaf measured at 25 days after sowing in the UCPH experiment. Loading plot (**b**) showing the loadings of the second principal component. Data are from three replicates of the P-fertilisation treatments P0 (control), TSP, SP (superphosphate), SS (sewage sludge), SSA (sewage sludge ash), FFPM (fibre fraction of pig manure) and Comp (compost of sewage sludge and garden/park waste). Pots were not amended with a BE (BE0)

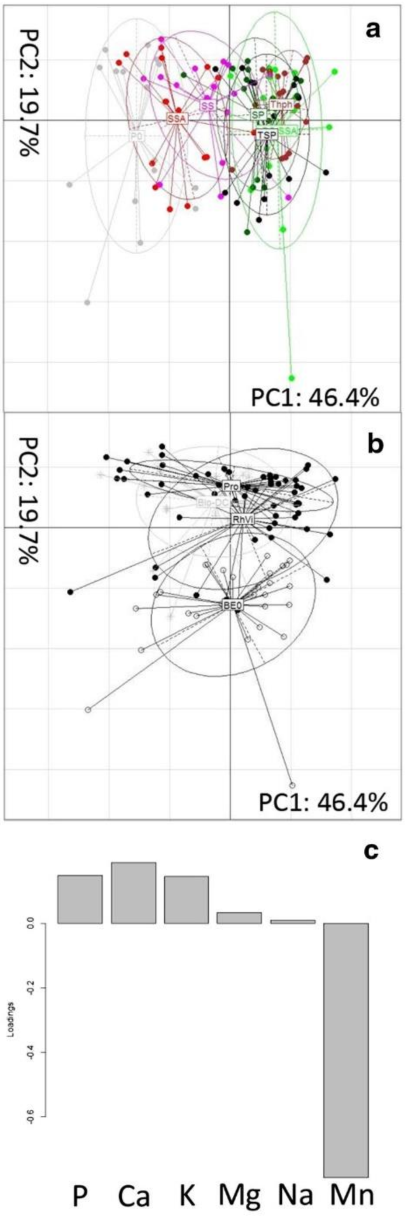

Fig. 5 Score plots (**a**, **b**) showing the result of a PCA on the elemental (Ca, K, Mg, Mn, Na, P) composition of the aboveground biomass grouped either according to P fertiliser applied (**a**) or BE inoculation (**b**). Loading plot (**c**) showing the loadings of the second principal component. Data are from the HK Kalke experiment. Codes for P fertilisers in **a** negative control (P0), TSP (TSP), superphosphate (SP), Thomas phosphate (Thph), sewage sludge (SS), sewage sludge ash (SSA), SSA-enriched LD slag (LDS/SSA, light green points)

Discussion

Was P the limiting factor in these experiments?

These pot experiments were undertaken on the assumption that P was the limiting factor in these trials. In the case of the UCPH experiment, the clear saturation-type relationship between P concentration in the youngest fully developed leaf during early growth and the subsequent biomass production (Fig. 3) served as validation that P limitation was in fact being studied in the UCPH experiment. Furthermore, the concentration of P recorded in leaves from the unfertilized treatment (Additional file 1: Table S3) was as low as 0.24 mg g^{-1} in one case and therefore probably within the deficiency

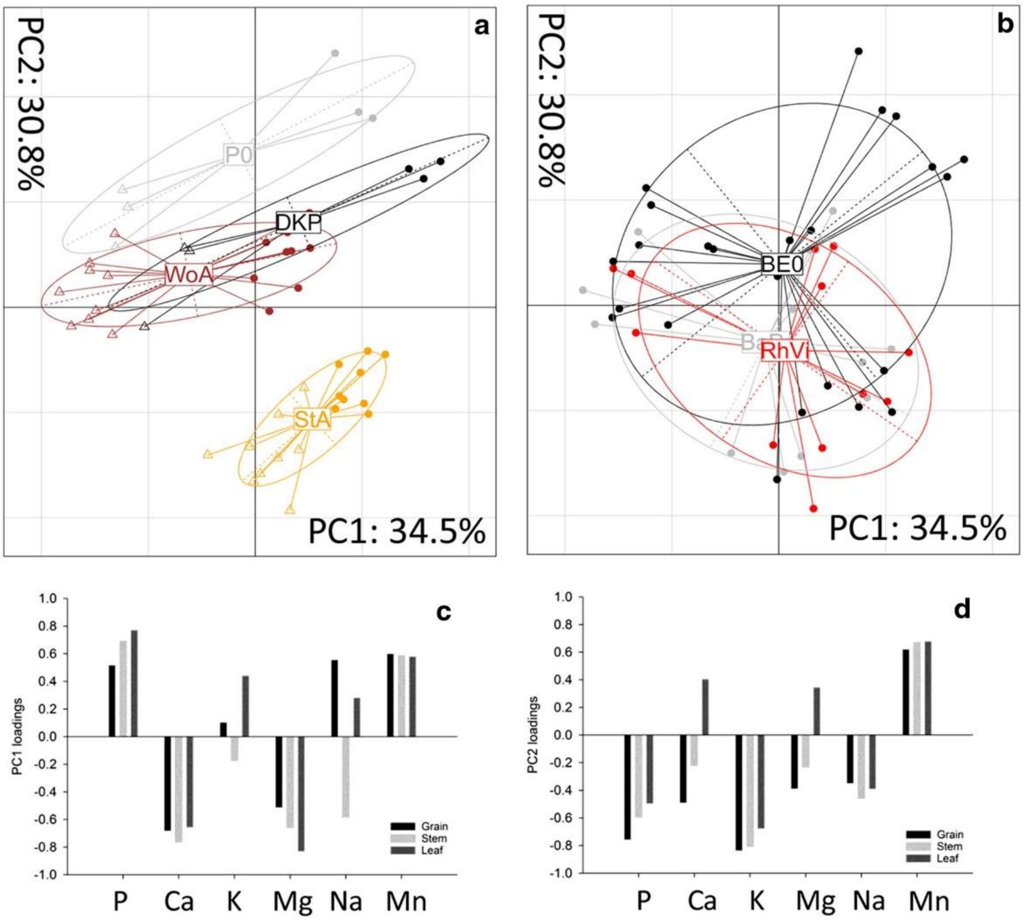

Fig. 6 Score plots (**a**, **b**) showing the result of a PCA on the elemental (Ca, K, Mg, Mn, Na, P) composition of the grain, stem and leaves. Samples are grouped either according to the P fertiliser applied (**a**) or BE inoculation (**b**). In **a** *filled circles* represent samples from the Humpolec soil, while *open triangles* represent samples from the Poděbrady soil. Loading plot (**c**) showing the loadings of the first principal component. Loading plot (**d**) showing the loadings of the second principal component. Data are from the CULS experiment

range at this stage [54]. Along the same lines, the clear relationships between soil P status and aboveground biomass in the HK Kalke experiment (Fig. 2) was validation that P was the limiting nutrient in the experiment. Here, we did not observe a positive response of P fertilisation on P concentration which shows that the P concentration of the whole shoot after 8 weeks of growth is not a robust measure of P deficiency. In contrast to the above, P could not be considered the sole limiting factor in the CULS experiment, since a positive growth response of adding readily soluble DKP (32 mg kg^{-1}) as the P fertiliser was not observed in this experiment. In general, the concentration of P was probably in the deficient range across all treatments (below 0.1 % in stem and leaves, Additional file 1: Table S4). This may partly be explained by a nitrogen limitation in the Humpolec soil, since soil solution nitrate levels in the Humpolec soil during the pot experiment were three times lower than those recorded in the Podêbrady soil (data not shown).

Were other nutrients limiting or present in toxic concentrations?

In the HK Kalke experiment, the concentrations of Ca, Mg, Ca, Mn and K (Additional file 1: Table S2) were in the adequate range for these elements in wheat shoots at the given growth stage [54].

In the UCPH experiment, the concentrations of Fe, K, S, Zn in the youngest fully developed leaf 25 days after sowing (Additional file 1: Table S3) were within the adequate range at this stage [54]. For B, Ca, Cu, S and Zn this was also generally the case (Additional file 1: Table S3), but the leaf from one of the three control plants analysed showed concentrations (see minimum in Additional file 1: Table S3) in the deficiency range [54]. For B, the highest concentration recorded (156 $\mu g\ g^{-1}$) might be at the limit of toxicity at this stage [54]. However, no clear symptoms were observed.

In the CULS experiment, the grain concentrations of K (Additional file 1: Table S4) indicated deficiency in this element across all treatments, while Mn (Additional file 1: Table S4) was in the adequate range for grain at maturity [54].

Did the added BEs enhance the availability of P from recycled fertiliser products?

As stated in the introduction, one possible mechanism for improving plant growth by a BE would be to increase the availability of P in the soil. When used in combination with recycled fertilisers, it is of interest whether or not the introduced organisms directly affect the solubilisation of the introduced P. In the HK Kalke experiment, no significant effect was observed for any of the tested BEs (Pro, RhVi, Bio-DC) on the level of available P in the soil (P_{H2O}). Since we do not have soil data for the other experiments, we cannot make claims regarding the soil P availability in these experiments. This is in accordance with previous studies showing that although microbial inoculants may demonstrate potential for solubilisation of sparingly soluble P sources (such as Ca-phosphates) in vitro, this does not necessarily translate into increased plant availability of P in the soil [55]. In the present study, there was no support for an increase of plant-available P in the soil as a result of inoculation with two bacterial products (Proradix and RhizoVital 42) and one fungal product (Biological fertiliser DC). There may be several possible explanations for the lack of a significant positive effect on P availability: (i) a limited proliferation of the introduced microorganisms in soil due to competition with native microorganisms, for example, (ii) the soil P level may not have been sufficiently low to promote the up-regulation of enzymes involved in P solubilisation, (iii) released P may have been taken up by the introduced microorganisms without subsequent release to the soil within the time frame of the experiments and finally (iv) the native microbial community of the soil and/or organic waste materials may have been optimal already in making P available from the introduced fertilizers.

Did the added bioeffectors affect the growth of plants and plant P uptake?

Despite previous reports that the tested organisms may enhance plant growth [30, 43, 46], only a small positive effect on aboveground biomass of Pro and RhVi in combination with TSP was found (Fig. 1a). The fact that there was only a positive effect in combination with TSP as a fertiliser may point towards a direct effect of the BEs on the plants rather than an effect on P availability in the soil. This interpretation was also supported by the fact that the uptake of P from TSP-fertilised soil was not significantly different between BE treatments (Table 6a). The direct effects of these microbial inoculants on the plants are in line with earlier work showing that Pro and RhVi may elicit defence responses in plants [41, 56], thus directly affecting the plant's metabolism. In the P0 treatment, a positive effect of Pro and RhVi was observed on the total P content of the aboveground biomass, which seemed to indicate that under these P-limited conditions the two BEs did improve plant P uptake, even though a BE-mediated increase in P_{H2O} was not observed.

As a prerequisite for an effect of BEs on the growth of wheat plants, the successful establishment of organisms in the rhizosphere may be required, and it has been stated that rhizosphere competence may be a key factor in the effectiveness of PGPM [57, 58]. On the other hand, there is also an example of a study where the supernatant of the culture medium in which *T. harzianum* T22 was grown resulted in a stronger effect on the growth of maize plants compared to inoculating with spores [30]. This indicates that active growth in the rhizosphere may not always be a prerequisite for an effect of a PGPM and that a direct hormonal effect on the plants is a possible mode of action of these organisms. The present study did not measure whether the microorganisms established themselves in the rhizosphere of the wheat plants, meaning that it cannot be ruled out that the lack of a plant growth-promoting effect of the added BEs was due to an unsuccessful colonisation of the wheat rhizosphere. On the other hand, the fact that a significant BE effect was seen on the elemental composition of the aboveground biomass in the HK Kalke experiment may be an indication that the added microorganisms were in fact able to establish in the wheat rhizosphere in these pot experiments. In the CULS experiment, the plant elemental composition of the aboveground biomass did not give any indication of a BE effect.

Do the different recycled fertiliser products tested have potential as P fertilisers?

A low availability of P in the soil after fertilisation with sewage sludge ash was observed, which translated into a relative fertiliser efficiency based on biomass production of 24–41 % and P uptake of 31 %. This result was in line with earlier work, showing that phosphorus in sewage sludge ash is generally not readily taken up by plants [9]. On the other hand, there may be considerable variations between different sewage sludge ashes, depending on the processing of sewage sludge in the water treatment plant [7]. Sewage sludge, Thomas phosphate and sewage sludge-enriched BOF slag (LDS/SSA) all resulted in levels of available P similar to or higher than TSP. In fact, fertilisation with LDS/SSA resulted in a significantly higher level of P_{H2O} compared to TSP. This was probably related to an increase in soil pH from ~5.6 in the TSP treatment to ~6.5 in the LDS/SSA treatment (Additional file 1: Table S1), since the availability of phosphates in soil is generally highest close to neutrality [59]. Severin et al. [9] found that the LDS/SSA product had high efficiency as a P fertiliser [9] in accordance with this study's results, yielding a P-fertilisation effect comparable to TSP. This shows the potential of this technology to produce a highly effective P fertiliser, partly based on sewage sludge devoid of any organic contaminants. However, the content of heavy metals could potentially be problematic. The content of Cr (1712 mg kg^{-1}, data not shown) for instance is above the current Danish limits [60], while in Germany contents above 300 mg kg^{-1} have to be declared [61]. An alternative to using sewage sludge ash could be to use sewage sludge as a fertiliser instead. Concerns may be raised regarding organic contaminants and problematic microorganisms, which are not relevant in the case of sewage sludge ash. However, organic contaminants probably do not pose a great threat here when the quality of present-day sewage sludge is taken into account [4]. In the present study, sewage sludge was observed to possess high potential as a P fertiliser, resulting in responses that are 76–106 % of those observed when using TSP. This was in relatively good agreement with a pot trial using English ryegrass in which the efficiency of different sludges was 62–86 % of monocalcium phosphate [62]. In the case of wood and straw ash, it was not possible to clearly evaluate their potential as P fertilisers based on the results presented here. This was due to the fact that (i) the CULS experiment lacked a positive control with the addition of a comparable level of total P and (ii) the input of P with the two different ash types was different. These problems aside, from the results presented here, it would not appear that wood ash and straw ash have great potential as P fertilisers, since the relative increase in biomass yield was not above 25 % in comparison to the HK Kalke and UCPH experiments showing yield increases of 50 % or more, even for sewage sludge ash. This result contradicted an earlier study in which a high P-fertilisation effect was found for rape meal, straw and cereal ashes [63]. However, as observed from the PCA plot, a small effect was observed on the plant elemental composition due to the wood ash and DKP treatments and greater effect of the straw ash treatment, but these differences were not clearly associated with differences in the aboveground biomass. These effects were observed to be independent of soil type. The fibre fraction of pig manure (FFPM) prepared using a decanter centrifuge was shown to have a high fertiliser efficiency that was not significantly lower than the positive TSP control. This was in accordance with previous results showing a high P availability after application of this solid manure fraction to soil [64].

Conclusions

Based on the results from the HK Kalke experiment, we did not find evidence to support the hypothesis that BE products increase the availability of P in the soil. Furthermore, the BE products only had a very limited effect on the growth of wheat plants across all experiments. Further work is therefore needed to elucidate whether inoculation with BEs has agronomic potential in wheat production. A number of the tested recycled P-fertiliser products (sewage sludge, P-enriched BOF slag and fibre fraction of pig manure) were shown in the HK Kalke and UCPH experiments to have a high potential as P fertilisers without a requirement for further processing.

Additional file

Additional file 1. Fig. S1. Biomass in the UCPH follow-up experiment. **Table S1.** Data on soil pH from the HK Kalke experiment and **Tables S2–S4**. Data on plant elemental composition from the HK Kalke (**Table S2**), UCPH (**Table S3**) and CULS (**Table S4**) experiments.

Authors' contributions

JDSL carried out the UCPH experiments, performed the majority of the data analysis in the paper and wrote the paper. MR carried out the HK Kalke experiment, contributed to data analysis and discussions of data. FM carried out the CULS experiment and performed the plant analyses for this experiment. MK supervised the analyses in the CULS experiment. PT supervised the experimental design in the CULS experiment. JM contributed to discussions regarding data interpretation. AN contributed to experimental design, data interpretation and the writing of the paper. All authors contributed to initial discussions of data. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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18) Soil pH changes in long-term field experiments with different fertilizing systems

Změny půdního pH v dlouhodobých pokusech s různými systémy hnojení

Změny půdního pH bezprostředně souvisí s přístupností fosforu. Proto byla do výběru publikací zařazena i tato studie, zabývající se změnami hodnot pH půdy způsobenými různými systémy hnojení v dlouhodobých přesných polních pokusech. Cílem bylo vyhodnotit změny pH způsobené zejména aplikací různých hnojiv. V literatuře je zmiňována jak negativní role zejména minerálních hnojiv (Hoyt et Hennig 1982, Malhi et al. 1998), tak pozitivní vliv aplikace organického hnojiva – chlévského hnoje (Repsiene et Skuodiene 2010, Leahu et al. 2011). Za tímto účelem byla do výzkumu zařazena 4 experimentální stanoviště – Červený Újezd, Hněvčeves, Lukavec a Praha-Suchdol. Na těchto stanovištích byly vyhodnoceny následující varianty: 1) nehnojená kontrola, 2) chlévský hnůj, 3) čistírenské kaly, 4) minerální dusík, 5) minerální N v kombinaci se slámou, 4) minerální NPK. U těchto variant bylo vždy stanoveno výměnné pH v ornici (0-30 cm). Z výsledků je zřejmý zejména silný vliv půdních podmínek stanoviště. Na černozemi stanoviště Suchdol (přirozeně vysoké pH a silná pufrační kapacita) prakticky nedošlo k acidifikaci půdy působením minerálního dusíku za 14 let trvání pokusu. Naopak u zbývajících stanovišť je patrný pokles pH u všech sledovaných variant (s výjimkou Hněvčevse, kde nedošlo ke změně pH u varianty hnojené minerálním dusíkem). Najvýraznější pokles hodnoty pH byl zaznamenán u variant minerální NPK a N + sláma na stanovišti Hněvčeves. V případě čistírenských kalů a chlévského hnoje došlo k výraznému poklesu pH půdy na stanovišti Červený Újezd. Stanoviště Lukavec vykazovalo nejvýraznější pokles pH u nehnojené kontroly. Výsledky studie jasně ukazují na fakt, že při hodnocení půdního pH musí být zahrnuta řada dalších působících faktorů (např. textura půdy, proces mineralizace, hnojení, obsah mobilního hliníku aj.).

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Soil pH Changes in Long-Term Field Experiments with Different Fertilizing Systems

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Abstract

Vašák F., Černý J., Buráňová Š., Kulhánek M., Balík J. (2015): Soil pH changes in long-term field experiments with different fertilizing systems. Soil & Water Res., 10: 19–23.

The changes of soil pH in long-term 14-year field experiments with different fertilizing systems are described. The field experiments were located at four sites of the Czech Republic with different soil and climatic conditions (Červený Újezd, Hněvčeves, Lukavec, and Prague-Suchdol). At each site, the same fertilizing systems and crop rotation (potatoes – winter wheat – spring barley) were established. Six experimental treatments were applied to crop rotation: (1) unfertilized treatments (control); treatments with organic fertilization: (2) farmyard manure (FYM), (3) sewage sludge (SS); treatments with mineral fertilizers: (4) nitrogen (N), (5) nitrogen with straw application (N + straw), and (6) nitrogen with phosphorus and potassium (NPK). The long-term effect of fertilizers significantly depends on soil conditions. At the site Prague-Suchdol minimal differences in the soil pH were observed by all treatments. This is caused by the high buffering capacity of Chernozems against the soil acidification. At Červený Újezd (Haplic Luvisol), Hněvčeves (Haplic Luvisol), and Lukavec (Stagnic Cambisol) sites, soil pH decreased by all treatments. Only at Hněvčeves site the soil pH did not change with N treatment. The highest soil pH decrease in the treatment with NPK (ΔpH –0.89) and N + straw (ΔpH –0.70) was observed at Hněvčeves site. By the treatments FYM and SS the highest decrease was registered at Červený Újezd (ΔpH of about –0.30 and –0.63, respectively). The highest decrease in control treatment (ΔpH of about –0.63) was observed at Lukavec site. The results showed that to evaluate long-term soil pH changes a whole complex of factors must be examined.

Keywords: acidification; crop rotation; mineral fertilization; organic fertilization; soil type

Soil pH is widely accepted as a dominant factor that regulates soil nutrient bioavailability, vegetation community structure, plant primary productivity, and a range of soil processes including soil microbial community structure and activity (Robson 1989). All the soil properties and the value of the soil pH can widely differ in reliance on soil type, topography, climate, vegetation, and anthropogenic activity, because all these factors influence the spatial variability of the observed soil types (Shi *et al.* 2009). The value of soil pH is directly influenced by all five soil-forming factors (parent rock, climatic conditions, organisms, topography, and time) and further the value of soil pH is dependent on the season influence, way of management, tested soil horizon, soil water contents, and time limit of sampling for analysis (Troeh & Thompson 2005).

The combined application of manure and mineral fertilizer has major effects on soil physical, chemical, and biological properties and it increases crop yields (Hou *et al.* 2012). Application of fertilizers is one of the causes of soil acidification (Hoyt & Hennig 1982). The acidification of soil by N fertilizer is caused by transformation of nitrogen in soil. The uptake of N as ammonium in the crop also contributes to soil acidification (Malhi *et al.* 1998).

Farmyard manure (FYM) is the main fertilizer maintaining soil potential fertility because with its application the soil receives biogenic elements that are assimilated by growing crops and thus removed from the soil (Repsiene & Skuodiene 2010). The manure application increases also soil pH (Ondrášek & Čunderlík 2008). Increasing of pH depends on the dose and timing of FYM application. Leahu *et al.* (2011) found during the experiment that the soil pH decreased for both versions: the control variant and the variants fertilized only with mineral fertilizers or using both mineral fertilizers and manure.

The aim of the study is to compare the change in the soil pH between treatments with mineral, organic fertilizers and unfertilized treatments applied during 14-years of experiments.

MATERIAL AND METHODS

Changes in the values of the soil reaction in the course of long-term field experiments at four sites – Červený Újezd, Prague-Suchdol, Hněvčeves, and Lukavec with different soil and climatic characteristics were monitored. Climatic and soil conditions are showed in Table 1. Contents of plant available nutrients in soil were published by Černý *et al.* (2010).

The same fertilizing systems with organic, mineral, and unfertilized control treatments are used at all sites. Potatoes, winter wheat, and spring barley are grown in crop rotation. Only at Červený Újezd silage maize is included to the crop rotation instead of potatoes because of agronomical conditions of the experimental site. Organic fertilizer (silage maize) is applied each autumn before planting the potatoes. Nitrogen is applied in the form of calcium ammonium nitrate. The area of the experimental plots at Hněvčeves and Lukavec is the same – 60 m^2, at Suchdol the area of the plot is 60.5 m^2, and at Červený Újezd it is 80 m^2. At all the sites crops are rotated in the sequence potatoes – winter wheat – spring barley. Fertilizing system and doses of nutrients during crop rotation are shown in Table 2.

Soil pH values were determined from the composite soil samples taken at establishment experiments before the first application of fertilizer in 1996. Soil pH was measured after the end of the crops rotation (i.e. after spring barley) in the years 2004 and 2010. Soil pH value of 2004 corresponds with the changes between 1996 and 2010 and therefore samples from 2001 and 2007 were not evaluated. The composite soil sample consisted of ten subsamples collected throughout the plot to the depth of 30 cm. Soil samples were oven-dried at the constant temperature of 30°C. Dry soil samples were sieved through a 2-mm mesh. Soil pH was determined in 0.2 mol/l KCl 2:5 w/v according to the methodology of the Central Institute for Supervising and Testing in Agriculture (Zbíral 2001). Each sample was measured four times. The soil reaction was measured using a pH meter WTW pH340i and a glass electrode WTW SenTix 21 (both devices WTW, Weilheim, Germany).

Table 1. Soil and climatic conditions

Sites	Červený Újezd	Hněvčeves	Lukavec	Prague-Suchdol
Location	50°4'22"N, 14°10'19"E	50°18'46"N, 15°43'3"E	49°33'23"N, 14°58'39"E	50°7'40"N, 14°22'33"E
Elevation (m)	398	265	610	286
Average annual temperature (°C)	7.7	8.2	7.7	9.1
Average annual rainfall (mm)	493	573	666	495
Soil type (WRB 2006)	Haplic Luvisol	Haplic Luvisol	Stagnic Cambisol	Haplic Chernozem
NRSC USDA	silty loam	silty loam	sandy loam	silty loam
CEC (mmol(+)/kg)	145	179	128	230
C_{ox} (%)	1.17	1.06	1.27	1.55
Clay (%) (< 0.002 mm)	5.4	4.4	3.2	2.2
Silt (%) (0.002–0.05 mm)	68.1	77.0	37.1	71.8
Sand (%) (0.05–2 mm)	26.5	18.7	59.7	26.0

CEC – cation exchange capacity

Table 2. Doses of nutrients during crop rotation (kg/ha)

Treatment	Potatoes/maize			Wheat			Barley		
	N	P	K	N	P	K	N	P	K
Control	–	–	–	–	–	–	–	–	–
SS	330[1]	201[2]	55[2]	0	0	0	0	0	0
FYM	330[1]	118[2]	374[2]	0	0	0	0	0	0
N	120	0	0	140	0	0	70	0	0
NPK	120	30	100	140	30	100	70	30	100
N + straw	138	6[2]	47[2]	140	0	0	70	0	0

[1]total nitrogen in organic fertilizers; [2]average dose of the fertilizers nutrient contents; SS – sewage sludge; FYM – farmyard manure; NPK – nitrogen with phosphorus and potassium

RESULTS AND DISCUSSION

The measured pH values of 1996 and 2004 from all experimental sites are presented in Table 3. Figure 1 shows minimal changes in soil pH of Chernozem at the site Prague-Suchdol. The minimal changes in all variants are caused by the higher buffering capacity against soil acidification. The same result was published by Yang *et al.* (2007). In his 22-year field experiment with mineral and organic fertilizers in soil with high carbonate content no change in the soil pH was observed.

In the control unfertilized treatments there was a decrease in the soil pH at all experimental sites (Table 4). The biggest decrease was ascertained at Lukavec, which is probably caused by lighter soil and lower sorption capacity. The decrease in pH is likely caused by leaching of basic ions and removal of basic ions by plants. For Ca and Mg leaching the annual rainfall is important. Lysimeter experiments conducted in the Czech Republic indicated that the annual rainfall higher than 600 mm may lead to calcium loss of about 27 kg/ha per year (Klement & Prchalová 2013). Lowering of pH in the control unfertilized treatment after a one-year observation was also documented by Arvas *et al.* (2011).

In the same study, pH reduction in treatments with sewage sludge application was traced after a one-year observation (Arvas *et al.* 2011). A slight reduction in pH was observed also by Tsadilas and Samaras (1999) after two years of experiments with sewage sludge applications. Forsberg and Ledin (2005) explain the pH decrease after the application of the sewage sludge by the buffering capacity reduction. The pH decrease magnitudes depend on the sludge application dose (Navas *et al.* 1998). This corresponds with the observed decreases of soil pH at the sites Červený Újezd, Hněvčeves, and Lukavec (Table 4).

At the present long-term field experiment the stabilizing effect of FYM on soil pH was not proved and after 14-years of FYM application pH decreased at all the sites (Table 4). The largest decrease was observed at Červený Újezd. However, the soil treated for 14 years with FYM exhibited higher pH than that treated with NPK at all the sites and with N at the sites Červený Újezd, Hněvčeves, and Lukavec. The lower soil pH decrease in treatments where organic fertilizers instead of mineral were applied is caused by the incorporation of organic matter in the soil minimizing pH fluctuation, which is consistent with the results of the 21-year-old experiment by Zhong *et al.* (2010). Bednarek *et al.* (2012), after 26 years of experiments, found higher pH and higher organic matter content by the soils with manure application

Table 3. Soil pH values detected at the time of setting up the experiment (1996) and in 2004

Treatments	Červený Újezd		Hněvčeves		Lukavec		Praha-Suchdol	
	1996	2004	1996	2004	1996	2004	1996	2004
Control	6.19	6.09	5.99	6.00	5.37	4.87	7.13	6.97
SS	6.73	6.29	6.16	5.98	5.09	5.02	7.26	7.21
FYM	6.54	6.37	6.17	6.04	5.25	4.89	7.29	7.26
N	6.41	6.34	5.77	5.83	4.98	4.67	7.21	7.29
NPK	6.66	6.44	6.31	6.07	5.29	4.71	7.27	7.31
N + straw	6.87	6.70	6.20	6.08	5.22	4.86	7.30	7.33

SS – sewage sludge; FYM – farmyard manure; NPK – nitrogen with phosphorus and potassium

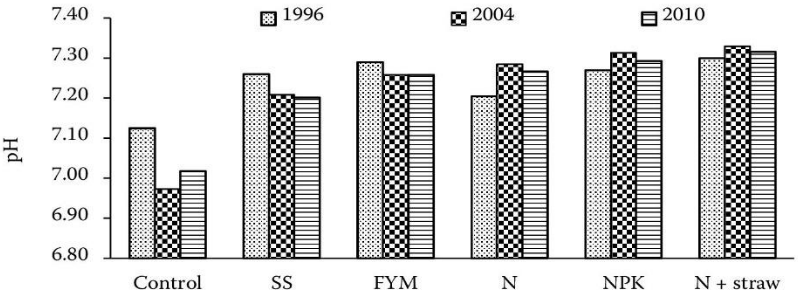

Figure 1. Soil pH_{KCl} at site Prague-Suchdol; SS – sewage sludge; FYM – farmyard manure; NPK – nitrogen with phosphorus and potassium

compared to those where mineral fertilizers (NPK) were applied.

Nitrogen fertilizers generally caused soil acidification. Irrespective of whether the NH_4^+-N is from ammonium nitrate, urea or anhydrous ammonia, the formed nitrate requires the same amount of compensating cations per unit of N applied. This requirement is theoretically 1.36 kg Ca/kg N (Gasser 1973). Practically Sluijsman (1970) calculated the acidity of nitrogen fertilizers separately for arable land and permanent grassland. The calculation includes only half the amount of nitrogen applied (50% utilization of N). Except for Lukavec, showing a quite significant pH reduction, no declines were recorded at the remaining sites (Table 4). This is probably caused by lower acidity of the nitrogen fertilizer with calcium. As for the nitrogen fertilizer acidity degree, Peryea and Burrows (1999) stated that ammonium nitrogen fertilizers containing calcium are among those causing the lowest acidification.

In the variant with the application of NPK, after 14 years of the experiments, there was a decrease in pH at all studied sites (Table 4) except for Prague-Suchdol. The highest decrease occurred at Hněvčeves. The soil acidification after the application of NPK corresponds with the results published by Makinde *et al.* (2009) who described the acidification after the application of NPK in Orthic Luvisol. The decrease in pH of 0.28 after the application dose of 300 kg NPK 15/15/15 on Luvisol was also published by Ayeni *et al.* (2012). Soil pH values during the experiment were also influenced by lowering sulphur deposition in the soil due to flue gas desulfurization in industry reducing the acidifying effect (Balík *et al.* 2009).

At Červený Újezd, Hněvčeves, and Lukavec sites, where nitrogen with straw was applied, the soil pH decreased (Table 4), which corresponds with the results stemming from the 27 long-term field experiments with ploughed straw with doses variants of 0, 25, 50, 75 kg N/ha/year. Soil pH decreased in all the variants, even in the variant with only ploughed straw (Malhi *et al.* 2011).

CONCLUSION

From the stated results it is evident that the evaluation of changes in soil pH must be assessed in relation to specific sites conditions. Chernozem at Prague-Suchdol site showed higher buffering capacity against acidification. The NPK treatment at Červený Újezd, Hněvčeves, and Lukavec sites caused higher soil pH decrease compared with the control treatment. The FYM treatment at the same sites led to lower soil pH decreases (or the N treatment at Hněvčeves) than the other treatments except for those with N. Mineral and organic fertilization proved to be one of the factors causing soil acidification.

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Table 4. Soil pH changes (ΔpH) after 14 years of experiments (1996–2010)

	Control	SS	FYM	N	NPK	N + straw
Červený Újezd	–0.38	–0.63	–0.30	–0.22	–0.46	–0.30
Hněvčeves	–0.25	–0.56	–0.21	0.03	–0.89	–0.70
Lukavec	–0.63	–0.22	–0.21	–0.46	–0.71	–0.40
Suchdol	–0.11	–0.06	–0.03	0.06	0.02	0.02

SS – sewage sludge; FYM – farmyard manure; NPK – nitrogen with phosphorus and potassium

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19) Influence of long-term fertilizer application on changes of the content of Mehlich 3 estimated soil macronutrients

Dlouhodobé hnojení a jeho vliv na obsah živin stanovených v půdě metodou Mehlich 3

Současný výzkum stále více ukazuje, jak důležité jsou výsledky z dlouhodobých pokusů (Černý et al. 2010, Hejcman et al. 2012 aj.). Přiložená publikace se proto zabývá vyhodnocením vlivu dlouhodobé aplikace různých hnojiv na obsah potenciálně přístupných makroprvků v půdě, stanovených metodou Mehlich 3. Pro vyhodnocení byla zvolena 2 stanoviště s rozdílnými půdně-klimaickými podmínkami (Humpolec a Praha-Suchdol), kde byly srovnávány 4 různé varianty: 1) nehnojená kontrola, 2) čistírenské kaly 3) chlévský hnůj a 4) minerální NPK. Metodou Mehlich 3 byly analyzovány půdy odebrané před založením pokusu a dále pak vzorky odebrané po třech letech (po dokončení osevní rotace brambory, pšwnice, ječmen), tzn. v letech 1999, 2002, 2005, 2008 a 2011. Výsledky potvrdily čistírenské kaly jako významný zdroj fosforu (Petersen 2003, Liu et al. 2010), a naopak nebyl zjištěn významný vstup K v tomto hnojivu. Hnůj se na obou půdách projevil jako důležitý zdroj potenciálně přístupného K a zároveň i hořčíku. Půdy po aplikaci čistírenských kalů vykazovaly i zvýšený obsah vápníku. To bylo pravděpodobně způsobeno přidáváním Ca při procesu stabilizace kalů. Souhrnně lze konstatovat, že chování aplikovaných hnojiv v půdě z hlediska obsahu potenciálně přístupných makroprvků bylo navzdory různým půdně-klimatickým podmínkám podobné. Při stejné hladině aplikovaného dusíku je zřejmé, že čistírenské kaly jsou významnějším zdrojem potenciálně přístupného fosforu a vápníku ve srovnání s chlévským hnojem. Hnůj byl naproti tomu lepším zdrojem hořčíku a draslíku. Varianta NPK v minerální podobě pravidelně vykazovala nejvyšší obsah potenciálně přístupného draslíku i přesto, že aplikovaná dávka K byla více než 3 krát nižší ve srovnání s chlévským hnojem.

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Influence of long-term fertilizer application on changes of the content of Mehlich-3 estimated soil macronutrients

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ABSTRACT

The aim of this work is to evaluate the changes of Mehlich 3 – P, K, Ca and Mg contents in soil during a long-term field experiments with sewage sludge, farmyard manure (FYM) and mineral NPK (NPK) application, compared to the control non-fertilized treatment. The experiment was established at the Humpolec and Suchdol sites (Czech Republic). Potatoes, wheat and barley were grown in crop rotation. Fertilizing system was based on the same nitrogen dose of 330 kg N/ha per one crop rotation. Archive soil samples from the beginning of the experiment (1996) and from the end of each year's crop rotation (1999, 2002, 2005, 2008 and 2011) were analyzed. In spite of the different soil-climatic conditions of the studied sites, very similar tendencies of P, K, Ca and Mg contents changes after the fertilizing systems used were observed in the soil. In case of the same nitrogen dose (330 kg N/ha), sewage sludge appeared to be better source of bioavailable soil phosphorus compared to the farmyard manure and NPK. On the contrary, FYM was a better source of bioavailable potassium and magnesium, despite the lower total magnesium content in FYM. The NPK treatment was the best long-term source of bioavailable potassium.

Keywords: soil; sewage sludge; farmyard manure; mineral NPK; bioavailable P, K, Ca, Mg

For the future it is important to understand the influence of fertilizer application on the nutrient dynamics in soil as well as its influence on the plant growth in more detail. The content of bioavailable nutrients in soils varies not only depending on the fertilizer used, but also on the seasonal influence, soil properties and many other factors. Therefore, the data of the long-term field experiments provide valuable information about the fertilizing optimization possibilities. The importance of long-term field experiments is mentioned in many papers (f.e. Johnston 1997, Černý et al. 2010, Hejcman et al. 2012).

This experiment is focused especially on the evaluation of long-term sewage sludge (SS) and farmyard manure (FYM) application on Mehlich-3 estimated macronutrients (P, K, Ca and Mg) content changes, compared to the mineral NPK (NPK) and control non-fertilized treatments. A positive effect of farmyard manure application on crop yield, soil properties and macronutrient content in soil was observed in numerous studies (Barzegar et al. 2002, Troeh and Thompson 2005, Bhattacharyya et al. 2007). Organic fertilizers may be replaced with straw left in the field after the harvest of cereals, although the effect of straw application on yield reported in literature is rather low (Powlson et al. 1985, Thomsen and Jensen 1994). Another alternative to the application of organic matter and nutrients may be fertilization with stabilized sewage sludge from waste water treatment plants. In numerous publications sewage sludge is described as a source of nitrogen and phosphorus (Petersen et al. 2003, Balík et al. 2007, Huang et al. 2012),

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which favor yield formation of crops (Christie et al. 2001). On the other hand, studies about the influence of sewage sludge on the other macronutrients content in soil and plants appear only seldom (Warman and Termeer 2005a,b, Chowaniak and Gondek 2009).

Mehlich-3 is a widely used method to extract plant-available phosphorus from soil over a wide range of pH value (Sims et al. 2002, Schroder et al. 2009, Zhang et al. 2009). It is one of the strongest extractants to estimate bioavailable nutrients from soils (Kulhánek et al. 2009). The method is also used by many laboratories to determine multiple plant-available nutrients simultaneously. Based on the interlaboratory tests it is possible to successfully use the method to determine K, Mg, Zn, Mn and Cu, respectively, but the results of Ca measuring are often not repeatable (Schroder et al. 2009).

The aim of this research work is to evaluate the influence of long-term application of different fertilizing systems (sewage sludge, farmyard manure and mineral NPK) on the Mehlich 3-bioavailable soil P, K, Ca and Mg contents changes.

MATERIAL AND METHODS

The long-term field experiment was based on experimental fields of the Czech University of Life Sciences in Prague and Crop Research Institute in Prague-Ruzyně, Czech Republic. The characteristic of the sites are given in Table 1.

Potatoes, winter wheat and spring barley were planted in crop rotation on small plots (60 m^2). Organic fertilizers were applied only to potatoes in the crop rotation. Therefore, the direct influence of organic fertilizers application could be observed on potatoes. For winter wheat and spring barley only the consequential influence could be observed. The total amounts of P, K, Ca and Mg applied per one crop rotation in different fertilizing treatments are summarized in Table 2.

For this experiment, sewage sludge from a wastewater treatment plant in Prague was used. The whole system of fertilizing is based on the uniform ratio of 330 kg N/ha per 3 years. That applies for organic and mineral fertilizers as well.

Archive air dried soil samples (< 2 mm) from the beginning of the experiment (1996) and from the end of each crop rotation (years 1999, 2002, 2005, 2008 and 2011) were chosen for the analysis. All the samples were taken after the harvest of the above mentioned crops and before organic fertilizer application. The soil samples were extracted according to the Mehlich-3 method (Mehlich 1984). Each sample was analyzed in two replications. The content of phosphorus was measured using inductively coupled plasma – optical emission spectroscopy (ICP-OES, Agilent Technologies 720, USA). The contents of K, Ca and Mg were measured using atomic adsorption spectroscopy (AAS, Varian 280 FS, Victoria, Australia).

RESULTS AND DISCUSSION

In order to eliminate the plots variability, the data from the year 1996 (before start of the experiment) were converted to 100%. The data from the following years were then compared with the year 1996. We usually did not find significant differences in the obtained results between the crops in crop rotation. Therefore the mean value of the P, K, Ca and Mg content in soil from all three crops together was calculated.

Figure 1 shows changes in soil P, K, Ca and Mg contents at the Humpolec site. The phosphorus contents in the year 1996 ranged between 59 and 89 mg P/kg. Sewage sludge appeared to be the best source of bioavailable phosphorus in soil. The P content increased from 100% in the year

Table 1. Characteristics of experimental fields

	Humpolec	Suchdol
Latitude	49°33'15''N	50°07'40''N
Longitude	15°21'02''E	14°22'33''E
Altitude (m a.s.l.)	525	286
Mean yearly temperature (°C)	7.0	9.1
Mean yearly rainfall (mm)	665	495
Soil type	cambisol	chernozem
Soil sort	sandy loam	loamy
pH^1	5.1	7.5
P (mg/kg)^2	77 (± 10)	74 (± 9)
K (mg/kg)^2	238 (± 47)	209 (± 18)
Ca (mg/kg)^2	1625 (± 187)	7803 (± 1760)
Mg (mg/kg)^2	112 (± 14)	209 (± 16)

^{1}Estimated in 0.01 mol/L $CaCl_2$, 1:10 w/v; ^{2}Average basic data estimated using Mehlich-3 method in archive samples (1996)

Table 2. Application rates of nutrients (kg/ha) – 3-year cycle

Treatment		Potatoes	Wheat	Barley
Control	N, P, K, Ca, Mg	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0
Sewage sludge	N	330[1]	0	0
	P	201[1]	0	0
	K	55[1]	0	0
	Ca	268[1]	0	0
	Mg	70[1]	0	0
Farmyard manure	N	330[1]	0	0
	P	70[1]	0	0
	K	352[1]	0	0
	Ca	250[1]	0	0
	Mg	30[1]	0	0
NPK	N	120	140	70
	P	30	30	30
	K	100	100	100
	Ca, Mg	35, 0	47, 0	21, 0

[1] Dose was calculated based on the average nutrient content in organic fertilizers; [2] Mineral fertilizers: N – calcium ammonium nitrate (27% N, 8% Ca); P – triple super phosphate (21% P); K – potassium chloride (50% K)

1996 to 233% in the year 2011. That was caused due to the high total amount of P added in sewage sludge compared to the other fertilizer treatments. Whole experiment was based on the same N dose. The average P contents in sewage sludge dry mass ranges between 1.71% and 2.35% (Mosquera-Losada et al. 2010). The sewage sludge applied to our experiment contained 2.20% P. On the contrary,

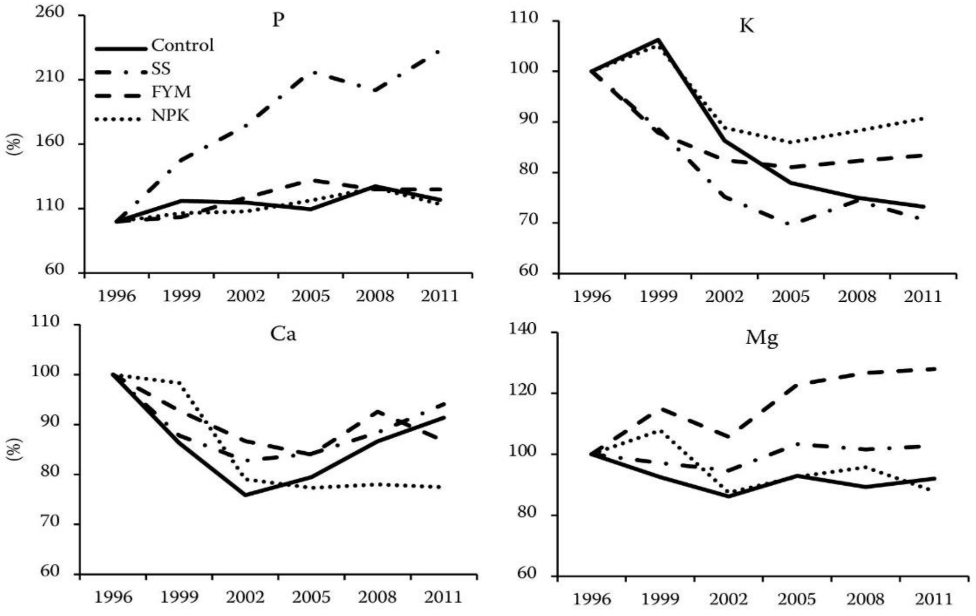

Figure 1. Changes of available soil P, K, Ca and Mg contents at Humpolec site. SS – sewage sludge; FYM – farmyard manure; NPK – mineral NPK

farmyard manure contained only 0.46% P in dry mass. That resulted in a total application dose of 201 kg P/ha per crop rotation (SS) and only 70 kg P/ha per crop rotation (FYM), respectively. Sewage sludge phosphorus availability is comparable with superphosphates (Coker and Carlton-Smith 1986). Therefore, such a big increase of bioavailable P content during the experiment was observed. The increasing tendency was visible at the other fertilizer treatments as well, but there were only slightly differences compared to the control non-fertilized treatment. Sewage sludge as a good source of available phosphorus compared to another organic fertilizers was reported by Petersen et al. (2003) as well. Based on the results of Huang et al. (2012) the P availability strongly depends on stabilization and P-precipitation processes.

Soil potassium contents ranged between 157 and 331 mg K/kg in the year 1996. A decreasing tendency in all treatments is visible. The K content in the NPK treatment decreased to 91% in the year 2011 compared to the 100% in the year 1996. The further decrease was observed at the treatment farmyard manure. Average K content gradually decreased to 83% in the year 2011. To the FYM treatment 17 kg K/ha more was yearly added than to the NPK treatment. However, the lower contents of bioavailable K in soil were found at the FYM treatment in the year 2011. Troeh and Thomson (2005) reported that the long-term farmyard manure application increase the soil sorption capacity. Potassium could be also more easily fixed to the non-available forms. Therefore, the estimated contents of bioavailable potassium were lower compared to the highly soluble K applied in NPK treatment at the end of experiment. However, Černý et al. (2010) published that NPK fertilizing resulted in higher crop yields. They reported in average about 29% higher yields of wheat and barley and 6% higher yield of potatoes compared to the FYM treatment at 5 experimental sites. This probably resulted in higher K uptake. The first mentioned fact had also probably stronger influence than the K uptake by plants.

The treatment amended with sewage sludge was comparable with control treatment in the year 2011. The measurements were only 73% (SS) and 71% (control), respectively. That was probably caused due to the low K content in sewage sludge compared to the high total N content (whole experiment was based on the same nitrogen dose). The total average content of nitrogen and potassium in sewage sludge applied to our experiments was 3.70% N and 0.60% K in dry mass, respectively. Similar N:K ratios are mentioned in other publications as well, for example 2.82% N and 0.44% K published by Bozkurt and Yarilgac (2003) or 2.20% N and 0.47% K (Antolín et al. 2005). Wen et al. (1999) estimated only 2.00% N and 0.10% K in sewage sludge dry mass. It was probably caused due to the less effective dewatering process used. On the contrary, the contents of nitrogen and potassium in farmyard manure are mutually comparable. Farmyard manure with average content of 2.00% N and 2.17% K in dry mass was applied in our experiment. Similar values are reported in many studies (Petersen 2003, Liu et al. 2010, Citak and Sonmez 2011 and others).

The total yearly potassium content added in the form of sewage sludge was also only 55 kg K/ha per 3 year crop rotation in our experiment, compared to the farmyard manure (352 kg K/ha/3 years) or NPK (300 kg K/ha/3 years).

The overall decreasing tendency of bioavailable potassium content in soil at the Humpolec site can be explained with the soil properties. There are much higher K losses in sandy soils compared to the loamy soils. Alfaro et al. (2004) reported the total K losses about 19 kg K/ha/6 months season on sandy soils and only 1 kg K/ha/6 months season on loamy soils, respectively. Their results were estimated at the fields with permanent grassland system, where are expected the lower K losses than in our experiment.

There were usually no visible tendencies between the calcium contents changes during the period of experiment. The measured values in the samples from the year 1996 ranged between 1532 and 2028 mg Ca/kg. Similar to the potassium content, a decreasing tendency was observed during the experiment.

Compared to the year 1996 (100%), the highest decrease (77%) was observed in the year 2011 in the NPK treatment. It can be partly explained due to the lower content of calcium applied (compared to FYM and NPK). The second reason are the higher calcium losses on loamy-sand compared to the loamy soil (Blume et al. 2002) and the third reason is acidifying effect of calcium ammonium nitrate applied (Vaněk et al. 2012), because of soil acidification is in positive correlation with Ca leaching (Pierson-Wickmann et al. 2009). These facts together with lower Ca uptake at control treatment resulted in the lower soil Ca contents at the NPK treatment in comparison with control non-fertilized treatment in the year 2011.

The magnesium content ranged between 93 and 132 mg Mg/kg at the start of the experiment. The content increased after the long-term farmyard manure application from 100% in 1996 to 128% in 2011. There were almost no changes in the Mg content of the treatment fertilized with sewage sludge during the experiment. On the contrary, a slightly decreasing tendency in soil Mg content of the NPK and control treatments was observed.

Figure 2 shows changes in soil P, K, Ca and Mg contents at the Suchdol site. Phosphorus contents increased always after fertilizer application. The average P value was 74 mg P/kg in the year 1996. The highest increase followed after long term sewage sludge application. Similarly to the Humpolec site, the P content in soil increased from 100% in the year 1996 up to 237% in the year 2011. After Kidd et al. (2007) the long-term application of sewage sludge led to the bioavailable P content increase as well. The application of farmyard manure resulted in an increased P content in soil. The average value in the year 2011 was 52% higher in comparison to 1996. Application of NPK in mineral form during the experiment led to the increase of P content by 25%. The average bioavailable P contents of the control treatment stayed almost unchanged during the experiment.

The contents of available potassium in soil ranged between 184 and 241 mg K/kg. On the contrary to the Humpolec site, there was an increasing tendency of the K content in the soil of all fertilizing treatments during the experiment. The highest increase compared to the year 1996 was observed in the NPK treatment (135%), followed by the FYM treatment (125%), control treatment (110%) and SS treatment (106%), respectively. On the contrary Warman and Termeer (2005b) reported the sewage sludge as a potentially good source of K for crop production. The overall increasing tendency in K content during the experiment can be explained by soil properties mentioned sooner at the Humpolec site description.

The calcium content at the Suchdol site was approximately 3.5 times higher than at the Humpolec site. The values in the wide range between 6348 and 8577 mg Ca/kg were obtained in the samples from the year 1996. Because of high natural soil Ca content, fertilizing probably did not have a significant influence on the bioavailable Ca contents changes during the experiment (on the contrary to the Humpolec site). There are no visible tendencies in soil Ca content changes during the experiment. One of the reasons can be the fact, that some authors do not recommend the Mehlich-3 method to estimate Ca content in soil as well (Schroder et al.

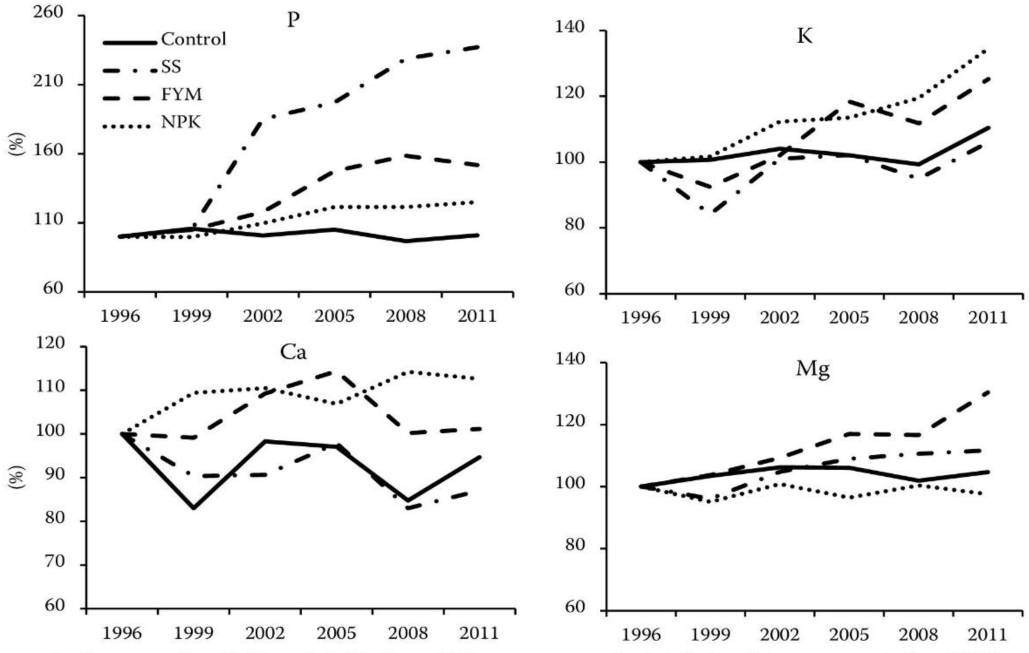

Figure 2. Changes of available soil P, K, Ca and Mg contents at Suchdol site. SS – sewage sludge; FYM – farmyard manure; NPK – mineral NPK

2009). However, the samples from our experiment were measured in one laboratory within two days in two replications. The measurement errors also should be eliminated.

The soil bioavailable magnesium contents had very similar tendencies to change as at the Humpolec site. The highest increase was observed in the FYM treatment. The Mg content increased by 30% in the year 2011 compared to 1996, followed by sewage sludge with 12% increase. The soil Mg amounts of the treatments NPK and control almost did not changed during the experiment. The interesting fact is that the highest increase was obtained in the FYM treatment at both experimental sites, although the higher total Mg content was applied to the SS treatment. Similar results were published by Chowaniak and Gondek (2009). They compared farmyard manure, sewage sludge and mineral fertilizers application in the incubation experiments. Based on their study, the intensity of changes related with Mg transformations strongly depends on the soil properties.

On the contrary to the different soil-climatic conditions of the studied sites, very similar tendencies in the soil of P, K, Ca and Mg contents changes after the fertilizing systems used were observed. At the same nitrogen dose (330 kg N/ha), sewage sludge appeared to be a better source of bioavailable soil phosphorus compared to the farmyard manure. On the contrary, FYM was a better source of bioavailable potassium and magnesium, despite the lower total magnesium content applied in FYM. It was not possible to deduce the tendency in soil calcium content changes during the experiment at both sites. The mineral NPK treatment was the best long-term source of potassium compared to the other studied fertilizing systems.

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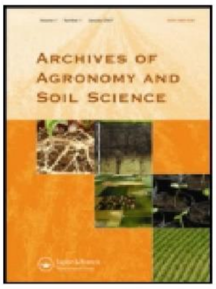
20) Winter wheat fertilizing using nitrogen-sulphur fertilizer

Hnojení pšenice ozimé dusíkato-sírovým hnojivem

Níže uvedený článek je zaměřen na sledování vlivu různých dávek síry při stejné dávce dusíku u pšenice ozimé. Přesné polní pokusy probíhaly v letech 2007-2010 na stanovištích Humpolec a Hněvčeves, přičemž celková dávka dusíku činila vždy 200 kg/ha. Hodnoceny byly různé dávky aplikované síry: 0, 10, 20 a 40 kg S/ha. Ta byla vždy dodávána ve formě $CaSO_4$ v ledku amonném se sírou (LAS). Dávka dusíku byla na finálních 200 kg/ha dorovnávána ledkem amonným s vápencem (LAV). Cílem práce bylo vyhodnotit vliv aplikace minerálního N-S hnojiva na výnos pšenice ozimé, obsah síry v nadzemní hmotě rostlin, zrnu a slámě a obsah přístupné síry v půdě. Půdní vzorky byly odebírány současně se vzorky nadzemní biomasy rostlin, tj. ve vegetačních fázích BBCH 26-28, 30-32, 37-39 a 49-51. Výnos zrna se pohyboval v rozmezí 7,2 – 10,9 t/ha a vykazoval zvyšující se tendenci se stoupající dávkou síry. To potvrzují i výsledky Scherer (2001), Salvagiotti et al. (2009) aj., kteří jako důvod uvádějí zlepšené využití dusíku díky dodané síře, a tím pádem i vyšší výnos. Přestože výsledky nebyly statisticky průkazné, vyšší obsahy přístupné síry v půdě byly zaznamenány vždy u variant hnojených S, což koresponduje s výsledky Ryant et Hřivna (2004), kteří rovněž aplikovali síru ve formě sádrovce ($CaSO_4$) k pšenici. Také Ercoli et al. (2011) dosáhli při aplikaci výrazně vyšších dávek síry (60 a 120 kg/ha) podobných výsledků. Při hodnocení obsahu S v půdě v jednotlivých vegetačních fázích je patrný postupný pokles obsahu přístupné S, způsobený pravděpodobně odběrem síry rostlinami. Dávka síry však neovlivnila obsah tohoto prvku v nadzemní hmotě rostlin. Na sírou hnojených variantách byly téměř vždy pozorovatelné zvýšené odběry S zrnem i slámou pšenice, avšak ve většině případů se jedná o statisticky neprůkazné rozdíly. Při srovnání obou stanovišť je patrný výrazný vliv ročníku i různých půdně-klimatických podmínek.



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Winter wheat fertilizing using nitrogen–sulphur fertilizer

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Winter wheat fertilizing using nitrogen–sulphur fertilizer

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Precise field experiments were established on two sites with winter wheat under different soil-climatic conditions in the Czech Republic. Four treatments were fertilized with same dose of nitrogen (200 kg N ha^{-1}) and increasing dose of sulphur (0, 10, 20 and 40 kg S ha^{-1}) using nitrogen–sulphur (N–S) fertilizer with calcium sulphate form. Soil and plant aboveground biomass samples were taken in the stages of development BBCH 26–28; 30–32; 37–39; 49–51. The winter wheat grain yield ranged between 7.20 and 10.86 t ha^{-1} and had an increasing trend with increasing sulphur dose. Although the differences were usually not statistically significant, there were found increasing tendencies of bioavailable sulphur content in soil with increasing S split doses. Soil S content decreased with time probably due to plant uptake. Sulphur dose did not influence the S content in plant aboveground biomass. The total S contents in grain after harvest ranged between 0.09% and 0.14% and were not significantly influenced with the fertilizing treatment. The same statement is valid for the S content in straw, which ranged between 0.03% and 0.11%. Both, S content in winter wheat seeds and straw were strongly influenced by the site conditions.

Keywords: winter wheat; sulphur; soil; plant; yields

Introduction

Sulphur deficiency is an actual problem in many sites, where recently S demand of plants was covered (Eriksen 2005; Lehmann et al. 2008; Skwierawska et al. 2008; Balík et al. 2009 and others). Although sulphur (S) has been known as an essential element for plant growth for over two centuries, S-containing fertilizers were not widely used for wheat crops before 1980s. At that time, wheat did not show evidence of S deficiency, because of air pollution from industry, intensive application of mineral fertilizers containing S and application of manure and cropping of plants with lower S uptake in crop rotation on the bigger area (Zhao et al. 1999; Scherer 2001; Blair 2002; Pacyna 2005).

From mentioned reasons, it is clear that sulphur becomes limiting factor for yield formation as well as for crop quality (Eriksen et al. 2004). Therefore, it is necessary to pay attention to sulphur-fertilizing and get more information about the different sulphur fractions in soil to optimize fertilizers split doses.

The optimum S content in plant tissue ranges between 0.1 and 0.5%. Sulphur demand increases in the following order: *Poaceae* < *Fabaceae* < *Brassicaceae* (Deloch 1960). Sulphur is mainly taken by roots up as SO_4^{2-} and the uptake is not influenced by other ions in soil solution and by soil conditions (Balík et al.2009).

Total content of S in soil usually ranges between 0.01 and 0.1% (Balik et al. 2009). The main portion of total S in soils is bound in soil organic matter (Eriksen et al. 1998;

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Kertesz & Mirleau 2004; Yang et al. 2007). Plant available S consists of (i) soluble inorganic SO_4^{2-}, (ii) sorbed inorganic SO_4^{2-} and (iii) the portion of organic S in soil that is mobilized during vegetation (Morche 2008).

The aim of this work is to investigate the influence of the application of S in different increments as calcium sulphate in combination with nitrogen on the yield of winter wheat and total S content in aboveground biomass as well as on the available S content in the soil.

Methods and materials

Precise field experiments were established on two sites with winter wheat (sort Sulamit) under different soil-climatic conditions in the Czech Republic (Humpolec and Hněvčeves) in the years 2008, 2009 and 2010. The sites are characterized in Table 1.

For this experiment, four fertilizing treatments were evaluated. Addition of nitrogen at all treatments reached 200 kg N ha^{-1}. Treatment A was fertilized only with nitrogen using only calcium ammonium nitrate (CAN), treatment B with 10 kg S ha^{-1}, treatment C with 20 kg S ha^{-1} and treatment D with 40 kg S ha^{-1} (Table 2). Each treatment comprised four replicates. Sulphur was applied in the form of $CaSO_4$ (N–S fertilizer – Lovofert® – ammonium nitrate and calcium sulphate – 24% N, 6% S). Fertilizers were applied in three split doses: (i) at the beginning of the spring vegetation, (ii) at the stage of development BBCH 30–32 and (iii) at the stage of development BBCH 49–51 (only nitrogen). Harvest was realised using small plot harvester.

Soil (0–30 cm) samples were taken up at the beginning of vegetation period and at the stages of development BBCH 26–28, BBCH 30–32, BBCH 37–39, BBCH 49–51. For the analyses, fresh soil samples (<5 mm) were analysed. The soil sample was extracted with demineralized water at 1:10 (w/v). After 60 min of shaking, each sample was centrifuged at 21,189 × g for the duration of 10 min in order to get bioavailable sulphur. This method was modified after Shan et al. (1992), who used the similar estimating of water extractable

Table 1. Characteristics of experimental fields.

		Average yearly				
Site	Altitude (m)	Rainfall (mm)	Temperature (°C)	Soil type	Texture	pH ($CaCl_2$)
Hněvčeves	265	573	8.2	Luvisol	Silty loam	6.3
Humpolec	525	665	7.0	Cambisol	Sandy loam	5.1

Table 2. Fertilizing scheme.

	Nutrients dose from Lovofert fertilizer			N + S doses (kg ha^{-1})			
Treatment	S (kg ha^{-1})	N (kg ha^{-1})	N dose from CAN N (kg ha^{-1})	1	2	3	Total N dose (kg ha^{-1})
A	0	0	200	80 + 0	80 + 0	40 + 0	200
B	10	40	160	80 + 10	80 + 0	40 + 0	200
C	20	80	120	80 + 20	80 + 0	40 + 0	200
D	40	160	40	80 + 20	80 + 20	40 + 0	200

sulphur as a part of fractionation. To be more precise, we used the ratio 10 g of sample and 100 ml of demineralized water.

Plant samples were taken at the stages of development BBCH 26–28, BBCH 30–32, BBCH 37–39, BBCH 49–51 and at maturity. Total concentrations of S in the above-ground biomass and in grain and straw of winter wheat were determined in the digests obtained by the following decomposition procedure: aliquots (0.5 g) of air-dried milled samples were decomposed in a digestion vessel with a mixture of 8 ml concentrated nitric acid, 5 ml of hydrochloric acid and 2 ml of concentrated hydrofluoric acid. The mixture was heated in an microwave assisted wet-digestion system (Ethos 1, MLS GmbH, Germany) for 33 min at 210°C. After cooling, the digest was quantitatively transferred into a 50 ml Teflon® vessel and evaporated to dryness at 160°C. The digest was then dissolved in a 3 ml nitric and hydrochloric acid mixture (1:3), transferred into a 25 ml glass tube, filled up by deionized water and kept at laboratory temperature until measurement. The concentrations of S in plant digests and soil extracts were determined using optical emission spectroscopy with inductively coupled plasma (ICP-OES) with axial plasma configuration (VistaPro, Varian, USA).

Statistical analysis were realized due to Statgraphics Centurion XVI and Microsoft Excel 2007.

Results and discussion

Wheat has a relatively low S requirement, amounting to about 20 kg S ha^{-1} with a grain yield of 8 t ha^{-1} (McGrath et al. 1996). This is in the order of magnitude of treatment C of our experiments (Table 3). Grain yield ranged between 7.20 and 10.86 t ha^{-1}. The highest yields were estimated in the years 2008 and 2010 at Hněvčeves site. The lowest yields were found in 2009 at Humpolec site, where the average reached only 7.20 t ha^{-1}. The highest yields were always found in the year 2008. It was due to more favourable rainfall and temperature conditions at both sites. Bigger differences between yields were found at Humpolec site, where we estimated of about 2 t ha^{-1} higher yields in the year 2008 compared with the following year. In the year 2009, there were less favourable conditions for winter wheat cropping. The precipitations between May and August were almost two times higher compared with the long-term average between 1971 and 2010. This resulted in the complication with winter wheat harvest at both experimental sites. There was also very dry March at Hněvčeves site (only 6 mm of precipitations), which led to lower tillering. The weather conditions were similar to long-term average in other experimental years.

Table 3. Winter wheat grain yield at 14% dryness (in t ha^{-1}).

	Hněvčeves			Humpolec		
Treatment	2008	2009	2010	2008	2009	2010
A	10.24^{a*}	9.49	10.61	9.72	7.20	8.52
B	10.86^{ab}	9.40	10.65	9.77	7.24	8.63
C	10.54^{b}	9.50	10.65	9.96	7.23	8.90
D	10.70^{b}	9.69	10.67	10.00	7.48	9.15
F-test	5.35	0.54	1.92	2.05	2.49	2.15
p-Value	0.01	ns	ns	ns	ns	ns

Note: *Among the treatments, those marked with different letters were found with statistically significant differences.

Comparing the different treatments, significant yield differences were only obtained at the Hněvčeves site in the year 2008. There were found significantly higher yields in the treatments C and D where 20 and 40 kg S ha^{-1}, respectively, were applied as compared with treatment A without S. The highest yield was found at the B treatment amended with 10 kg S ha^{-1}, but the difference was not statistically significant. It was probably due to very low solubility of the calcium sulphate form in soil.

In the other cases, we did not found significant differences between studied treatments, but there are visible some tendencies to the yield increase with increasing S rate. The highest yields in the years 2008 and 2009 at the Hněvčeves site and in the years 2009 and 2010 at Humpolec site were always obtained at the treatment D where the highest amount of S was applied. This corresponds with the results of Ryant & Hřivna (2004), who also did not found significant differences between treatments fertilized using nitrogen fertilizer and the treatment fertilized using nitrogen fertilizer together with gypsum, but they found increasing tendencies with increasing S rate. Similar results were found by Ercoli et al. (2011), who applicated higher S rates (60 and 120 kg S ha^{-1}) in $CaSO_4$ form to winter wheat together with nitrogen.

The increasing tendencies in yields are caused with increasing nitrogen efficiency due to the sulphur-fertilizing that is mentioned with many authors (e.g. Scherer 2001; Salvagiotti et al. 2009 and others).

Total sulphur uptake with grain and straw mentioned in Table 4 was in close relation with grain yield. The amount of sulphur taken up by plants ranged between 10.37 and 31.45 kg S ha^{-1}. That corresponds with results of Ercoli et al. (2012), who get similar results in the experiments with durum wheat. The highest sulphur uptakes were estimated at the Hněvčeves site in the years 2008 and 2010 and the lowest at Humpolec site in the year 2008. The lowest S uptakes were usually found at the treatments without S or with the rate of 10 kg S ha^{-1}. On the contrary, the highest S uptake was usually obtained at the C and D treatment, where 20 and 40 kg S ha^{-1}, respectively, were applied. The only statistically significant differences were found at Hněvčeves site in the year 2008, where the S uptake at the A treatment without S was significantly lower.

The third aim of this work was to estimate the influence of N–S fertilizing on sulphur content in the aboveground biomass, grain and straw of winter wheat (Table 5). Similar to the soil, the differences between observed treatments were usually not statistically significant due to the low solubility of calcium sulphate in soil. The measured S values ranged between 0.04 and 0.53% S. The highest S contents were usually found at the stage of development BBCH 26–28 during the vegetation, followed by decreasing trend due to the dilution effect.

Higher S amounts were usually found at Hněvčeves site. Significant differences between studied fertilizing treatments were found only in 6 cases from 30. It was in the

Table 4. Sulphur uptake with grain and straw (in kg S ha^{-1}).

	Hněvčeves			Humpolec		
Treatment	2008	2009	2010	2008	2009	2010
A	22.63^{a}*	16.13	18.67	22.16	10.37	15.51
B	28.24^{b}	15.98	19.92	21.10	11.08	16.91
C	29.51^{b}	17.10	17.57	22.71	11.71	16.20
D	31.46^{b}	16.47	19.21	22.00	11.44	17.93
F-test	8.25	2.34	1.19	0.71	0.46	2.87
p-Value	0.01	ns	ns	ns	ns	ns

Note: *Among the treatments, those marked with different letters were found with statistically significant differences.

Table 5. S contents in dry matter of aboveground biomass of winter wheat (in %).

Stage of development	Treatment	Hněvčeves			Humpolec			Current S dose kg ha^{-1}
		2008	2009	2010	2008	2009	2010	
BBCH 26–28	A	0.27	0.18	0.29	0.26	0.25	0.42^{a}*	0
	B	0.28	0.18	0.31	0.32	0.24	0.43^{a}	10
	C	0.27	0.18	0.28	0.22	0.26	0.53^{b}	20
	D	0.31	0.19	0.28	0.30	0.25	0.55^{b}	20
	F-test	0.96	0.03	0.71	1.17	0.13	10.53	–
	p-Value	ns	ns	ns	ns	ns	0.01	–
BBCH 30–32	A	0.34	0.15	0.32	0.14	0.25	0.39^{a}	0
	B	0.36	0.16	0.30	0.14	0.20	0.40^{a}	10
	C	0.30	0.15	0.31	0.15	0.24	0.29^{b}	20
	D	0.35	0.16	0.31	0.19	0.22	0.28^{b}	20
	F-test	2.48	1.33	0.71	2.84	2.31	43.28	–
	p-Value	ns	ns	ns	ns	ns	0.01	–
BBCH 37–39	A	0.10	0.07	0.28	0.10	0.08^{a}	0.29	0
	B	0.13	0.07	0.30	0.09	0.08^{a}	0.28	10
	C	0.12	0.06	0.27	0.09	0.12^{b}	0.32	20
	D	0.12	0.05	0.28	0.10	0.12^{b}	0.29	40
	F-test	2.90	1.26	0.30	2.90	17.04	0.79	–
	p-Value	ns	ns	ns	ns	0.01	ns	–
BBCH 49–51	A	0.10	0.04	0.13^{ab}	0.08	0.08	0.18^{ab}	0
	B	0.12	0.04	0.08^{a}	0.09	0.08	0.20^{a}	10
	C	0.11	0.05	0.14^{ab}	0.08	0.06	0.13^{b}	20
	D	0.12	0.04	0.15^{b}	0.08	0.06	0.21^{a}	40
	F-test	0.87	0.14	2.27	0.24	4.45	3.19	–
	p-Value	ns	ns	0.01	ns	ns	0.05	–
S content in grain after harvest	A	0.13^{a}	0.10	0.11	0.12	0.09	0.14	0
	B	0.13^{a}	0.10	0.11	0.12	0.09	0.14	10
	C	0.14^{b}	0.10	0.11	0.12	0.09	0.13	20
	D	0.14^{b}	0.10	0.12	0.11	0.09	0.14	40
	F-test	11.51	0.65	1.65	1.96	1.33	1.65	–
	p-Value	0.01	ns	ns	ns	ns	ns	–
S content in straw	A	0.07	0.07	0.06	0.09	0.06	0.03	0
	B	0.10	0.07	0.07	0.08	0.07	0.04	10
	C	0.10	0.08	0.05	0.09	0.08	0.04	20
	D	0.11	0.07	0.05	0.10	0.07	0.04	40
	F-test	1.32	1.15	0.98	1.80	1.68	1.92	–
	p-Value	ns	ns	ns	ns	ns	ns	–

Note: *Among the treatments, those marked with different letters were found with statistically significant differences.

winter wheat grain 2008 at Hněvčeves site, where was found significantly higher S content in the treatments C and D (with S rates 20 and 40 kg S ha^{-1}, respectively). Significantly higher S content at the treatment D was also found at the stage of development BBCH 49–51 in the year 2010 at the same site.

Significantly higher S contents at the treatments C and D were found in the stage of development BBCH 37–39 in the year 2009 at Humpolec site. Similar trend was found in the stage of development BBCH 26–28 in the year 2010. But in this year, we found also opposite tendencies in the case of BBCH 30–32, where the S contents in aboveground biomass were significantly lower at the more fertilized treatments.

In the most of cases, in contrast to the soil, increasing tendencies of S content in aboveground biomass with increasing rates of N–S fertilizer were only seldom observed.

We calculated the correlation coefficient between bioavailable S contents in soil and percentage S in aboveground biomass and got correlations of about $r = 0.30$. Obtained results did not correspond with results of Matula (1999), who obtained correlation coefficient $r = 0.82$.

The differences between sulphur content in grain and straw were not statistically significant. The S contents in grain were very similar and ranged between 0.09 and 0.14% S in all studied years. Györi (2005) estimated in long-term field experiments with winter wheat similar values (in average 0.15% S). The total sulphur contents in straw varied between 0.03 and 0.11% S and were not significantly influenced with fertilizing treatment.

The changes in sulphur contents in soil after using increasing S rates in the $CaSO_4$ form were evaluated in different stages of development of winter wheat at Humpolec and Hněvčeves site. The results are mentioned in Table 6. Obtained values of bioavailable sulphur in topsoil (0–30 cm) ranged between 0.4 and 17.0 mg S kg^{-1}. That corresponds with the results of Matula and Pechová (2005), who estimated 4–13 mg S kg^{-1}. Relative big

Table 6. The contents of bioavailable sulphur (mg S kg^{-1}) in topsoil dry matter (0–30 cm).

		Hněvčeves			Humpolec			Current S dose kg ha^{-1}
Stage of development	Treatment	2008	2009	2010	2008	2009	2010	
BBCH 26–28	A	3.4^{a}*	15.4	14.2	3.7	7.9	2.2^{a}	0
	B	5.2^{a}	14.8	19.0	6.0	14.9	5.4^{b}	10
	C	10.4^{b}	12.6	20.0	4.8	12.4	6.6^{b}	20
	D	10.2^{b}	12.8	19.8	7.1	12.7	4.9^{b}	20
	F-test	5.6	0.4	0.4	2.0	2.0	8.5	–
	p-Value	0.01	ns	ns	ns	ns	0.01	–
BBCH 30-32	A	3.7^{a}	4.2	8.5	2.3	7.9	8.0	0
	B	4.5^{a}	4.5	8.3	3.4	4.9	8.8	10
	C	6.7^{b}	4.0	22.6	4.1	9.6	5.2	20
	D	8.4^{b}	6.5	11.1	5.1	9.3	8.7	20
	F-test	14.4	1.3	2.6	2.0	4.6	0.4	–
	p-Value	0.01	ns	ns	ns	ns	ns	–
BBCH 37-39	A	3.6	4.5	13.0	4.8^{a}	4.5	6.9	0
	B	3.5	6.0	10.7	3.1^{a}	9.2	5.1	10
	C	4.2	8.0	12.0	3.4^{a}	15.1	6.4	20
	D	5.0	16.6	11.8	9.7^{b}	16.0	12.7	40
	F-test	2.0	4.2	1.1	8.4	4.2	1.3	–
	p-Value	ns	ns	ns	0.01	ns	ns	–
BBCH 49-51	A	1.4	3.6	8.3	1.1^{a}	5.9	3.0^{a}	0
	B	1.3	10.1	11.3	3.6^{a}	8.5	4.6^{ab}	10
	C	0.9	17.6	9.4	3.7^{a}	8.9	7.2^{b}	20
	D	4.5	5.0	7.9	17.0^{b}	14.1	4.6^{ab}	40
	F-test	0.9	1.8	0.5	13.4	4.1	3.3	–
	p-Value	ns	ns	ns	0.01	ns	0.05	–
After harvest	A	0.4^{a}	5.8	8.0	0.5^{a}	7.8	3.1^{a}	0
	B	0.6^{a}	7.2	6.1	0.6^{a}	8.9	3.0^{a}	10
	C	0.8^{a}	9.4	4.6	0.9^{b}	10.9	3.3^{a}	20
	D	1.7^{b}	15.5	6.1	1.4^{c}	9.3	4.6^{b}	40
	F-test	11.8	4.7	1.4	15.4	0.6	10.2	–
	p-Value	0.01	ns	ns	0.01	ns	0.01	–

Note: *Among the treatments, those marked with different letters were found with statistically significant differences.

differences in S contents in soils between the studied stages of development point to the statement of Schnug & Haneklaus (1998) that any analytical value on SO_4^{2-} concentration of a soil sample is more or less only for the moment the sample has been taken.

From Table 6, it is clear that there are usually no significant differences between observed treatments. These were found only in the years 2008 and 2010 with significantly higher S contents at the treatments C and D at the stages of development BBCH 26–28 and 30–32 (S rate – 20 kg ha^{-1}) at Hněvčeves site and after the harvest at D treatment (40 kg S ha^{-1}) in the year 2008 at both sites.

Significant differences between studied treatments in the year 2010 were found only at Humpolec site. The significantly lowest contents were found at the control treatment (A) in the stages of development BBCH 26–28 and 49–51, where these were measured only 2.2 mg S kg^{-1} and 3.0 mg S kg^{-1}, respectively.

Despite this, in most cases, the differences between treatments were not significant; there are visible trends in increase of the S content in soil with increasing S rate. The lowest bioavailable S contents were usually found at control treatment and the highest at the treatments C and D fertilized with 20 and 40 kg S ha^{-1}, respectively.

Conclusions

Despite the worse sulphur availability from calcium sulphate form, S-fertilizing positively influenced the yield of winter wheat grain. We got an increasing trend of yield and S uptake with grain and straw with increasing S rate.

In the most of cases, there were no visible tendencies to the changes of aboveground biomass, grain and straw sulphur contents depending on N–S fertilizer application.

Fertilizing with sulphur using N–S fertilizer led usually to increasing tendencies of the bioavailable S content in the soils with increasing S rate.

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21) Influence of nitrogen-sulfur fertilizing on the content of different sulfur fractions in soil

Hnojení minerálním dusíkem a sírou a jeho vliv na různé frakce síry v půdě

Studie je zaměřena na sledování vlivu různých dávek síry při stejné dávce dusíku u ozimé řepky. Přesné polní pokusy probíhaly v letech 2007-2010 na stanovištích Humpolec, Hněvčeves a Uhříněves přičemž celková dávka dusíku činila vždy 200 kg/ha. Hodnoceny byly různé dávky aplikované síry: 0; 12,5; 20 a 50 kg S/ha. Ta byla vždy dodávána ve formě $CaSO_4$ v ledku amonném se sírou (LAS). Dávka dusíku byla na finálních 200 kg/ha dorovnávána ledkem amonným s vápencem (LAV). Cílem práce bylo vyhodnotit vliv aplikace minerálního N-S hnojiva na výnos řepky ozimé a obsah přístupné síry v půdě. Půdní vzorky byly odebírány současně se vzorky nadzemní biomasy rostlin, tj. na začátku jarní vegetace a ve vegetačních fázích BBCH 30-32 a 50-52. Zde byl měřen obsah vodorozpustné, adsorbované, okludované antericky vázané síry. Obsah přístupných forem síry v půdě (vodorozpustné a adsorbované) před založením pokusu byl na všech stanovištích velmi podobný a ani jedna z frakcí nepřesahovala hodnotu 10 mg S/kg. Obsah estericky vázané síry se v rámci stanovišť výrazně lišil, což ukazuje na rozdílné rezervy potenciálně uvolnitelné síry v závislosti na půdně-klimatických podmínkách. Obsah přístupné síry v půdě se po aplikaci hnojiv téměř vždy zvyšoval úměrně k aplikované dávce, což koresponduje i s výsledky Morche (2008). Naopak obsah estericky vázané síry nebyl hnojením téměř ovlivněn. Reakce rostlin na hnojení sírou se na jednotlivých stanovištích poměrně lišily. Zatímco na stanovišti Hněvčeves vedla ke zvýšení výnosu už i nejmenší dávka síry (12,5 kg S/ha), a to ve všech pokusných letech, u stanoviště Humpolec byl zaznamenán nejvyšší efekt hnojení sírou v roce 2010 a u stanoviště Uhříněves v roce 2009. Ukázalo se tak, že síra se v poslední době podílí stále více na zvýšení i stabilizaci výnosů. Důvodem jsou pravděpodobně nízké obsahy S v půdě z důvodu poklesu vstupů S v atmosférických depozicích (Balík et al. 2009). Hnojení sírou mělo pozitivní vliv na výnosy řepky i přes stabilní formu S dodávanou v $CaSO_4$.

Influence of the nitrogen-sulfur fertilizing on the content of different sulfur fractions in soil

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ABSTRACT

Precise field experiments were established at 3 sites with oilseed rape under different soil-climatic conditions in the Czech Republic (Humpolec, Hněvčeves and Uhříněves) in the years 2008–2010. In this experiment, four fertilizing treatments with increasing S rate were evaluated. The contents of bioavailable (S_W), adsorbed (S_{ads}), occluded (S_{ocl}), and hydroiodic acid (HI) reducible (S_{HI}) sulfur were measured. The contents of the fractions within the studied sites in the samples collected before fertilizers application were comparable. The S_W, S_{ads} a S_{ocl} contents did not exceed 10 mg S/kg. The S_{HI} contents differed depending on site and year. Fertilizing using S as $CaSO_4$ positively influenced the contents of S_W, S_{ads}, and S_{HI} in soil. Sulfur fertilizing had also the positive tendencies to increase the winter rape yields but the differences between studied treatments were not statistically significant.

Keywords: N-S fertilizing; bioavailable; adsorbed; occluded and estersulfate bounded sulfur; rape yield

Bioavailable sulfur deficiency in soils is an actual problem in the Czech Republic and in other European countries. It applies to the countries with a recent good sulfur supply as well (Eriksen 2004, Lehmann et al. 2008, Skwierawska et al. 2008, Balík 2009 and others).

It can be attributed to the (i) decrease of atmospheric emissions; (ii) less intensive application of mineral fertilizers together with changes of their composition; (iii) restriction of manure, and (iv) cropping of plants with high S uptake in crop rotation (e.g. rape) on a bigger area (Scherer 2001, Blair 2002, Pacyna 2005).

From the mentioned reasons it is clear that sulfur is becoming a limiting factor for the harvest and production quality (Eriksen et al. 2004). Therefore, it is necessary to pay attention to sulfur fertilizing and get more information about different sulfur fractions in soil to optimize fertilizers split doses.

Inorganic sulfur represents usually 5–10% from total S (Tisdale et al. 1993). It can appear in many different forms. The most important form for S uptake by plants is its bioavailable sulfate form (SO_4^{2-}). This form represents usually only about 1% of total S (Knauff 2000). Another form represents the sulfates adsorbed due to the weak bind on the surface of soil particles. These have a direct influence on the perseveration of sulfate balance with soil solution (McLaren and Cameron 1996). The most stable sulfur form is S occluded with carbonates, especially with calcium carbonate (Chen et al. 1997). The mentioned forms are possible to estimate using different sequential extraction methods, for example after Shan et al. (1997).

The content of organic sulfur ranges usually about 90% of total S in soil (Eriksen et al. 1998, Solomon et al. 2001) and includes (i) sulfur directly bound with carbon; (ii) sulfur indirectly bound with carbon, for example C-O-S and C-N-S bounds (Morche 2008) which can be approximately estimated using the extraction method according to Shan et al. (1992). This method can be also used for estimating some part of inorganic sulfur (Havlin et al. 2005). Both mentioned groups are potential sources of bioavailable sulfur.

The aim of this work is to evaluate different S forms in soils amended using different fertilizing systems in precise field experiments.

MATERIAL A METHODS

Precise field experiments were established at three sites with oilseed rape under different

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Table 1. Characteristic of experimental fields

Site	Altitude (m)	Mean yearly		Soil type	Soil sort	pH ($CaCl_2$)
		rainfall (mm)	temperature (°C)			
Uhříněves	295	575	8.3	luvisol	clayey	6.5
Hněvčeves	265	573	8.2	luvisol	silty loam	6.3
Humpolec	525	665	7.0	cambisol	sandy loam	5.1

soil-climatic conditions in the Czech Republic (Humpolec, Hněvčeves and Uhříněves) in the years 2008–2010 (except 2010 at Uhříněves site, where the plants were damaged due to strong frost in spring). The sites are characterized in Table 1.

For this experiment, four fertilizing treatments were evaluated. Addition of nitrogen (calcium ammonium nitrate – 27% N and calcium sulfate ammonium nitrate – 24% N, 6% S) at all treatments reached 200 kg N/ha. Treatment A was fertilized only with nitrogen, treatment B with 12.5 kg S/ha, treatment C with 25 kg S/ha and treatment D with 50 kg S/ha (Table 2). Each treatment was conducted in 4 replicates. Sulfur was applied in the form of $CaSO_4$ (as a component of N-S fertilizer Lovofert® – ammonium nitrate calcium sulfate). Fertilizers were applied in three split doses: (i) at the beginning of the spring vegetation; (ii) at the vegetation period BBCH 30-32, and (iii) at the vegetation period BBCH 50-52 (only nitrogen).

For the analyses, fine (< 2 mm) topsoil (0–30 cm) samples of the spring vegetation beginning and from the BBCH 50-52 period were chosen. The samples were extracted using the following S-fractionation (Morche 2008): the soil sample was first extracted with demineralized water at 1:10 (w/v). After 30 min of shaking, each sample was centrifuged at 10 000 rpm for the duration of 10 min in order to get bioavailable sulfur (S_W). Following the centrifugation, the samples were extracted with 0.032 mol/L NaH_2PO_4 at the 1:10 (w/v) ratio. After 30 min of shaking, each sample was again centrifuged at 10 000 rpm for the duration of 10 min to get adsorbed sulfur (S_{ads}). The rest of the soil sample was extracted with 1 mol/L HCl. The extraction ratio was 1:20 (w/v). After 60 min of shaking, the samples were centrifuged for 10 min at 10 000 rpm to get occluded sulfur (S_{ocl}). For the HI-reducible sulfur (S_{HI}), the method after Shan et al. (1992 adapted by Morche 2008) was used.

RESULTS AND DISCUSSION

Precise field experiments with oilseed rape were established at three sites under different soil-climatic conditions in the Czech Republic in the years 2008–2010. Soil samples taken up before fertilizing and after adding the whole dose of S-fertilizers (vegetation period BBCH 50-52) were analyzed. In these samples, water extractable (bioavailable) sulfur (S_w), adsorbed sulfur (S_{ads}), occluded sulfur (S_{ocl}) and HI reducible sulfur (S_{HI}) was determined. Estersulfate bound sulfur (S_{es}) was subsequently calculated from these results. Regarding the fact that reached values did not fit the parameters of normal distribution, only basic statistical parameters were calculated. Table 3 mentions the different sulfur fractions amounts, estimated from the soil samples taken up before beginning of oilseed rape spring vegetation. The contents of the estimated fractions were comparable and the significant differences did not exceed the value of 0.5 (S_w); 0.4 (S_{ads}); 1.1 (S_{ocl}) and 7.7 (S_{HI}). For this reason it is possible to evaluate these sites as suitable for such experiments. It is also clear that the bioavailable S content was in very close range 4.1–6.8 mg S/kg (Table 3). This corresponds to the results of Matula

Table 2. Fertilizing of the experiment

Fertilizing treatment	1st rate (regeneration)	2nd rate (BBCH 30–32)	3rd rate (BBCH 50–52)	Total	
	(kg N/ha + kg S/ha)			(kg N/ha)	(kg S/ha)
A	80 + 0	80 + 0	40 + 0	200	0
B	80 + 12.5	80 + 0	40 + 0	200	12.5
C	80 + 25	80 + 0	40 + 0	200	25
D	80 + 25	80 + 25	40 + 0	200	50

Table 3. The contents of different S fractions in soils before fertilizing (mg S/kg)

Fraction	Hněvčeves			Humpolec			Uhříněves	
	2008	2009	2010	2008	2009	2010	2008	2009
S_W	6.3	6.2	6.3	4.1	4.4	4.5	6.3	6.8
S_{ads}	4.1	4.4	4.3	4.3	4.5	4.5	5.1	5.3
S_{ocl}	3.5	3.1	3.7	7.5	8.1	7.8	8.6	8.4
S_{in}*	13.8	13.7	14.3	15.9	17.0	16.8	20.0	20.5
S_{HI}	56.9	35.1	54.3	105.4	168.3	172.1	70.4	113.8
S_{es}**	43.1	21.4	50.0	89.5	151.3	155.3	50.4	93.3

*Σ $S_{W,}$ $S_{ads,}$ S_{ocl}, **calculated as $S_{es} = S_{HI} - S_i$

(2007), who estimated the range of bioavailable sulfur between 3.7 and 29.3 mg S/kg (estimated using 0.5 mol/L ammonium acetate) on 36 sites in the Czech Republic. A similar range was found for the S_{ads} and S_{ocl} values, where the contents reached 4.1–5.1 mg S/kg and 3.1–8.6 mg S/kg, respectively. The lowest contents of the bioavailable sulfur were always found at the Humpolec site. The contents of adsorbed sulfur were comparable at all sites and studied years.

Using the HCl extraction as a part of the fractionation should mobilize a major portion of S occluded with carbonates (Chen et al. 1997, Shan et al. 1997). In this case differences were observable between studied sites. The highest S_{ocl} contents were found at the Uhříněves site, where the values reached 7.5–8.6 mg S/kg and the lowest at Hněvčeves site, where they reached only 3.1–3.7 mg S/kg. Similar results were obtained by Balík et al. (2009), who estimated the average values of 7.2 mg S/kg (S_w), 5.2 mg S/kg (S_{ads}) and 9.9 mg S/kg (S_{ocl}) on 10 long-term non-fertilized sites in the year 2007.

The sum of the fractions S_w, S_{ads}, and S_{ocl} represents the so-called 'inorganic S' (S_{in}) (Morche 2008). Although the use of the extractants is not fully selective, it is possible to assume that most of the extracted S is inorganic S (Shan et al. 1992, Morche 2008). The observed values ranged between 13.7 and 20.5 mg S/kg.

The S_{HI} method should estimate the rate of organic sulfur bound with estersulfates. However, it is important to pay attention to the fact that using this method does not only estimate this S form. Sulfur dominates in estersulfate form, but it also estimates some ratio of inorganic sulfur (Havlin et al. 2005). Using the S_{HI} method estimates the whole content of inorganic sulfur (Morche 2008). Therefore, it is better to calculate estersulfate bound sulfur content like $S_{es} = S_{HI} - S_{in}$.

From Table 4 is clear that sulfur fertilizing influenced the amounts of almost all of estimated sulfur fractions. The differences were usually not statistically significant, but there are visible increasing tendencies with increasing S-fertilizer dose.

The lowest bioavailable sulfur content was always found on control sulfur non-amended treatment where the values reached about 5.7 mg S/kg. With the increasing S-dose the S_w content increased as well. The highest contents of bioavailable sulfur were found at the treatment fertilized with 50 kg S/ha. The average value of this treatment was 14.4 mg S/kg. Measured data correspond with the results published by Shan et al. (1997), who estimated the ratio of S_W between 5.8–44.4 in the pot experiments (13 soils) and 8.0–34.2 mg S/kg in the field survey (25 soils) with soils using different fertilizing systems.

Similar trends were observable for the S_{ads} values as well. Values between 5.0 and 5.6 mg S/kg were found in the year 2008, but the differences in 2009 and 2010 were obvious. Almost the same results were obtained by Balík et al. (2009), who observed an increasing trend of S_w and S_{ads} after a long term application of farmyard manure and farmyard manure together with NPK, where the part of N was added using ammonium sulfate. Shan et al. (1997) observed the S_{ads} range between 5.2 and 176 mg S/kg in a pot experiment and a closer range between 11.5 and 29.7 mg S/kg in the field survey.

Different fertilizing system probably did not have an influence on the content of sulfur occluded with carbonates. The increasing S_{ocl} trend with increasing S dose was observed only in the year 2010. Morche (2008) compared the changes in S_{ocl} contents after a long term usage of composts and mineral fertilizers. She gets in average about 16.0–16.3 mg S/kg higher contents at the soils

Table 4. The contents of different S fractions after S fertilizing (average from observed sites)

S fraction	Treatment	2008		2009		2010	
		(mg S/kg)	SD	(mg S/kg)	SD	(mg S/kg)	SD
S_w	A	6.0	0.9	5.5	0.6	5.5	1.1
	B	7.2	1.0	7.5	1.4	6.1	1.6
	C	7.9	2.9	11.0	6.7	9.4	1.4
	D	12.4	3.9	16.5	8.0	14.2	4.9
S_{ads}	A	5.0	0.5	4.9	0.7	4.1	0.8
	B	5.0	0.4	5.6	0.4	5.3	0.6
	C	5.1	0.5	6.7	1.3	6.2	1.0
	D	5.6	0.8	7.9	1.8	8.2	2.1
S_{ocl}	A	6.6	1.1	7.4	2.2	6.2	0.9
	B	6.3	1.0	6.8	1.3	6.2	1.5
	C	6.3	0.8	9.6	3.1	8.2	1.1
	D	7.1	1.2	8.3	2.5	8.5	0.8
S_{in}	A	17.6	1.3	17.9	0.7	15.8	1.2
	B	18.4	0.9	19.9	1.6	17.5	0.7
	C	19.4	2.1	27.3	1.3	23.7	1.5
	D	25.1	2.5	32.7	2.5	30.8	2.0
S_{HI}	A	103.7	8.3	100.9	13.9	113.3	7.6
	B	104.9	12.5	109.2	5.3	115.5	3.3
	C	110.4	15.1	111.4	6.9	123.2	3.6
	D	114.0	13.3	135.2	7.1	128.8	7.6
S_{es}**	A	86.1	7.2	83.0	8.5	97.5	4.3
	B	86.5	6.3	89.3	4.6	98.0	6.5
	C	91.0	9.8	84.1	5.1	99.5	6.5
	D	88.9	8.2	102.5	15.8	98.0	7.3

SD – standard deviation; **$S_{es} = S_{HI} - S_{in}$; A – 0 kg S/ha; B – 12.5 kg S/ha; C – 25 kg S/ha; D – 50 kg S/ha

amended with composts. Similar tendencies were observed for S_{ads} as well.

The S_{in} ($S_W + S_{ads} + S_{ocl}$) content confirmed the above mentioned tendencies. Calculated values ranged between 15.8 and 32.7 mg S/kg, whereas the lowest contents were always estimated at control sulfur non-fertilized treatment (A) and the highest at the treatment fertilized using 50 kg S/ha (D).

The highest S_{HI} amount was observed at D treatment and the lowest at A treatment. There, in contrast of inorganic sulfur fractions, outstanding differences between studied treatments were found. Values between 32.8 and 97.7 mg S/kg were found at the Hněvčeves site and values between 107.5 and 198.3 mg S/kg were even found at the Humpolec site.

From the results it is clear that the increase of S_{HI} content was caused by the parallel estimating of the S_{in} fraction. After calculating the estersulfate bound S content ($S_{HI} - S_{in}$) it is obvious, that using different fertilizers did not probably influence the content of S_{es} in soil. A signifficant S_{es} increase was observed only at treatment D in the year 2009. The values of S_{es} found corresponds to the results of Shan et al. (1997) who got the S_{es} range between 41 and 205 mg S/kg in pot experiments and between 61 and 274 mg S/kg in a field survey.

The aim of our experiment was also to evaluate the yields of oilseed rape (Table 5). There were no statistically significant differences between the studied treatments. Despite that, it is possible to assume that S-fertilizing using $CaSO_4$ S-form

Table 5. The yield of oilseed rape seeds at 12% moisture

Site	Treatment	2008		2009		2010	
		(t/ha)	SD	(t/ha)	SD	(t/ha)	SD
Hněvčeves	A	3.93	0.18	3.79	0.41	6.84	0.68
	B	4.21	0.14	4.13	0.07	6.88	0.88
	C	4.03	0.26	4.08	0.16	6.85	0.66
	D	4.33	0.14	4.31	0.19	7.02	0.85
Humpolec	A	3.06	0.18	3.17	0.05	2.70	0.11
	B	3.27	0.18	3.27	0.11	4.00	0.12
	C	3.15	0.13	3.27	0.14	3.59	0.11
	D	3.25	0.15	3.06	0.07	4.48	0.12
Uhříněves	A	3.65	0.21	4.69	0.37	–	–
	B	3.49	0.26	5.08	0.17	–	–
	C	3.59	0.14	5.41	0.07	–	–
	D	3.64	0.16	5.29	0.13	–	–

SD – standard deviation; A – 0 kg S/ha; B – 12.5 kg S/ha; C – 25 kg S/ha; D – 50 kg S/ha

positively influenced the oilseed rape yield. There are visible tendencies: the seed yields on non-S-fertilized treatments are usually the lowest and at the treatment fertilized using the highest S dose (50 kg S/ha) the highest yields were obtained. The highest yields were reached in the year 2010, where we got a range between 6.84 and 7.02 t of oilseed rape seed per ha at the Hněvčeves site. The least favourable was the year 2008, where we got the highest yields at the Hněvčeves site as well, but the values ranged only between 3.93 and 4.33 t/ha. The lowest yields were usually found at the Humpolec site, where are the worst soil-climatic conditions for oilseed rape cropping.

From our experiment is possible to conclude following facts: the S_w content ranged between 4.1 and 6.8 mg S/kg, the S_{ads} between 4.1 and 5.3 mg S/kg, the S_{ocl} between 3.1 and 8.6 mg S/kg and the S_{HI} between 35.1 and 175 mg S/kg.

The N-S fertilizer application (with the increasing S dose) increased the S_w and S_{ads} contents. The contents of S_{ocl} and S_{es} were probably not influenced by the chosen fertilizers.

The highest oilseed rape yields were always found at the Hněvčeves site and the lowest yields were usually observed at the Humpolec site.

Increasing S dose led usually to an increase of oilseed rape seed yield, but the differences between the observed treatments were not statistically significant.

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22) Mineral and organic fertilization efficiency in long-term stationary experiments

Efektivita minerálního a organického hnojení v dlouhodobých polních pokusech

V poslední době je stále větší pozornost věnována dlouhodobým polním pokusům. Je zřejmé, že provedená opatření mohou mít značné sezónní výkyvy a pozitivní výsledek v jednom roce nemusí zákonitě znamenat totéž pro další sezónu. Nejen proto dlouhodobé sledování přináší cenné informace o vlivu jednotlivých systémů hnojení a jiných opatření. V této publikaci byly hodnoceny výnosy plodin (brambory nebo kukuřice, pšenice ozimá a ječmen jarní) v pokusech založených v roce 1996. Hlavním sledovaným parametrem byl vliv různých systémů hnojení v různých půdně-klimatických podmínkách pěti stanovišť, vše po 14-ti letech od založení pokusu. Z výsledků je zřejmé, že samotná půdní úrodnost nemůže dlouhodobě zajistit vyšší výnosy. Logicky zde dochází k postupnému odčerpání živin a degradaci organické hmoty, která v některých variantách nebyla doplňována. Kontrolní nehnojená varianta tak vždy vykazovala nižší výnosy obilnin i sušiny hlíz brambor. V případě obilnin se jednalo o statisticky průkazné rozdíly, u brambor byly vzhledem k variabilitě výnosů (Hamouz et al. 2005) rozdíly neprůkazné. Z výsledků je dále zřejmé, že efekt hnojení se mnohem výrazněji projevil na méně úrodných stanovištích. Aplikace chlévského hnoje vedla k průměrnému zvýšení výnosů pšenice o 30 % a ječmene o 22 %. Ještě lepších výsledků bylo dosaženo po aplikaci čistírenských kalů, kde jejich aplikace zvýšila výnos pšenice dokonce o 41 % a ječmene o 26 %. Hnůj i čistírenské kaly zvýšily i výnos brambor, a to v průměru o 30 %. Nejvyšších výnosů bylo dosaženo vždy aplikací minerálních hnojiv, kde u pšenice došlo ke zvýšení o 59 %, u ječmene o 50 % a u brambor o 36 % (vždy oproti kontrole). Hnojení minerálními hnojivy rovněž vykazovalo vyšší variabilitu výnosů na jednotlivých stanovištích, což potvrzuje i studie Haberle et Ivičic (2006). Nižší efekt organických hnojiv (hnoje) ve srovnání s minerálním hnojením je udáván i ve studii Berzsenyi et al. (2000). Přesto však lze aplikaci hnoje jednoznačně doporučit, neboť pozitivně ovlivňuje bilanci organické hmoty, mikrobiální aktivitu půdy, vodní režim, stabilitu půdních agregátů a další (Ruan et al. 1993, Karlen et Doran 1993, Černý et al. 2008). Je možné konstatovat, že relativní zvýšení výnosů v důsledku hnojení bylo na všech stanovištích srovnatelné. Výjimku tvoří pouze stanoviště Lukavec, kde byl efekt hnojení výrazně vyšší. Důvodem byla pravděpodobně nízká přirozená úrodnost stanoviště.

Mineral and organic fertilization efficiency in long-term stationary experiments

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ABSTRACT

In long-term stationary experiments under different soil-climatic conditions, an influence of mineral and organic fertilization on yield of winter wheat, spring barley and potato tubers was evaluated. Statistically significantly lowest grain yields of winter wheat (4.00 t/ha) and spring barley (2.81 t/ha) were obtained in non-fertilized plots at all experimental sites. In the case of potatoes, the lowest yield of dry matter (5.71 t/ha) was recorded in the control plot, but the result was not statistically significant. The manure-fertilized plot gave the average yield of wheat higher by 30%, of barley by 22%. Application of sewage sludge resulted in wheat yield higher by 41% and barley yield higher by 26% over control. On average, application of sewage sludge and manure increased the yield of potatoes by 30% over control. The highest yield was obtained after application of mineral fertilizers; average yield increased by 59, 50 and 36% in winter wheat, spring barley and potatoes, respectively. No statistically significant differences among the plots with mineral fertilizers were observed. At different sites, the yield of studied crops varied; however, the effect of fertilization on yield increments was similar at all experimental sites except for Lukavec. It is the site with the lowest natural soil fertility, and it showed the highest effect of the applied fertilizers.

Keywords: long-term field experiment; winter wheat; spring barley; potatoes; manure; sewage sludge; mineral fertilizers

One of the most important intensification factors of crop production is plant nutrition and fertilization. This factor has a significant impact on yield and quality of harvested products. Cereals belong among crops that are important from the viewpoint of growing area. The ratio of winter wheat on agricultural soils presents an important part; it is an intensive crop with high demands and its yield is limited with appropriate agrotechnical equipment, site conditions and especially nutrition. Another important crop is spring barley; it is sensitive to weather conditions in spring period (Trnka et al. 2007). Barley can compensate weather changes mainly at balanced fertilization. Potatoes are described as a crop with high precrop value which is, however, influenced by the type of fertilization. Stable yields are obtained from potatoes fertilized with organic fertilizers, especially farmyard manure.

Yet, most agricultural companies in the Czech Republic limit fertilization to usage of mineral nitrogen fertilizers; the amount of nutrients uptaken by main and secondary products is often higher than the input of nutrients to soil in form of organic and mineral fertilizers. Comparison of conditions in the Czech Republic and European countries was described by Chloupek et al. (2004). A positive effect of farmyard manure application on crop yield was observed in numerous studies (Vaněk et al. 1997a, Balík et al. 1999, Barzegar et al. 2002). Nevertheless, many agricultural companies have no animal production or have significantly decreased production of farmyard fertilizers. Organic fertilization then may be replaced with straw left in the field after the harvest of cereals, although the effect of straw application on yield reported in literature is rather low (Powlson et al. 1985, Thomsen and Jensen 1994,

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Procházková et al. 2003). Yet, a positive influence of straw on soil properties was observed (Christensen 1986, Powlson et al. 1987, Ocio et al. 1991, Černý et al. 2003). An alternative to the application of organic matter and nutrients may be fertilization with stabilized sewage sludges from waste water treatment plants. Numerous publications describe advantages of sewage sludge application to agricultural soils, mainly for its influence on yield of fertilized crops (Christie et al. 2001), but also with reference to soil properties (Johansson et al. 1999, Balík et al. 2007). Compared to organic fertilizers, the reported influence of mineral, mainly nitrogen fertilizers on grain yield of cereals is often higher (Hansen 1996).

The effect of fertilization on crop yield was observed in many studies; the trials however show that the strength of the effect may differ. Soil conditions, climatic factors and weather conditions in a given year also significantly influence yield production (Benjamin et al. 2003, Cai and Qin 2006). Locally-specific effects of field trials are often a limiting factor for drawing correct conclusions. A short-term effect can be inconsistent with the results of long-term observations. Hence, agricultural practices need the results of both short-term and long-term experiments. Field trials are the most relevant source of such information and may help to balance the inconsistence of outputs of e.g. pot experiments (Leikam et al. 1983).

The aim of this work is to evaluate long-term effects of different fertilizers on yield of winter wheat, spring barley and potato tubers in different soil-climatic conditions.

MATERIAL AND METHODS

The effect of fertilization on grain yield of winter wheat and spring barley was observed in precise long-term field trials. These trials were established in 1996 at five sites of the Czech Republic with different soil-climatic conditions: Červený Újezd, Hněvčeves, Humpolec, Lukavec, Praha - Suchdol (Table 1). Within the trials three crops were rotated in the following order: potatoes, winter wheat, spring barley. Because of the agrotechnical conditions of the Červený Újezd site, potatoes as the experimental crop were replaced there by silage maize. The size of experimental plots was 60 m^2, at Červený Újezd site it was 80 m^2. The trial comprised 7 treatments: 1. no fertilization (control); 2. sewage sludges (SS); 3. farmyard manure (FYM); 4. half dose of farmyard manure + N in mineral nitrogen fertilizers (FYM + N); 5. mineral nitrogen fertilizers (N); 6. NPK in mineral fertilizers (NPK); 7. straw of spring barley + N in mineral nitrogen fertilizers (ST + N). Organic fertilizers – sewage sludges (SS), farmyard manure (FYM) and straw (ST) were always applied in autumn (October) to potatoes (maize). Mineral phosphorus and potassium fertilizers were applied to each crop in autumn; mineral nitrogen fertilizers were applied to potatoes and spring barley in spring prior to crop establishment. In the case of winter wheat the nitrogen dose was divided into halves, the first one was applied as regenerative fertilization, the second one as productive fertilization (Table 2). The content of nitrogen was 140 kg N/ha for wheat and 70 kg N/ha for spring barley. The NPK treat-

Table 1. Experimental sites characteristics

Site	Červený Újezd	Hněvčeves	Humpolec	Lukavec	Suchdol
Location	50°4'22"N, 14°10'19"E	50°18'46"N, 15°43'3"E	49°33'16"N, 15°21'2"E	49°33'23"N, 14°58'39"E	50°7'40"N, 14°22'33"E
Altitude (m a.s.l.)	410	265	525	610	286
Mean annual temperature (°C)	7.7	8.2	7.0	7.7	9.1
Mean annual precipitation (mm)	493	573	665	666	495
Soil type	Luvisol	Luvisol	Cambisol	Cambisol	Chernozem
Soil texture	loam	clay-loam	sandy-loam	sandy-loam	loam
pH ($CaCl_2$)	6.5	5.9	5.1	4.3	7.5
C_{ox} (%)	1.7	1.6	2.3	1.7	2.6
CEC (mmol$^{(+)}$/kg)	145	179	159	128	230
P Mehlich III (mg/kg)	100	84	90	124	91
K Mehlich III (mg/kg)	80	150	190	213	230
Mg Mehlich III (mg/kg)	110	130	100	80	240
Ca Mehlich III (mg/kg)	3600	3600	1300	1100	9000

Table 2. Application rates of nutrients (kg/ha) – (3-year cycle)

Treatment	Potatoes			Wheat			Barley		
	N	P	K	N	P	K	N	P	K
Control	–	–	–	–	–	–	–	–	–
Sewage sludge (SS)	330[1]	201[2]	55[2]	0	0	0	0	0	0
Farmyard manure (FYM)	330[1]	118[2]	374[2]	0	0	0	0	0	0
FYM + N[3]	165[1]	59[2]	187[2]	110	0	0	55	0	0
N[3]	120	0	0	140	0	0	70	0	0
NPK[3]	120	30	100	140	30	100	70	30	100
Straw ST + N[3]	138	6[2]	47[2]	140	0	0	70	0	0

[1]nitrogen as total nitrogen in organic fertilizers; [2]average yearly dose, depends on nutrient content in organic fertilizers (Table 3); [3]mineral fertilizers: N – calcium ammonium nitrate (27% N); P – triple super phosphate (21% P); K – potassium salt (50% K)

ment of winter wheat and spring barley included phosphorus at a rate of 30 kg P/ha (Triple Super Phosphate) and potassium at a rate of 100 kg K/ha (60% potassium salt). At the other treatments, the dosage of P and K depended on the content of nutrients in applied organic fertilizers (Table 3). At all sites, the sewage sludges from the Waste Water Treatment Plant in Prague-Troja were used. Plant harvesting was done with an experimental combine harvester. The average size of harvested plots was 20 m^2 in Humpolec and Hněvčeves, 40 m^2 in Lukavec and Suchdol and at Červený Újezd site it was 60 m^2. The obtained fresh matter yield was converted into dry matter content for spring barley, winter wheat and potato. Samples for dry matter determination were dried at 105°C. The yield data sets were recalculated to dry matter yield per one hectare area.

The results were assessed using the ANOVA statistical analysis. The differences in yield caused by the type of fertilization at individual sites for the whole experimental period (1997–2008) were compared. To evaluate the obtained results, the STATISTICA programme was used.

RESULTS AND DISCUSSION

The presented results were obtained during the whole period of the trial, i.e. from 1997 to 2008. The aim being to document effects of different forms of fertilization, the graphical forms use relative values related to the control non-fertilized treatment. Variability of the obtained results is quantified using the coefficient of variation. Higher variability is influenced mainly by the interannual yield variability at individual sites.

Potatoes and maize. Average yields of potatoes and silage maize dry matter are shown in Table 4. The lowest yield was obtained at control plots at all sites. Lower values of potato tubers dry matter, namely 4.89 and 5.17 t/ha were recorded at control plots at Suchdol and Hněvčeves sites, respectively. At Humpolec and Lukavec sites, the yields of potato tubers dry matter were 6.30 and 6.49 t/ha, respectively. Site conditions and type of fertilization have a great effect on potato tubers yield formation as well as on their qualitative parameters (Hamouz et al. 2005). Compared with cereals, potatoes gave a lower yield increment as

Table 3. Average characteristics of organic fertilizers and their dry matter (DM) application rates

	Dose (t/ha/y)	DM content (%)	Nutrients content (% DM)				
			N	P	K	Ca	Mg
Sewage sludge (SS)	9.00	30.6	3.66	2.23	0.61	3.00	0.78
FYM Červený Újezd	14.48	30.3	2.48	0.81	2.14	2.28	0.58
FYM Hněvčeves	16.63	25.6	2.15	0.75	2.49	2.51	0.59
FYM Humpolec	14.92	24.6	2.28	0.72	2.24	1.55	0.62
FYM Lukavec	17.77	26.3	1.90	0.63	2.72	1.71	0.51
FYM Suchdol	16.83	34.2	2.05	0.76	1.94	2.20	0.60
Straw (ST)	5.00	95.0	0.35	0.11	0.93	0.49	0.04

Table 4. Mean dry matter yield (t/ha) of potato tubers (silage maize) at different sites (average per 12 years)

Treatment	Červený Újezd^1		Hněvčeves^2		Humpolec^2		Lukavec^2		Suchdol^2	
	yield	s	yield	s	yield	s	yield	s	yield	s
Control	12.47	2.34	5.17	1.51	6.30	1.90	6.49	1.97	4.89	1.48
SS	15.32	2.45	7.55	2.50	8.02	2.35	8.56	2.70	5.94	1.67
FYM	14.09	3.56	7.67	2.89	8.65	2.61	8.34	2.73	5.84	1.38
FYM + N	13.98	3.33	7.02	2.31	8.17	2.23	7.92	2.42	5.77	1.42
N	14.70	3.78	8.14	2.89	9.02	2.24	9.77	2.95	5.54	1.51
NPK	15.89	4.14	8.16	3.60	8.04	1.80	10.18	3.04	6.12	1.63
ST + N	14.95	3.40	8.18	2.44	8.15	2.23	9.18	2.58	5.63	1.44

^{1}yield of silage maize DM; ^{2}yield of potato tubers DM

a response to fertilization. At Suchdol site, the average yield, compared to control, was higher by 13–18% at plots treated with nitrogen fertilizers, by 19% with manure treatment, by 21% with sewage sludges and by 25% at plots treated with NPK fertilizer (Figure 1). Similar results were obtained at Hněvčeves and Humpolec sites, where the yield increments were even higher. In Hněvčeves, the increase of yield at plots treated with half dose of manure was 36% over control. At plots with manure and sewage sludges, the increase was 46 and 48%, respectively. The other treatments gave an increase by 58% compared to control. At Humpolec site, the highest increments, compared to control, were recorded at N treatment (43%) and FYM treatment (37%). The other treatments resulted in an increase of yield over control within the range of 27–29%. At Lukavec site, the yield increment was lowest at treatment with half dose of farmyard manure, i.e. 22% over control; the treatment with manure and sewage sludges resulted in an increase by 28 and 32%, respectively. Plots amended with mineral fertilizers showed yield higher by 41–57% than control. In the case of potatoes, we observed high interannual variability of yield in the course of the trial, and thus the specific differences in yields were not regarded as statistically significant (Figure 2). Interannual yield variability is also influenced by type of fertilization; the treatments with mineral fertilizers showed higher yield variability compared to organic fertilizers. Similar results are reported by Haberle and Ivičic (2006). Average yield of silage maize dry matter at Červený Újezd site was 12.47 t/ha at non-fertilized plot. Plots fertilized with manure had yield higher by 12–13% than control. Nitrogen application increased the yield by 18% and N with straw by 20%. The application of sewage sludges resulted in an increase of silage maize dry matter by 23% compared to control. The greatest effect was recorded at application of NPK fertilizers, with the yield increased by 27% over control.

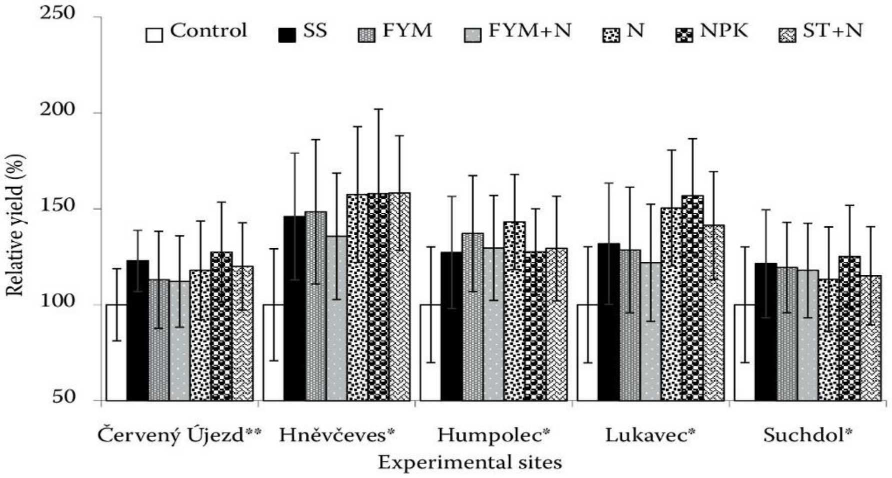

Figure 1. Relative dry matter yield (%) of potato tubers* (silage maize**) at different sites

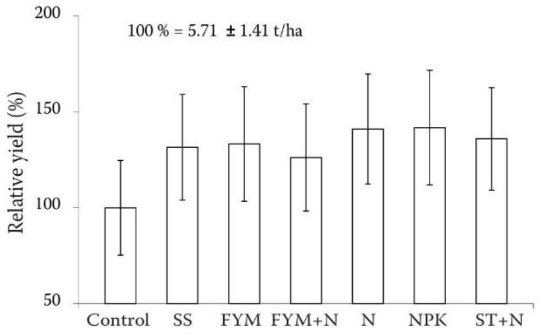

Figure 2. Relative dry matter yield (%) of potato tubers – all sites average per 12 years

Winter wheat. Average yields of winter wheat at all experimental sites are shown in Table 5. Winter wheat grain yield was affected by site conditions, year and fertilization treatment.

The lowest yields were obtained at all sites at the non-fertilized control plots; the significantly lowest average yield, namely 2.74 t/ha, was recorded at Lukavec site, the highest average yield of 4.92 t/ha was recorded at Humpolec site. The Lukavec site trials, compared to the other sites, gave the lowest values of wheat grain yield at plots treated with manure and sewage sludges. Both the treatments had the average yield of 3.80 t/ha for the whole experimental period. At other sites, the differences in grain yield of winter wheat among fertilized plots were not significant; however, the treatments with only manure or sewage sludges always resulted in lower yields. Lower yields of winter wheat after farmyard manure application in long-term trials were described by Berzsenyi et al. (2000), who compared the effect of manure application and combinations of NPK fertilization. Although the effect of manure application on yield is lower than that of NPK fertilization, usage of farmyard manure brings about other positive aspects, especially to soil properties. It serves, for instance as a source of organic matter, it increases stability of soil aggregates, influences soil water regime, activity of soil microorganisms and amount of microbial biomass (Raun et al. 1993, Karlen and Doran 1993, Černý et al. 2008). A greater effect on grain yield of cereals is reported at combined application of manure and mineral fertilizers (Manna et al. 2005). It is in compliance with our results; the average grain yield of winter wheat at application of manure and mineral nitrogen fertilizers was higher by 0.59–2.47 t compared to manure application only.

In a comparison of treatments with sewage sludges and manure, a greater effect on yield was recorded at sewage sludges application; the average yield was higher by 0.41 t/ha compared to manure application. The results indicate that the treatment with sewage sludges had a positive effect on winter wheat yield, which is in accordance with the results of other long-term trials (Mantovi et al. 2005). As for the application of mineral nitrogen fertilizers, the obtained values of winter wheat grain yield were relatively balanced; the differences in yield were influenced mainly by site soil-climatic conditions. Average yield of treatments with mineral nitrogen fertilizers ranged between 5.42 and 6.01 t/ha at Červený Újezd and Suchdol sites, between 6.04 and 6.50 t/ha at Lukavec site and between 6.95 and 7.24 t/ha at Humpolec and Hněvčeves sites.

In a comparison of effects of different types of fertilization on grain yield of winter wheat, a significant difference was recorded between the control and fertilized plots. Only at Suchdol site, this difference was not statistically significant. Inasmuch as it is a fertile site with good reserve of available nutrients, the effect of fertilization was rather low. At more fertile sites, the absence of fertilization results in a much slower yield reduction compared to less fertile sites (Vaněk et al. 1997b). This is documented by the results from

Table 5. Mean dry matter yield (t/ha) of winter wheat grain at different sites (average per 12 years)

Treatment	Červený Újezd		Hněvčeves		Humpolec		Lukavec		Suchdol	
	yield	s	yield	s	yield	s	yield	s	yield	s
Control	3.39	0.53	4.54	1.22	4.92	0.74	2.74	0.48	4.43	1.05
SS	5.07	0.74	7.01	1.65	6.82	0.98	3.80	0.96	5.53	1.61
FYM	4.79	0.97	6.64	1.07	5.96	1.00	3.80	0.82	5.00	1.84
FYM + N	5.54	0.89	7.32	1.26	6.91	1.33	6.27	0.70	5.59	1.41
N	5.65	0.75	7.24	1.17	6.95	1.25	6.41	0.68	5.75	1.45
NPK	5.55	0.67	7.17	1.18	7.13	1.16	6.50	0.74	6.01	1.38
ST + N	5.42	0.99	7.23	1.24	7.20	1.44	6.04	0.76	5.57	1.47

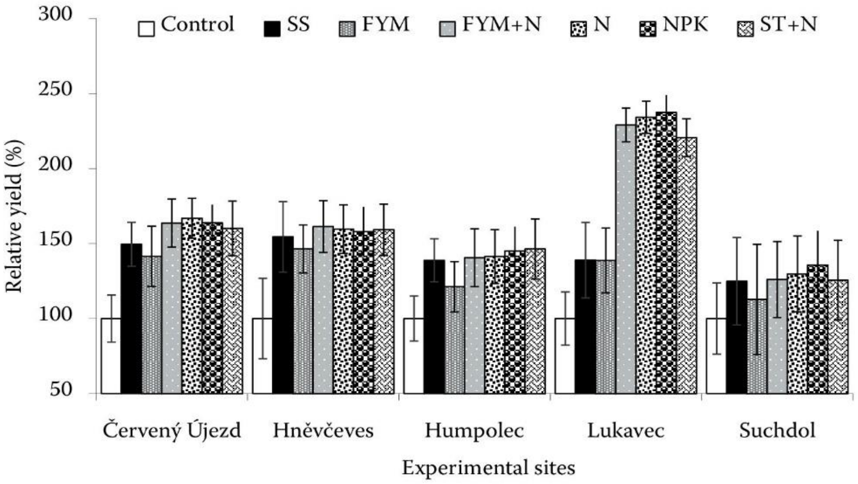

Figure 3. Relative yield of winter wheat grain (%) at different sites

Lukavec site; the treatments with mineral fertilizers resulted in yields higher by 120 to 137% over control (Figure 3). At the other sites, the yields at plots treated with mineral fertilizers were higher by 40–66% compared to control. Application of sewage sludges increased grain yields by 25% at Suchdol site, by 39% at Humpolec and Lukavec sites, and the highest increase was recorded at Červený Újezd and Hněvčeves sites, where the increase reached 50 and 55%, respectively. A lower effect on yield was observed in the case of manure application (Figure 4); the increase was higher by 13, 21 and 39% at Suchdol, Humpolec and Lukavec sites. The greatest effect of manure application on winter wheat grain yield was recorded at Červený Újezd and Hněvčeves sites, where the yield increased by 41 and 46%, respectively.

Spring barley. Average yield of spring barley at studied sites is shown in Table 6. Spring barley grain yield was, as that of winter wheat, influenced by site conditions, year and fertilization treatment. However, a higher interannual variability of yields was observed at spring barley. In a comparison of effect of fertilization on spring barley grain yield, the lowest values were obtained at all sites at control non-fertilized plots; the lowest average yield was 1.92 t/ha in trials at Lukavec site, whereas the highest average grain yield in control was obtained at Suchdol and Hněvčeves sites, with the values of 3.27 and 3.30 t/ha. At Červený Újezd and Humpolec experimental sites, the average grain yields of spring barley at control plots were 2.73 and 2.85 t/ha, respectively. As in the case of winter wheat, the lowest yield of fertilized plots was obtained at Lukavec site; average spring barley yield after treatment with sewage sludges reached 2.39 t/ha, whereas it was 2.59 t/ha in the case of manure application. The treatments with mineral fertilizers in Lukavec resulted in average values of 3.39–3.57 t/ha. Among fertilized plots no statistically significant differences were obtained.

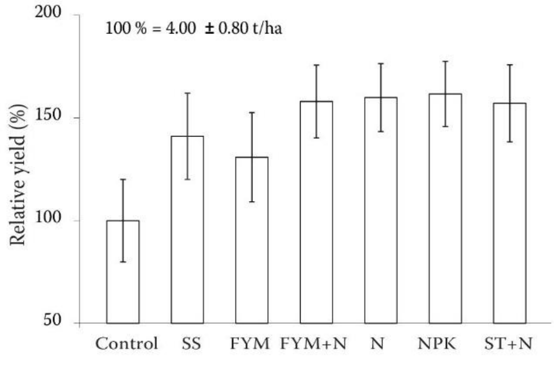

Figure 4. Relative yield (%) of winter grain – all sites average per 12 years

At other sites, the differences among spring barley grain yields in fertilized plots were not significant, either; however, lower yields were always obtained in treatments with only manure or sewage sludges compared to plots with mineral fertilization. Yield increment of spring barley after application of different doses of manure was also described by Hansen (1996); differences among yields were not significant, whereas he reported significantly higher yield after the application of NPK.

In a comparison of plots treated with sewage sludges and manure, the application of sewage sludges had a greater effect on yield; the average values were by 0.22 t/ha higher than with manure

Table 6: Mean dry matter yield (t/ha) of spring barley grain at different sites (average per 12 years)

Treatment	Červený Újezd		Hněvčeves		Humpolec		Lukavec		Suchdol	
	yield	s	yield	s	yield	s	yield	s	yield	s
Control	2.73	0.71	3.30	0.47	2.85	0.81	1.92	0.31	3.27	0.80
SS	3.80	0.91	4.30	0.91	3.61	0.80	2.39	0.54	3.68	0.87
FYM	3.65	0.89	3.88	0.78	3.57	0.71	2.59	0.59	3.42	0.91
FYM + N	4.27	0.79	4.53	1.37	4.52	0.57	3.44	0.59	4.26	0.71
N	4.22	0.63	4.53	1.49	4.43	0.73	3.52	0.75	4.13	1.05
NPK	4.40	0.77	5.15	1.71	4.70	0.69	3.57	0.84	4.30	0.81
ST + N	4.22	1.03	4.78	1.30	4.60	0.89	3.39	0.61	4.39	0.81

application. If these results are compared to wheat values the yield reaction of spring barley to sludge application is lower; it is caused by a longer period after sludges application, as barley was the last crop in rotation. A fast process of mineralization of organic matter from sewage sludges releases nutrients for crops after application and the influence of fertilization in following years decreases (Antolín et al. 2005). The half-life of nitrogen in organic bonds of sewage sludges is reported to be one year (Hall 1984). This is documented by the results from the sites with light soils; at Humpolec site, yields of the plots treated with sewage sludges and manure were equal, and at Lukavec site, the long-term yield of the plot treated with sewage sludges was by 0.20 t/ha lower compared to manure. Organic matter applied in the farmyard manure is more stable compared to sewage sludges (Saviozzi et al. 1999) and has a greater effect on the content of organic C and N in soil (Nedvěd et al. 2008).

In a comparison of plots treated with mineral nitrogen fertilizers, the obtained grain yields of spring barley were relatively stable; the differences in yields were influenced mainly by site soil-climatic conditions. Values of average yields at plots treated with mineral nitrogen fertilizers ranged between 4.52–5.15 t/ha at Humpolec and Hněvčeves sites, between 4.13–4.40 t/ha at Červený Újezd and Suchdol sites, and 3.44–3.57 t/ha at Lukavec site.

In a comparison of effect of fertilization on spring barley grain yield, a significant difference was obtained between control and fertilized plots. The effect of fertilization was, however, lower than in the case of winter wheat. A greater difference between control and fertilized plots was observed at Červený Újezd, Humpolec and Lukavec sites; the difference was statistically significant only between control and application of mineral fertilizers (Figure 5). At plots treated with organic fertilizers, higher yield, compared to control, was

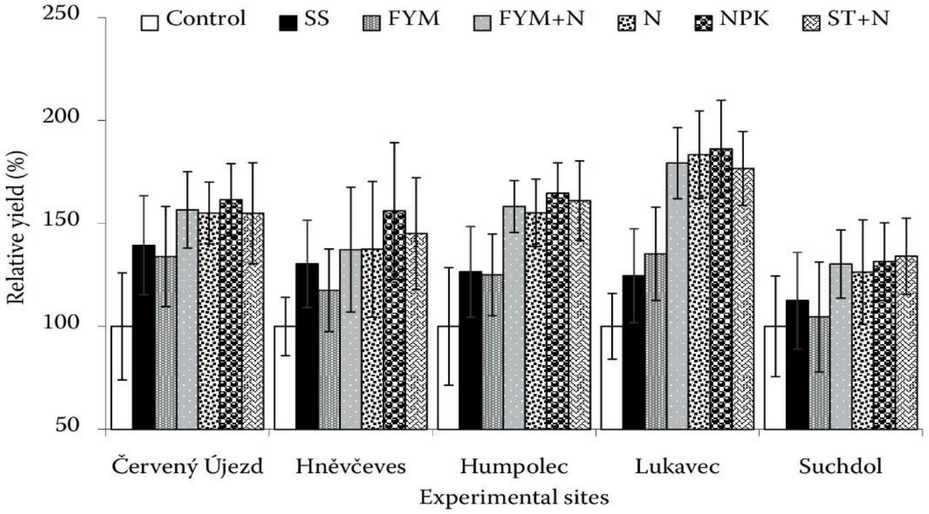

Figure 5. Relative yield of spring barley grain (%) at different sites

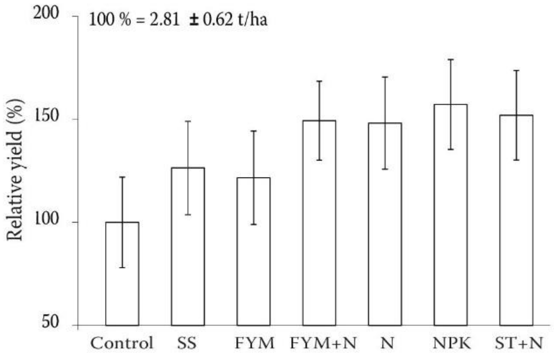

Figure 6. Relative yield (%) of spring barley grain – all sites average per 12 years

obtained only in the case of sewage sludge treatments. Their application increased grain yield by 13, 25, 27 and 30% at Suchdol, Lukavec, Humpolec and Hněvčeves sites, respectively; the greatest effect was recorded at Červený Újezd site, where the yield increased by 39%. A lower influence was observed at manure application. The yield, compared to control, was higher by 5, 18, 25, and 34% at Suchdol, Hněvčeves, Humpolec and Červený Újezd sites, respectively. The greatest effect on spring barley grain yield after manure application was obtained at Lukavec site, where the yield increased by 35% over control.

In treatments with mineral fertilizers the increment of spring barley grain yield was higher. As in the case of winter wheat, the grain yield of spring barley showed a strongest reaction to applied fertilizers at Lukavec site where the treatments with mineral fertilizers resulted in an increase by 77 to 86% over control. The lowest effect of mineral fertilization was obtained at Suchdol site; the average yield was by 26–34% higher than control. At other sites, the plots fertilized with mineral fertilizers gave the yield higher by 37–65% compared to control.

The results of the evaluation of cereals show a more significant effect on yield at plots treated with mineral fertilizers; the most important nutrient was applied nitrogen. A lower effect was reported after farmyard manure and sewage sludges application (Figures 4 and 6). This effect was more important for winter wheat; in the case of spring barley, with the longer interval after application, the yield decreased. At all sites, the lowest yields were obtained at non-fertilized plots.

The overall comparison of all three studied crops and all sites indicates the importance of combining mineral and organic fertilization. The yields after application of organic fertilizers only (though at relatively high doses) did not reach the values obtained at balanced mineral fertilization. No significant differences between farmyard manure and sewage sludges treatment were recorded in either crop (providing that the amount of supplied nitrogen was the same).

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23) Evaluation of phosphorus mobility in soil using different extraction methods

Vyhodnocení mobility fosforu v půdě pomocí různých extrakčních metod

V níže uvedené publikaci jsou hodnoceny různé formy půdního fosforu z rozdílných půdně klimatických podmínek dlouhodobých pokusů realizovaných v České republice a v Německu. Z těchto pokusů bylo celkem odebráno více než 200 půdních vzorků z 22 stanovišť, ve kterých byly porovnávány následující frakce fosforu: 1) okamžitě přístupný fosfor extrahovaný 0.01 mol/l $CaCl_2$, 2) výměnný fosfor stanovený iontovýměnnými (AEM) membránami, 3) a 4) potenciálně přístupný fosfor extrahovaný metodami Mehlich 3 a DL (dříve Egner), 5) celkový minerální fosfor, 6) organický podíl P, 7) fosfor vázaný na železo a hliník, 9) celkový P stanovený v rámci frakcionace Marks (1977) a 8) reziduální P v lučavce královské. Uvedené pořadí metod odpovídá i jejich extrakční účinnosti, kdy nejnižší výsledky byly dosahovány v případě metody $CaCl_2$ a nejvyšší u lučavky královské. Z toho je zřejmé, že se v případě fosforu získaného frakcionací dle Marks (1977) nejedná o skutečně celkový fosfor, ale pouze o určitý podíl reziduálního P. Při statistickém vyhodnocení byly podle předpokladů získány velmi těsné korelace ($p<0{,}001$) mezi metodami zaměřenými na přístupné a potenciálně přístupné formy P. Při stejné hladině významnosti byly potvrzeny i vztahy mezi celkovým minerálním P, fosforem vázaným na železo a hliník, P v lučavce královské i fosforem stanoveným frakcionací dle Marks (1977). Je nutno uvést, že takto vysoké korelace byly částečně způsobeny i různými variantami hnojení. Zde se nárůst obsahu jedné frakce P v důsledku hnojení projevil zpravidla i nárůstem ostatních frakcí P. proto byly korelační vztahy těsnější. Obsahy okamžitě přístupného P v půdě byly podobné jako v dříve publikované studii Kulhánek et al. (2007). V rámci vyhodnocení extrakční účinnosti metod pro stanovení přístupného P bylo nejvyšších hodnot dosaženo u metody Mehlich 3. Vzhledem k nízkému pH extraktantu a poměrně vysoké koncentraci jednotlivých složek zde lze předpokládat i extrakci některých nepřístupných forem P (Tiessen et Moir 1993). Obsahy reziduálního fosforu se pohybovaly v širokém rozmezí 273 – 2024 mg P/kg. To je v souladu s výsledky řady publikací (mj. Mengel 1991, Schilling 2000, Blume et al. 2002), kde je tato variabilita vysvětlována především vlivem stanoviště.

Evaluation of phosphorus mobility in soil using different extraction methods

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ABSTRACT

Soil samples (from Czech and German long-term field experiments) were used to estimate different soil phosphorus (P) fractions. More than 200 topsoil (0–30 cm) samples from different fertilizing treatments were taken. These were analyzed for P in soil solution (P_{CaCl_2}) [0.01M $CaCl_2$ extract], exchangeable sorbed P (P_{ex}) [anion exchange (AE) membranes] and bioavailable P [Doppel-Lactat and Mehlich 3 (P_{DL} and P_{M3})]. Other fractions analyzed were total inorganic (P_{in}), total ($P_{M\text{-}tot}$) and organic (P_{org}) P [fractionation after Marks], P sorbed on Fe and Al (P_{FeAl}) [fractionation after Schwertmann] and residual P (P_{ar}) [aqua regia extract]. Comparison of medians appeared to be better for evaluating extraction abilities. Phosphorus fractions were in the following order: (P_{ar} = 100%); P_{CaCl_2} (0.2%) < P_{ex} (9%) < P_{DL} (10%) < P_{M3} (16%) < P_{in} (24%) < P_{org} (37%) < P_{FeAl} (55%) < $P_{M\text{-}tot}$ (59%). Low amounts of P_{in}, P_{org} and $P_{M\text{-}tot}$ did not verify the applicability of the Marks' fractionation for the set of studied soils. Close correlations at $P \leq 0.001$ were found for all methods for estimating the fractions of bioavailable phosphates (P_{CaCl_2}, P_{ex}, P_{DL} and P_{M3}). Statistically significant relations were observed between P_{in} with P_{ar}, $P_{M\text{-}tot}$ and P_{FeAl}.

Keywords: phosphorus; soil; bioavailability; P fractions

Total P amounts, which range between 0.02 and 0.20%, depend on the composition and type of the soil (e.g. Mengel 1991, Schilling 2000, Blume et al. 2002) and are divided in different fractions. All of them may have an impact on the availability of P for plants. Therefore, it is important to have a good knowledge of all P fractions to gain a better insight of processes taking place in soils.

Phosphorus concentration is normally lowest in soil solution. This fraction is usually estimated by extraction with water (Luscombe et al. 1979) or with 0.01M $CaCl_2$ (Houba et al. 1994) and ranges between 0.8–8 mg P/kg (Marschner 1995). Exchangeable sorbed P (P_{ex}) is another important source of bioavailable P. It is the part of soil P that is exchangeable and adsorbed on the surface of soil particles and can be easily released to the soil solution. This fraction can be estimated for example with anion resins (Tiessen and Moir 1993, Lewis and McGechan 2002), or recently, via anion exchange (AE) membranes (Kuono et al. 1995, Sato and Comerford 2006).

The most commonly used methods are those for estimating total bioavailable P in soils: e.g. Mehlich 3 (Mehlich 1984), Olsen (Olsen et al. 1954), CAL and Doppel-Lactat (DL) (Hoffman 1991), Bray 1 and 2 (Bray and Kurtz 1945), etc. These extractions are focused on the determination of potentially quickly bioavailable P, i.e. P in soil solution and weakly bound P.

One of the most important fractions in non-calcareous soils are Fe and Al phosphates (P_{FeAl}). Adsorption runs commonly on the sesquioxides (Fe, Al oxides), allofans, clay minerals and organic Fe and Al complexes. Adsorption increases with increasing amorphous phases (Sharpley 1995). Adsorbed phosphates are released back to the soil solution with increasing pH value (desorption), resulting thus into plant available fractions (Schilling 2000). Fe and Al phosphates are estimated

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with different fractionation methods, e.g., Chang and Jackson (1957), Hedley et al. (1982) or separately with the method according to Schwertmann (1964).

Several fractionation methods, e.g., Chang and Jackson (1957), Marks (1977), Hedley et al. (1982) etc., are commonly used for estimating total inorganic P (P_{in}). One part of these fractionations is ever estimating of total P (P_{tot}). Based on data from these fractionation methods, it is possible to calculate the amount of total organic P (P_{org}) as the difference between P_{tot} and P_{in} (Olsen and Sommers 1982).

To estimate total P (P_{tot}) in soils many methods are available. Some of them are described in detail in the work of Kara et al. (1997). Commonly used extraction procedure is the aqua regia extraction (P_{ar}). According to Crossland et al. (1995) this method correlates well with alkaline fusion. Therefore, the aqua regia extraction for estimating of residual P is useful for indirect estimation of total P in soils.

The aim of this work was to evaluate selected methods used for estimating of different P forms in soil. The methods studied included 0.01M $CaCl_2$ extraction, use of anion exchange membranes, Mehlich 3 and Doppel-Lactat methods, estimating of Fe-, Al-P, and fractionation after Marks (1977) and the extraction with aqua regia.

MATERIALS AND METHODS

Soil samples (0–30 cm) were taken from 22 sites of long-term field experiments in the Czech Republic (16 sites) and Germany (6 sites). Characteristics of these sites are summarized in **Table 1**. The experiments have been running for more than 20 years at the time of sampling, except Lukavec, Humpolec, Červený Újezd and Hněvčeves sites,

Table 1. Characteristics of the experimental sites

Site	Soil type	Soil texture	pH (0.01M $CaCl_2$)
Horažďovice	Cambisol	loamy sand	5.9
Chrastava	Cambisol	loamy sand	5.8
Jaroměřice	Haplic luvisol	loamy	6.3
Krásné Údolí	Cambisol	sandy loam	5.5
Libějovice	Haplic luvisol	sandy loam	6.2
Lípa	Cambisol	sandy loam	5.9
Staňkov	Haplic luvisol	loamy	6.0
Svitavy	Haplic luvisol	sandy loam	6.2
Vysoká	Stagnosol	loamy	6.3
Pusté Jakartice	Haplic luvisol	loamy	5.8
Uherský Ostroh	Haplic luvisol	loamy	6.6
Žatec	Chernozem	silty clay	6.4
Červený Újezd	Haplic luvisol	loamy	6.5
Hněvčeves	Mollic luvisol	clay loamy	5.9
Humpolec	Cambisol	sandy loam	5.1
Lukavec	Cambisol	sandy loam	5.3
Dahlem (Germany)	Albic luvisol	loamy sand	5.4
Lauchstädt (Germany)	Chernozem	silt loam	6.9
Lauterbach (Germany)	Dystric cambisol	silt loam	5.3
Dikopshof (Germany)	Haplic luvisol	silt loam	5.6
Thyrow (Germany)	Albic luvisol	loamy sand	5.0
Halle (Germany)	Chernozem	loamy sand	6.0

where the samples were taken 6 years after experiment set up. Only sites with an average pH value under 7.0 were chosen. Commonly 228 samples from different fertilizing treatments were taken. More fertilizing treatments were chosen, to get higher variability of studied samples. Samples were air-dried and passed through a 2-mm sieve.

Soil samples were analyzed for different P fractions. The following methods were used: 0.01M $CaCl_2$ extraction (Houba et al. 1994) for readily available P (P_{CaCl_2}), anion exchange (AE) membranes (adapted after Kuono et al. 1995) for the extraction of exchangeable P (P_{ex}), Mehlich 3 (Mehlich 1984) and Doppel-Lactat (DL) (Hoffman 1991) methods for bioavailable P (P_{M3} and P_{DL}), P sorbed on Fe and Al compounds (P_{FeAl}) according to Schwertmann (1964), extraction with 0.2M $C_2H_8N_2O_4 \cdot H_2O$ and afterwards digestion with H_2SO_4 and H_2O_2 at 250°C for total inorganic (P_{in}), total organic (P_{org}) and total P ($P_{M\text{-}tot}$) contents according to Marks (1977). To estimate P_{in} soil samples were extracted with 1M NaOH after the DL extraction. Five ml of 1M NaOH extract were then digested with 1M H_2SO_4 at 120°C to obtain the total P ($P_{M\text{-}tot}$) content. Aqua regia extracts (EN 13346) were used for measuring residual P (P_{ar}). All reagents used were in per analysis quality. All extracts were measured spectrophotometrically after Murphy and Riley (1962) with SKALAR SANPLUS SYSTEM. Spearman correlation test, average, median, maximal and minimal values were used for the statistical evaluation.

RESULTS AND DISCUSSION

The obtained data did not meet normal distribution with the statistical tests. Therefore, basic statistical characteristics (average, median, maximal and minimal contents) were used for the evaluation of results. These are depicted in Table 2.

The comparison of extraction ratios of all methods yielded almost always statistically significant differences. Only between P_{ex} and P_{DL}; $P_{M\text{-}tot}$ and P_{FeAl} no statistically significant differences were obtained.

The lowest content of P was, as expected, measured in soil solution with 0.01M $CaCl_2$ (P_{CaCl_2}). Maximal content of P was 8.90 mg P/kg. The value of median was only 1.70 mg P/kg. It corresponds with our previous results (Kulhánek et al. 2007), where we obtained values between 0.20 and 2.80 g P/kg.

A relatively low content of P was also measured with AE membranes (P_{ex}) where similar values of average and median were reached, which varied around 60.0 mg P/kg. This method should estimate exchangeable sorbed P, which has the ability to easily pass to the soil solution and become available for plants.

No significant differences (based on the box-plot comparison) were found between the methods Mehlich 3 and Doppel-Lactat commonly used for estimating total bioavailable P. With both of them we did not reach any outlaying observations. The median of P_{DL} was 70.3 mg P/kg and when using P_{M3} the estimate was about 35.5 mg P/kg higher. Both methods yielded higher values than P_{ex}. It is also likely that with the Doppel-Lactat and especially Mehlich 3 method results into nonbioavailable fractions of P. This does not, however, limit the possibility of using these methods to estimate bioavailable P, because according to Tiessen and Moir (1993) the Mehlich 3 and Doppel-Lactat methods should not only estimate significant contents of nonbioavailable P fractions.

The fractionation after Marks (1977) was used for estimating the contents of total inorganic (P_{in}), organic (P_{org}) and total P ($P_{M\text{-}tot}$). The results showed that in the analyzed soils, usually higher contents of P were bound in organic matter. The median of P_{in} was 190 mg/kg and 250 mg P/kg for P_{org}. Analyzing the median values appeared to be more suitable in this case, because of the presence of outlaying observations that inadequately influenced the values of averages.

The content of P_{FeAl} averaged 446 mg P/kg. Regarding the outlaying observations in the area of extremely high values, it appeared to be more

Table 2. Portions of different P fractions in soils (mg P/kg)

	P_{CaCl_2}	P_{ex}	P_{DL}	P_{M3}	P_{in}	P_{org}	$P_{M\text{-}tot}$	P_{FeAl}	P_{ar}
Average	1.70	61.4	84.4	116	190	317	506	446	705
Median	1.30	59.1	70.3	106	162	250	397	374	676
Max	8.90	147	326	297	696	910	1440	1430	2024
Min	0.00	8.6	12.2	19.8	47.0	19.5	130	142	273

Table 3. Correlations between observed P fractions (Spearman correlation coefficient *r*)

	P_{CaCl_2}	P_{ex}	P_{DL}	P_{M3}	P_{in}	P_{org}	P_{FeAl}	$P_{M\text{-}tot}$	P_{ar}
P_{CaCl_2}	1.00	0.50**	0.50**	0.58**	–0.08	0.22	–0.08	–0.19	–0.05
P_{ex}		1.00	0.79**	0.58**	0.28*	0.10	0.27*	0.18	0.36*
P_{DL}			1.00	0.65**	0.25*	–0.02	0.24	0.08	0.37*
P_{M3}				1.00	0.52**	0.26*	0.53**	0.38*	0.43**
P_{in}					1.00	0.63**	0.88**	0.84**	0.87**
P_{org}						1.00	0.70**	0.96**	0.49**
P_{FeAl}							1.00	0.84**	0.75**
$P_{M\text{-}tot}$								1.00	0.68**
P_{ar}									1.00

**highly significant relations at $P \leq 0.001$, *significant relations at $P \leq 0.01$

suitable to use the median as well. The value of median was 374 mg P/kg.

The highest contents were obtained with the estimation of residual P (P_{ar}). Here the average value was 705 mg P/kg and the median 676 mg P/kg. The highest content (2024 mg P/kg) was found at the Lauterbach site and the lowest (273 mg P/kg) at the Thyrow site. All of the measured values ranged between 0.02 and 0.20%. This range has been determined for total P by many authors (e.g. Mengel 1991, Schilling 2000, Blume et al. 2002, etc.). Due to the high extraction efficiency of the aqua regia extraction, it is possible to assume, that the obtained values were close to the total content of P in soil. This corresponds with the results of Crossland et al. (1995). These authors obtained very low differences between P_{ar} and total P estimated using the alkaline fusion.

Our results showed that the Marks fractionation did not probably estimate the corresponding P fractions. This is possible to derive from the fact that the P_{FeAl} content was very close to $P_{M\text{-}tot}$. Therefore almost all P was sorbed to Fe and Al compounds, which is not probable. Additionally, the $P_{M\text{-}tot}$ content was significantly lower compared to P_{ar} content. It is clear, that the Marks' fractionation is usable only as a rough tool for estimating the ratios between P_{in} and P_{org} in the analyzed soils. A similar ratio between P_{in} and P_{org} was published by Bünemann et al. (2007) using Saunders and Williams (1957) extraction method.

Figure 1 shows the percentual distribution of different P fractions in soils based on median comparisons. According to the low contents of $P_{M\text{-}tot}$, P_{ar} was chosen as 100%.

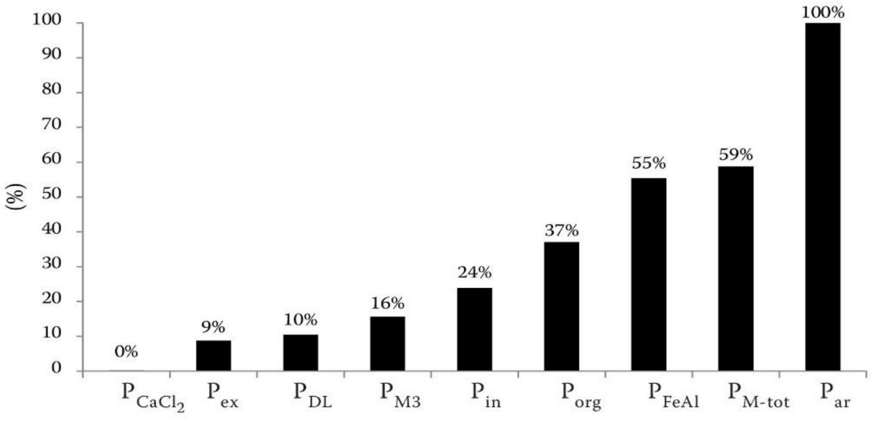

Figure 1. Percentage distribution of different P fractions in soils

The lowest percentage of different fractions represented P in soil solution (0.2%). Similar values of 9% and 10% were reached with AE membranes and the Doppel-Lactat method, respectively. The amount estimated with the Mehlich 3 method was 16%. That corresponds well with results of many authors, that the content of bioavailable phosphates in soil is relatively low and reaches usually about 10% of total P content (Mengel 1991, Marschner 1995, Blume et al. 2002, Bünemann et al. 2007). The P_{org} content was about 13% higher compared to the content of P_{in}. More than 50% of P_{ar} constituted of phosphates adsorbed on iron and aluminum compounds.

Correlation analysis was used for comparison of different P fractions (Table 3). Spearman correlation coefficients, which are suggested for comparison of data with non-normal distribution, were used for the calculating of relations. The relations were calculated at $P \leq 0.01$ and $P \leq 0.001$.

Comparing the different methods for estimating bioavailable P fractions in soils (P_{CaCl_2}, P_{ex}, P_{DL} and P_{M3}) led to close correlations at the $P \leq 0.001$. It means that with the mentioned methods it is possible to estimate mutually dependent P fractions and in this case it shows close relations between P in soil solution and exchangeable and total bioavailable P, respectively. Tightness of relations between P_{ex} and phosphates estimated with the Mehlich 3 and Doppel-Lactat methods shows that these methods probably did not estimate significant contents of nonbioavailable P. Significant relations between P_{M3} and P_{ex} were obtained in the work of Zheng et al. (2003). Close correlations between P in soil solution and P_{M3} was also found by Magdoff et al. (1999), who reached $r = 0.8$ at $P \leq 0.01$ in 28 noncalcareous soils. Zbíral and Němec (2002) compared the P_{M3} and P_{CaCl_2} fractions using regression analysis and obtained significant relations as well (coefficient of determination $r^2 = 0.56$).

Close correlations at $P \leq 0.001$ were obtained between P_{M3} and $P_{in.}$ Therefore, it is probable that the Mehlich 3 method estimated a significant content of total mineral P. Despite the low extraction efficiency of the Marks fractionation it is possible to presume that this method did not estimate the fraction of total inorganic P, but only a fraction of it. Relations between P_{in} with P_{ex}, P_{CaCl_2} and P_{DL} were not statistically significant at $P \leq 0.001$.

In most of cases, statistically significant relations between bioavailable forms of P and P_{org} were not reached.

High correlation coefficients between P_{in}, P_{org} and $P_{M\text{-}tot}$ were obtained, because these fractions originated from one fractionation. The correlation coefficient between P_{org} and $P_{M\text{-}tot}$ was even 0.96.

Close relations between P_{FeAl} and $P_{M\text{-}tot}$ and P_{ar} showed that a considerable content of P in noncalcareous soils can be adsorbed on Fe and Al compounds.

Regression analysis was used for evaluating the results of all the studied P fractions. Similar results to correlation analysis were obtained. In most cases linear regression appeared to be the most suitable.

As a conclusion it is possible to state that P contents in different fractions in soils can be written as: $P_{CaCl_2} < P_{ex} < P_{DL} < P_{M3} < P_{in} < P_{org} < P_{FeAl} < P_{M\text{-}tot} < P_{ar}$.

The Marks' fractionation probably does not estimate the corresponding contents of different P fractions. Therefore, this fractionation is only usable as guidance about ratios between P_{in}, P_{org} and P_{tot} in the analyzed soils.

Close correlations between the methods estimating bioavailable phosphates (P_{DL} and P_{M3}) with P in soil solution (P_{CaCl_2}) and exchangeable P (P_{ex}) verified the usability of the Mehlich 3 and Doppel-Lactat extraction schemes in noncalcareous soils.

High contents of phosphates in analyzed noncalcareous soils were bound in Fe and Al compounds. This was verified by the high percentage of this fraction compared to P_{ar}.

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24) Differences in soil sulfur fractions due to limitation of atmospheric depositions

Změny obsahů různých frakcí půdní síry v důsledku poklesu vstupů S v atmosférických depozicích

Po roce 1989 došlo v rámci snahy o zlepšení životního prostředí k výraznému snížení emisí potenciálně škodlivých prvků při spalování hnědého uhlí zejména z tepelných elektráren. To se týká i síry, jejíž imise před rokem 1989 přesahovaly 115 kg/ha. Během postupného snižování emisí došlo k poklesu vstupů síry z atmosférických depozic o více než 100 kg/ha ve srovnání s rokem 1989. Podobná změna se odehrála i v řadě ostatních evropských států. Snížení vstupů S v atmosférických depozicích tak bylo hlavním důvodem poklesu obsahu přístupné síry v půdě, což dokládá řada studií (Scherer 2001, Zhao et al. 2003, Lehmann et al. 2008). Cílem této práce bylo vyhodnotit změny obsahu síry v půdách v podmínkách České republiky. Za tímto účelem byly analyzovány půdy odebrané z dlouhodobých pokusů ÚKZÚZ, založených většinou před rokem 1980. K dispozici byly archivní vzorky z roku 1981 a vzorky odebrané v roce 2007 z 10 stanovišť v ČR. V těchto vzorcích byla stanovena vodorozpustná, adsorbovaná a „okludovaná“ síra (v součtu minerální S), dále estersulfátová a celková organická S, včetně celkového obsahu síry. Z výsledků jasně vyplývá, že poklesy vstupů síry z atmosférických depozic se nejvíce projevily na obsahu přístupných forem síry v půdě (vodorozpustné a adsorbované). Obsah vodorozpustné síry poklesl dokonce o více než 60 %, a to prakticky bez ohledu na varianty hnojení (hnůj, hnůj + NPK). Naproti tomu obsahy organické síry a celkové S zůstaly téměř nezměněny. To pravděpodobně značí, že uvolňování S, zejména z organických forem, je velmi pozvolným procesem. Potvrdily se tak výsledky ostatních studií (Zhao et al. 2003, Lehmann et al. 2008, Morche 2008), kde byly zjištěny velmi podobné výsledky u všech sledovaných frakcí půdní síry. Závěrem lze konstatovat, že pokles obsahu přístupné síry v půdě za období 1981-2007 byl velmi výrazný a do budoucna lze očekávat stoupající význam síry v řízené výživě rostlin. Proto se tato publikace stala základem pro navazující výzkum, z něhož některé výsledky jsou uvedeny výše.

Differences in soil sulfur fractions due to limitation of atmospheric deposition

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ABSTRACT

The aim of this work was to estimate the changes in contents of different sulfur (S) fractions in soils under conditions of lowering inputs of S from emissions together with the influence of application of manure and mineral fertilizers. Soil samples from long-term field experiments were used for this purpose. The samples were taken from 10 sites from precise long-term field experiments with different soil-climatic conditions in the Czech Republic. The samples were analyzed using the following fractionation: (i) water soluble S (H_2O extracts), (ii) sorbed S (0.032M NaH_2PO_4 extracts) and (iii) S occluded with carbonates (1M HCl extract). Furthermore, the concentration of total S (S_{tot}) and organic S (S_{org}) was determined. Soil samples were taken in the years 1981 and 2007. During 26 years a decrease of S_{tot} by about 3–8%, water soluble S by 65–68% and sorbed S by 39–44% were observed in the topsoil of the evaluated soils. Furthermore, a low increase in the content of organic S was observed. The estimated ratio of S_{org} reached 78.7–80.9% from S_{tot} in the year 1981 and 87.7–89.8% in 2007. Farmyard manure (40 t/ha) applied every 4 years did not have a significant influence on S fractions and S_{tot} contents in soils; intensive S fertilizing increased S_{tot} and mobile S forms contents in soils. Very close correlations were obtained especially between S_{tot} and water soluble S and organic S.

Keywords: soil; S; long term stationary experiments; S-fractionation; fertilizing

Sulfur deficiency in plant nutrition is an actual problem in the Czech Republic (Matula 1999, Matula and Pechová 2005, Vaněk et al. 2008) and in other European states (Scherer 2001, Zhao et al. 2003, Yang et al. 2007, Lehmann et al. 2008). Agricultural and horticultural crops are more susceptible to sulfur deficiency. Sulfur deficiency can be attributed to the decrease of atmospheric emissions, less intensive application of mineral fertilizers together with change of their composition, restriction of manure and cropping of plants with high S uptake in crop rotation (e.g. rape) on bigger areas.

The total content of S in soil usually ranges between 0.01 and 0.1% (Stevenson and Cole 1999, Balík et al. 2007, Morche 2008). The main portion of total S in soils is bound in soil organic matter (Eriksen et al. 1998, Kertesz and Mirleau 2004, Yang et al. 2007).

Plant available S consists of: (i) soluble inorganic SO_4^{2-}, (ii) sorbed inorganic SO_4^{2-}, (iii) the portion of organic S in soil that is mobilized during vegetation periods.

Although it is possible to estimate the content of inorganic fractions using different extractants, a satisfactory method for estimating mobile organic S is currently not known. For the quantification of inorganic fraction the following extractants are used: H_2O, $CaCl_2$, $Ca(H_2PO_4)_2$, NaH_2PO_4, KH_2PO_4, $NaHSO_3$ (Zhao and McGrath 1994, Matula 1999, Hu et al. 2005, Lehmann et al. 2008). The SO_4^{2-} ions in soil solution and weakly sorbed SO_4^{2-} are estimated using water extracts (Prietzel et al. 2001). The P-containing extractants are used to release

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water soluble forms and sorbed S. The extractants on the Cl$^-$ basis mobilize water soluble SO_4^{2-} (Hu et al. 2005). The $CaCl_2$ solution has a lower extraction power because of the formation of lowly soluble $CaSO_4$ (Matula 1999); therefore, the water extraction is more suitable. The number of studies for estimating mobile fractions in soils has increased after the restriction of S emissions. The results of Lehmann et al. (2008) showed that during 150 years on the Rothamsted (UK) site, the content of different S fractions varied according to S emissions.

The aim of our experiment was to estimate the changes in different S fractions in soils that were influenced by lowering S inputs from emissions. Furthermore, the influence of manure and mineral fertilization was assessed.

MATERIAL AND METHODS

Topsoil (0–30 cm) samples from stationary long-term field experiments were used for the experiment. The experimental sites are owned by the Central Institute for Supervising and Testing in Agriculture (CISTA) Brno, Czech Republic. Samples from the years 1981 and 2007 were chosen. The stationary experiments on 10 sites with different soil-climatic conditions in the Czech Republic were established in the years 1966–1977. Basic agrochemical characteristics of the soil samples before establishing the experiments are presented in Table 1. Periodic crop rotation was used during the experiments (Table 2).

Three fertilization treatments were chosen: (i) control – not fertilized, (ii) farmyard manure, (iii) farmyard manure + mineral NPK fertilizing (Table 3). Each treatment was conducted in 6 replicates and then mixed together to get one representative soil sample from each treatment. The samples were always taken after the harvest of winter wheat in the years 1981 and 2007.

Fine soil was used for the analysis (< 2 mm). The samples were extracted using the following S-fractionation (Morche 2008): the soil sample was first extracted with demineralized water at 1:10 (w/v). After 30 min of shaking, each sample was centrifuged at 10 000 rpm for the duration of 10 min. Following the centrifugation, the samples were extracted with 0.032M NaH_2PO_4 at the 1:10 (w/v) ratio. After 30 min of shaking, each sample was again centrifuged at 10 000 rpm for the duration of 10 min. The rest of the soil sample was extracted with 1M HCl. The extraction ratio was 1:20 (w/v). After 60 min of shaking, the samples were centrifuged 10 min at 10 000 rpm.

Total concentrations of S in soils were determined in the digests obtained by the following decomposition procedure: Aliquots (0.5 g) of air-dried soil samples were decomposed in a digestion vessel with a mixture of 8 ml of concentrated nitric acid, 5 ml of hydrochloric acid, and 2 ml of concentrated hydrofluoric acid. The mixture was heated in the Ethos 1 (MLS GmbH, Germany) microwave-as-

Table 1. Agrochemical characteristics of the soils in the year of the experiments establishment

Site	Soil type	pH (KCl)	Nutrients-Mehlich 2 (mg/kg)				Content (%)		Texture classes (%)				
			P	K	Mg	Ca	N_{tot}	C_{ox}	< 0.001	0.001 –0.01	0.01 –0.05	0.05 –0.25	0.25 –2 mm
Horažďovice	cambisol	6.2	79	172	155	1648	0.12	0.80	6.70	13.7	16.6	18.8	44.0
Chrastava	cambisol	5.3	42	144	125	1425	0.08	0.99	10.5	17.8	56.1	13.8	1.80
Jaroměřice	haplic luvisol	6.1	73	201	199	2413	0.12	1.03	18.1	20.7	38.5	14.1	8.70
Krásné Údolí	cambisol	6.2	50	135	155	2134	0.14	1.09	5.90	16.6	20.5	25.6	21.4
Libějovice	haplic luvisol	5.7	79	150	105	1685	0.14	0.88	10.8	16.9	16.1	19.3	37.0
Lípa	cambisol	6.6	68	135	82	2075	0.13	0.99	7.90	17.8	24.5	20.9	28.9
Uherský Ostroh	haplic luvisol	6.9	63	293	165	3820	0.12	1.06	17.3	14.8	26.4	30.4	11.5
Pusté Jakartice	haplic luvisol	6.7	70	108	76	2142	0.12	0.97	10.1	18.0	61.8	8.70	1.50
Sedlec	chernozem	7.4	95	270	196	4501	0.12	1.34	16.2	16.0	40.9	23.9	3.10
Žatec	chernozem	7.3	91	265	277	4001	0.17	1.53	21.7	16.5	26.6	24.0	11.2

Table 2. Crop rotations on the different sites

Year	1	2
1	oat – alfalfa	oat – clover
2	alfalfa	clover
3	winter wheat	winter wheat
4	silage maize	potatoes
5	winter wheat	winter wheat
6	spring barley	spring barley
7	sugar beet	potatoes
8	spring barley	spring barley

1 – Uherský Ostroh, Pusté Jakartice, Sedlec, Žatec; 2 – Horažďovice, Chrastava, Jaroměřice, Krásné Údolí, Libějovice, Lípa

sisted wet digestion system for 33 min at 210°C. After cooling, the digest was quantitatively transferred into a 50 ml Teflon® vessel and evaporated to dryness at 160°C. The digest was then dissolved in a 3 ml nitric and hydrochloric acid mixture (1:3), transferred into a 25 ml glass tube, filled up with deionized water, and kept at laboratory temperature until measurement. The certified reference material RM 7003 Loam was applied for the quality assurance of analytical data.

The concentrations of S in soil digests and extracts were determined using optical emission spectroscopy with inductively coupled plasma (ICP-OES) with axial plasma configuration, Varian, VistaPro, equipped with autosampler SPS-5 (Australia). Operating measurement wavelength for ICP-OES was 180.7 nm for S. Measurement conditions were: power 1.2 kW, plasma flow 15.0 l/min, auxillary flow 0.75 l/min, nebulizer flow 0.9 l/min.

RESULTS AND DISCUSSION

The Czech Republic was known as an important S emitting country from the 1950s to the beginning of the 1990s. For example in the year 1991, the emissions in Prague region reached 394 kg S/ha, in the north Bohemian region 484 kg S/ha and in the rest of the Czech Republic they reached almost 100 kg S/ha (Tlustoš et al. 2001). New technologies of thermal power plant desulfurization significantly decreased SO_2 emissions. Therefore, dry + wet deposition is currently approximately 15–30 kg S/ha. It is possible to deduce that this change could significantly influence the total content of S in soils and its different fractions (Zhao et al. 2003).

Table 1 summarizes the agrochemical characteristics of the soils from different sites at the beginning of the long-term field experiments. Ten of the chosen sites represent well different soil-climatic conditions in the Czech Republic. These soils are usually loamy and the pH ranges between acidic and neutral values. In these long-term experiments stabile crop rotation was performed (Table 2). In this study, 3 treatments were chosen. The control treatment was not fertilized. Quality farmyard manure was periodically applied to treatments 2 and 3. Based on manure analysis in the last 10 years, it is possible to deduce that S input is approximately 8 kg/ha year (Table 3), which also corresponds to the results of Eriksen et al. (1998). Ammonium sulfate and simple superphosphate application before sowing to treatment 3 added high amounts of mineral S (99.2 kg S/ha year).

The plant material was not archived in the previous years of experiment. It is thus problematic to estimate S export through harvest. Zhao et al.

Table 3. Design of the experiment

No.	Treatment	Description	Average yearly S dose added with fertilizers (kg S/ha)
1	control	con	0
2	farmyard manure[1]	$FYM_{(+Ca)}$	8
3	farmyard manure + NPK[2] in mineral fertilizers	$FYM_{(+Ca)}$ + NPK	8 + 91.2

[1] 40 t farmyard manure/ha is periodically applied to the root crops (potatoes, sugar beet) in the crop rotation. The average farmyard manure dose is 10 t/ha year

[2] the amount of farmyard manure is same with the treatment 2. The average yearly dose of nutrients is: 69 kg N/ha, 50 kg P/ha, 59 kg K/ha. The nitrogen is applied together with the soil preparation in the ammonium sulfate form; to the fertilizing during vegetation nitrochalk is used. The source of phosphorus is simple granulated superphosphate (7.5% P). Potassium is applied in the potassium salt (50% K). The application of liming was effected by pH/$CaCl_2$ value and soil-climatic conditions at treatments 2 and 3

(2003) estimated that S ratio originating from the atmosphere accounted for 60–78% of total S uptake with harvest of winter wheat during maximal atmospheric deposition (year 1970). Therefore, we can assume that in our experiments this factor dominated between 1970 and 1995. From the exactly registered harvest results, from the different sites and average S uptakes from the literature, the yearly uptake of S was estimated to 13.1 kg S/ha at the control treatment, 14.0 kg S/ha at the $FYM_{(+Ca)}$ treatment and 22.0 kg S/ha at the $FYM_{(+Ca)}$ + NPK treatment. Higher contents (approximately by 1/3) of S due to intensive fertilization with mineral S form were calculated at the treatment 3.

Tables 4 and 5 summarize S contents using the fractionation of the soil samples from the years 1981 and 2007. The contents of plant available S (S_{H_2O}), exchangeable sorbed S ($S_{NaH_2PO_4}$) and occluded S (S_{HCl}) were estimated after Morche (2008). The content of organic S (S_{org}) was calculated as a difference between total S (S_{tot}) and sum of mineral S (S*). A decrease of total S content in soils was observed in time. Average contents observed at the control treatment were 221 mg S/kg in the year 1981 and 204 mg S/kg in 2007. Similar decreasing trends were observed at the treatment 2 (from 216 mg S/kg to 210 mg S/kg) and 3 (from 235 mg S/kg to 219 mg S/kg). The contents of S_{tot} ranged in dependence on the site and treatment between 133 and 334 mg S/kg in 1981 and between 105 and 321 mg S/kg in 2007. These soils follow similar S_{tot} trends as most European soils. Similar results in other European experiments were published also by Zhao et al. (2003), Lehmann et al. (2008) or Morche (2008).

The content of S_{H_2O} reached 22.6 mg S/kg at the control treatment in 1981 and was only about 7.20 mg S/kg in 2007, which represents only a fraction of 32% (Figure 1). Similar trends were obtained at the treatments 2 and 3 where it accounted for 33% and 35%, respectively. The measured results varied on the majority of sites (except Žatec) between 4–9 mg S/kg. This corresponds with the results of Balík et al. (2006) in other soil-climatic

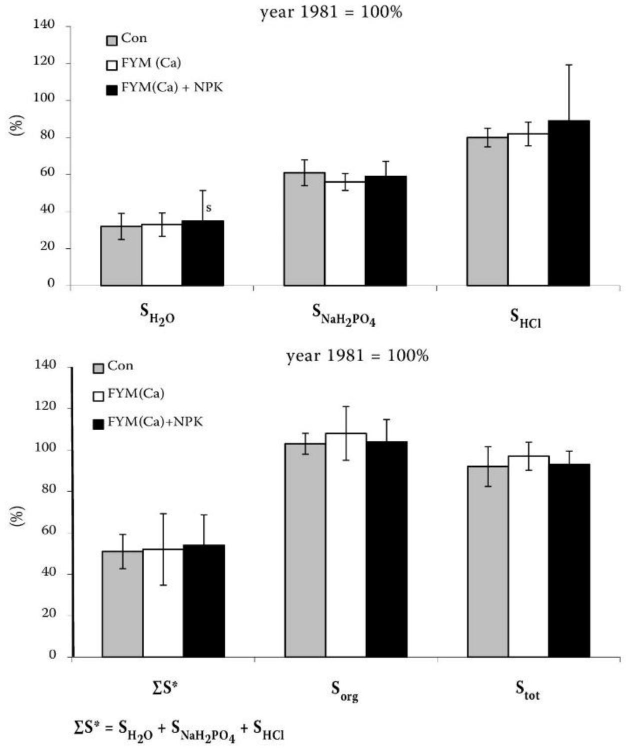

Figure 1. The average ratio (in %) of different S fractions in soils in the samples of the year 1981 compared to 2007

Table 4. The content of different S fractions, year 1981

Site	Treatment	S_{H_2O}	$S_{NaH_2PO_4}$	S_{HCl}	S*	S_{org}	S_{tot}
		(mg S/kg)					
Horažďovice	Con	14.4	6.10	12.4	32.9	187	219
	$FYM_{(+Ca)}$	15.6	7.10	15.2	37.9	170	208
	$FYM_{(+Ca)}$ + NPK	21.5	6.70	18.2	46.4	162	208
Chrastava	Con	41.2	16.0	21.7	79.3	244	323
	$FYM_{(+Ca)}$	35.7	13.0	19.5	68.5	229	297
	$FYM_{(+Ca)}$ + NPK	40.1	15.0	23.7	79.0	239	318
Jaroměřice	Con	18.9	7.50	8.27	34.7	228	262
	$FYM_{(+Ca)}$	19.3	7.40	9.13	35.8	172	207
	$FYM_{(+Ca)}$ + NPK	31.4	8.50	10.9	50.7	211	262
Krásné Údolí	Con	29.8	11.0	16.9	57.4	227	284
	$FYM_{(+Ca)}$	22.2	9.00	16.1	47.3	231	279
	$FYM_{(+Ca)}$ + NPK	25.5	9.00	25.1	59.6	215	274
Libějovice	Con	21.3	5.60	10.6	37.4	171	209
	$FYM_{(+Ca)}$	20.5	5.60	10.5	36.5	187	223
	$FYM_{(+Ca)}$ + NPK	24.9	6.60	13.9	45.5	175	221
Lípa	Con	18.3	9.40	11.4	39.1	147	186
	$FYM_{(+Ca)}$	19.1	8.70	10.8	38.6	120	159
	$FYM_{(+Ca)}$ + NPK	18.3	8.30	11.6	38.2	108	146
Ostroh	Con	14.1	5.40	10.7	30.2	134	164
	$FYM_{(+Ca)}$	15.4	5.80	8.90	30.0	124	154
	$FYM_{(+Ca)}$ + NPK	21.3	6.30	8.80	36.4	134	170
Pusté Jakartice	Con	19.2	6.40	8.40	33.9	99.2	133
	$FYM_{(+Ca)}$	16.7	5.80	7.30	29.8	104	133
	$FYM_{(+Ca)}$ + NPK	20.1	7.30	7.00	34.4	149	183
Sedlec	Con	24.0	8.30	14.7	47.1	187	234
	$FYM_{(+Ca)}$	22.2	9.00	11.2	42.4	168	210
	$FYM_{(+Ca)}$ + NPK	32.2	9.70	10.7	52.6	181	234
Žatec	Con	24.6	9.60	9.20	43.4	149	192
	$FYM_{(+Ca)}$	27.5	9.20	10.3	47.0	245	292
	$FYM_{(+Ca)}$ + NPK	38.5	9.00	11.1	58.6	275	334
Average of the sites	Con	22.6	8.50	12.4	43.5	177	221
	$FYM_{(+Ca)}$	21.4	8.10	11.9	41.4	175	216
	$FYM_{(+Ca)}$ + NPK	27.4	8.70	14.1	50.1	185	235

$S^* = \Sigma S_{H_2O} + S_{NaH_2PO_4} + S_{HCl}$

conditions in the Czech Republic and it confirms the need for S application through mineral fertilizers for sulfur-intensive crops. It is also necessary to mention that S analyses using ICP-OES estimate total extractable S and the ratio of total S with mineral S ranges between 50 and 90% (Kotková et al. 2008). The content of exchangeable sorbed S also decreased through the 26 years of experiment

Table 5. The content of different S fractions, year 2007

Site	Treatment	S_{H_2O}	$S_{NaH_2PO_4}$	S_{HCl}	S*	S_{org}	S_{tot}
		(mg S/kg)					
Horažďovice	Con	4.55	3.80	13.7	22.0	197	219
	$FYM_{(+Ca)}$	4.18	3.30	14.5	22.0	174	196
	$FYM_{(+Ca)}$ + NPK	7.49	3.40	9.00	19.9	179	199
Chrastava	Con	5.87	8.20	14.8	28.9	159	188
	$FYM_{(+Ca)}$	4.93	5.80	13.6	24.3	200	224
	$FYM_{(+Ca)}$ + NPK	8.37	6.00	12.7	27.0	253	280
Jaroměřice	Con	5.85	5.90	7.20	19.0	158	177
	$FYM_{(+Ca)}$	5.29	4.50	6.50	16.3	198	214
	$FYM_{(+Ca)}$ + NPK	7.05	5.00	7.40	19.4	178	197
Krásné Údolí	Con	9.55	4.70	10.8	25.1	265	290
	$FYM_{(+Ca)}$	8.12	4.50	10.8	23.3	233	256
	$FYM_{(+Ca)}$ + NPK	13.5	6.60	13.6	33.6	224	258
Libějovice	Con	4.90	2.60	7.50	15.0	155	170
	$FYM_{(+Ca)}$	5.68	4.00	12.3	21.9	178	200
	$FYM_{(+Ca)}$ + NPK	6.08	3.50	8.30	17.9	170	188
Lípa	Con	6.44	5.50	10.3	22.3	120	143
	$FYM_{(+Ca)}$	5.26	4.70	6.90	16.9	88.0	105
	$FYM_{(+Ca)}$ + NPK	6.88	4.20	10.0	21.0	97.0	118
Ostroh	Con	4.91	3.70	6.60	15.2	118	134
	$FYM_{(+Ca)}$	4.66	3.60	7.50	15.7	125	140
	$FYM_{(+Ca)}$ + NPK	7.84	4.90	8.00	20.7	178	199
Pusté Jakartice	Con	5.94	3.90	5.20	15.0	115	130
	$FYM_{(+Ca)}$	7.14	3.90	7.40	18.5	175	193
	$FYM_{(+Ca)}$ + NPK	8.88	4.10	8.10	21.1	160	181
Sedlec	Con	6.67	5.20	7.60	19.5	251	270
	$FYM_{(+Ca)}$	6.47	5.30	9.30	21.1	235	256
	$FYM_{(+Ca)}$ + NPK	11.0	5.90	28.2	55.1	215	270
Žatec	Con	17.6	8.20	15.5	41.3	280	321
	$FYM_{(+Ca)}$	19.1	6.20	9.00	34.3	284	318
	$FYM_{(+Ca)}$ + NPK	17.6	7.30	9.80	34.8	266	301
Average Of the sites	Con	7.20	5.20	9.90	22.3	182	204
	$FYM_{(+Ca)}$	7.10	4.60	9.80	21.4	189	210
	$FYM_{(+Ca)}$ + NPK	9.50	5.10	11.5	27.0	192	219

$S^* = \Sigma\, S_{H_2O} + S_{NaH_2PO_4} + S_{HCl}$

duration. Contents in the year 2007 decreased to 61% at the treatment 3 (Figure 1). From the results of Prietzel et al. (2001) it is clear that the SO_4^{2-} sorption intensity strongly depends on soil pH value and increases with increasing acidity. This was not verified in our experiments. With liming, the OH^- ion displaces the SO_4^{2-} from the sorption sites on the Fe and Al oxides and the

Table 6. The ratio (in %) of the different S fractions compared to the S_{tot} content in soils

Treatment	S_{H_2O}	$S_{NaH_2PO_4}$	S_{HCl}	S*	S_{org}	S_{tot}
Year 1981						
Con	10.2	3.9	5.6	19.7	80.3	100
$FYM_{(+Ca)}$	9.90	3.7	5.5	19.2	80.9	100
$FYM_{(+Ca)}$ + NPK	11.7	3.7	6.0	21.3	78.7	100
Year 2007						
Con	3.5	2.5	4.9	10.9	89.1	100
$FYM_{(+Ca)}$	3.4	2.2	4.6	10.2	89.8	100
$FYM_{(+Ca)}$ + NPK	4.3	2.3	5.3	12.3	87.7	100

$S^* = \Sigma\, S_{H_2O} + S_{NaH_2PO_4} + S_{HCl}$

sorption values of sulfates are underestimated (Evans 1986). In our experiment, lower contents at the treatment 2 (limed on some sites) compare to the treatment 1 were calculated, but the differences were not significant. Therefore, we can only speculate about some trends without significant relevance. The sorption could as well decrease due to increased sorption of organic anions after farmyard manure application. Other works are not uniform in this question. Although Haque and Walnsley (1973) estimated a positive correlation between the sorption of SO_4^{2-} and organic matter, Singh and Johnson (1986) obtained a negative correlation. The results of Kaiser and Zech (1996) show that organic acids anions block the sorption sites on hydrated Fe and Al oxides. Furthermore, the increasing concentration of phosphorus anions in soil solution decreases SO_4^{2-} sorption (Bohn et al. 1986). Therefore, application of phosphorus fertilizers increases the mobility of SO_4^{2-}. We cannot quantify how these factors influenced our experiments, although at the treatment 3, intensive phosphorus and S fertilization was performed. Our measured values correspond well with the results of Scott (1976), who found similar value in Australian soils, but are higher than Schnug's (1988) results from northern Germany.

Using the HCl extraction as a part of the fractionation should mobilize a major portion of S occluded with carbonates (Shan et al. 1992, Chen et al. 1997).

From the data presented in Tables 4 and 5 it is clear that high S contents on the Sedlec and Žatec sites were not found, which was previously expected (based on the soils characteristics). The values of pH/KCl were 7.3 (Žatec) and 7.4 (Sedlec). Calcium contents (Mehlich 2) were 4001 mg Ca/kg (Žatec) and 4501 mg Ca/kg (Sedlec). On the other hand, on the Chrastava site (acidic soils); the highest S_{HCl} contents were obtained. Therefore, using HCl does not selectively estimate only S occluded with carbonates. That confirms the results of Morche (2008); the data on occluded S obtained from this extraction are thus overestimated. From the comparison of the data from 1981 and 2007 a decrease of occluded S is apparent. The values of this fraction in the year 2007 reached only 80–89% from the year 1981.

The sum of the fractions S_{H_2O}, $S_{NaH_2PO_4}$ and S_{HCl} represents the so-called 'inorganic S' (Morche 2008). Although the use of the extractants is not fully selective, it is possible to assume that most of the extracted S is inorganic S (Shan et al. 1992, Morche 2008). Due to the significant restriction of atmospheric depositions during the last 20 years

Table 7. The values of the correlation coefficients between S_{tot} content and the different S fractions contents in soils (n = 30)

	1981				2007			
	S_{H_2O}	$S_{NaH_2PO_4}$	S_{HCl}	S_{org}	S_{H_2O}	$S_{NaH_2PO_4}$	S_{HCl}	S_{org}
Correlations with S_{tot}	0.811	0.685	0.622	0.987	0.720	0.558	0.373	0.993
P-value	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01

in the Czech Republic the ratio of inorganic S decreased by 50% (Figure 1). Intensive S fertilization (treatment 3) led to increased contents of inorganic S in soil (27 mg S/kg) in the year 2007. The ratio of different fractions (in %) from the total S content is mentioned in Table 6. Similar to the results of Chapmann (2001), organic S is the major fraction. It accounted for 78.7–80.9% from S_{tot} in the year 1981 and 87.7–89.8% in 2007. The experimentally estimated data in the year 2007 are close to the data presented by Kertesz and Mirleau (2004), who published a ratio of 95%. From the comparison of the results from 1981 and 2007, the content of inorganic S fractions decreased during 26 years. While the ratio ranged between 9.9 and 11.7% in the year 1981, it was only between 3.4 and 4.3% in 2007. The ratio of S occluded with carbonates ranged between 5.5–6.0% in the year 1981 and between 4.6–5.3% in 2007. Similar results (6.9% from total S) were mentioned also by Chen et al. (1997). Our results are significantly lower then the values from Roberts and Bettany (1985) for calcareous soils in Canada. The decreasing content of inorganic S was probably influenced by decreasing inputs and with elutriation losses. The content of S_{org} did not change. That corresponds also with a stable C_{ox} content. The average value of C_{ox} was 1.02% at the control treatment in the year 1981 and 1.04% in 2007, at the treatment $FYM_{(+Ca)}$ 1.04% in 1981 and 1.07% in 2007 and at the treatment $FYM_{(+Ca)}$ + NPK the content decreased from 1.15% in 1981 to 1.09% in 2007.

The values of correlation coefficients between different S fractions with S_{tot} are presented in Table 7. It is necessary to point out, that the high values were partly influenced by the evaluation of the long-term field experiments, where the closer relation can be expected. For both sets of samples a close relations between S_{H_2O} and S_{tot} were found. That indirectly confirms the usefulness of the S_{H_2O} extraction for S fertilizing optimization. The close relations between S_{org} and S_{tot} were influenced by the procedure of S_{org} estimation.

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25) Evaluating of phosphorus quantity/intensity parameters in soils with different systems of organic fertilising

Vyhodnocení parametrů kvantity/intenzity (Q/I) fosforu v půdách hnojených různými organickými hnojivy

Statické analytické metody pro stanovení přístupného fosforu zahrnují pouze jeden parametr. Vzhledem ke komplikovanému chování fosforu v půdě však tento parametr nemusí charakterizovat skutečně přístupný fosfor. Zejména sorpční schopnost některých půd může znamenat bariéru komplikující příjem fosforu rostlinami. Proto existují i tzv. dynamické metody umožňující stanovit míru sorpce fosforu v půdě. Jednou z nich je stanovení sorpce P pomocí sorpčních izoterm. Z těchto analýz je možné vypočítat stupeň nasycení půdy fosforem, a také a schopnost půdy poutat P. Cílem níže uvedené publikace bylo zjistit míru sorpce P v dlouhodobých přesných polních pokusech s různými systémy hnojení. Za tímto účelem byly stanoveny sorpční izotermy fosforu výluhem v 0,01 mol/l chloridu vápenatého s různými přídavky fosforu (0, 1, 2, 3, 4, 6, 8, 10 mg P/l) (Self-Davis et al. 2000). Míra sorpce fosforu byla vypočtena na základě změn poměrů množství výměnně sorbovaného fosforu (ΔQ) a tzv.intenzity P v půdním roztoku (ΔI). Regresní analýzou pak bylo možné dopočítat rovnovážnou koncentraci mezi fosforem v půdním roztoku a výměnně sorbovaným P, a dále pak i pufrační kapacitu fosforu. Vše bylo sledováno u různých organických hnojiv porovnávaných s kontrolou. Pro vyhodnocení sorpčních ukazatelů půdy vykazovaly nejvhodnější parametry kořenová ($\Delta Q = a\sqrt{\Delta I} + b$) a logaritmická funkce ($\Delta Q = a\ln(\Delta I) + b$). Nejnižší rovnovážná koncentrace pro logaritmickou funkci byla vždy naměřena u kontrolní nehnojené varianty. Nízké hodnoty byly stanoveny rovněž u variant hnojených ječnou slámou s minerálním dusíkem. Následovaly obě varianty hnojené čistírenskými kaly (240 a 720 kg P/ha ve formě kalů) s podobnými výsledky rovnovážné koncentrace okolo 0,25 mg P/l. Nejvyšší rovnovážné koncentrace P bylo vždy dosaženo u variant hnojených chlévským hnojem (0,45 mg P/l). Opačný trend byl logicky zaznamenán při výpočtu pufrační kapacity P. Zde bylo nejvyšších hodnot dosahováno u kontrolních variant a nejnižších naopak u varianty hnojené chlévským hnojem, což potvrzují i výsledky studie McDowell et al. (2003). Podobné tendence byly zaznamenány i při výpočtu z kořenové funkce. Ukazuje se tak, že hnojení chlévským hnojem hraje významnou roli při zlepšení sorpčních schopností půdy i z hlediska fosforu.

Evaluating of phosphorus quantity/intensity parameters in soil with different systems of organic fertilising

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ABSTRACT

One of the refinement methods for estimating the parameters of phosphorus dynamics in soil is the construction of sorption isotherms in dependence on changes of exchangeable sorbed phosphorus in soil (ΔQ) and changes of phosphorus amount in soil solution (ΔI). Regression analysis allows to calculate equilibrium concentration (c_{equ}) and phosphorus buffering capacity (BC). The mentioned analyses were realised on soils from the long-term field experiments of the Czech University of Life Sciences (CULS) in Prague and Crop Research Institute (CRI) in Ruzyně, Czech Republic. The influence of different organic fertilisers compared to the control (not amended) treatment was tested. For the evaluating of parameters, the root and logarithmic functions were used. The lowest c_{equ} of the logarithmic function was always found on not amended treatment. Low amounts were found in the treatments amended with barley straw as well. The highest amounts were found in soil after potatoes cropping fertilised with farmyard manure (FYM). In the FYM variant fertilised with 70 kg P/ha, the c_{equ} value reached 0.45 mg P/l. Both treatments fertilised with sewage sludge (720 kg P/ha and 240 kg P/ha) showed similar values of about 0.25 mg P/l. A different trend was found for the phosphorus buffering capacity (BC); this was the highest at the control treatment and at the treatment fertilised with straw. The lowest BC was observed in both soils after potatoes fertilised with FYM, where it reached 61 mg P/kg and 65 mg P/kg, respectively. Similar trends were found when evaluating root function.

Keywords: long-term experiments; soil; phosphorus dynamics; equilibrium concentration; buffering capacity

Currently, supplemental parameters for estimating phosphorus in soils obtained with static and kinetic methods are often used (Macháček 1999). Evaluating phosphorus dynamics in soils is important for obtaining deeper insight into phosphorus transformations in soils in dependence on their properties. Different methods can be used for a long-term characterisation of phosphates releasing processes from soil matrix to the soil solution (McGechan 2002). Evaluating dynamic indices is an ideal variant for estimating fertiliser amounts needed (e.g. sewage sludge) (Barry et al. 1995).

For the realization of these methods we start from several basic parameters: intensity – I – level of instantaneous phosphorus availability to the plants. Extraction with 0.01M $CaCl_2$ or solution of other weak salts are most commonly used. Furthermore, electroultrafiltration is sometimes used (Self-Davis et al. 2000). The intensity can be only calculated on the basis of the phosphorus buffering capacity (BC).

The phosphorus buffering capacity is also termed differential, phosphate and equilibrium capacity. It can be defined as the capability of a soil to compensate concentration changes of different phosphorus forms (Hartikainen 1991). These data are commonly used to evaluate phosphorus amount that is desorbed by dilution of solution, but not phosphate, which is bound through slow and strong sorption (McGechan and Lewis 2002). Total BC is calculated from adsorption-desorption dependence as a portion of change of quantity (ΔQ) and intensity (ΔI). The advantage when using these relations is that it is possible to predict the sorption and mobilisation of phosphorus in soils (Morel et al. 1996) and to more accurately

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evaluate phosphorus availability in soils in comparison with other methods estimating available phosphorus (Hartikainen 1991).

The most commonly used methods for evaluating phosphorus dynamics in soils are the adsorption-desorption isotherms (Fox and Kramparth 1970). They are usually used to correct obtained data on adsorption of phosphorus and other reactive solutions. Some formulas are calculated with nonlinear relations (Chien and Clayton 1980, McGechan and Lewis 2002, Eckholm et al. 2005). They are defining an immediate effort to reach equilibrium in their simplest form, but they can be modified to describe the time dependent approximation of equilibrium.

MATERIAL AND METHODS

The long-term field experiment was based on experimental fields of CULS and CRI in Prague-Ruzyně, Czech Republic. The characteristic of the sites is given in Table 1.

Potatoes (cv. Karin), winter wheat (cv. Samanta) and spring barley (cv. Akcent) were cropped in crop rotation on small plots (60 m^2). Maize (hybrid Rivaldo) was cropped instead of potatoes on the site Červený Újezd. Organic fertilisers were applied only to the first crop in crop rotation. Because of that, the direct influence of organic fertilisers application could be observed on potatoes and maize. On winter wheat and spring barley it was possible to observe the consequential influence. The amounts of phosphorus in different treatments are summarised in Table 2. For this experiment, sewage sludge from wastewater treatment plant in Prague was used. Barley straw is always ploughed in before potatoes and maize cropping.

The whole system of fertilising is based on the uniform ratio of 330 kg N/ha per 3 years. That applies for organic and mineral fertilisers or their combinations. Only to the sludge 3 treatment 990 kg N/ha per 3 years was added in the form of sewage sludge.

The modified methodology of Self-Davis et al. (2000) was used for the analysis. The stock solution consisted of 0.01M $CaCl_2$. Different amounts of phosphorus in the form of KH_2PO_4 were added to the solution so that the resulting gradient of extractants had a phosphorus concentration of 0, 1, 2, 3, 4, 6, 8, 10 mg P/l. Five g of the sample was used for the extraction. An amount of 50 ml of the extraction solution was added to each sample. Each sample was also extracted with all of the mentioned phosphorus concentrations. After 120 min of shaking the samples were filtered and the content of phosphorus was measured spectrophotometrically using the SKALAR SAN^{PLUS} SYSTEM.

Table 1. Characteristic of experimental fields

Site	Altitude (m)	Mean yearly rainfall (mm)	Mean yearly temperature (°C)	Soil type	Soil sort	pH ($CaCl_2$)
Červený Újezd	410	493	7.7	luvisol	loam	6.5
Hněvčeves	265	573	8.2	luvisol	silty loam	5.9
Humpolec	525	665	7.0	cambisol	sandy loam	5.1
Lukavec	610	666	7.7	cambisol	sandy loam	5.3

Table 2. System of fertilising of the long-term field experiment (amount of added nutrients per ha)

Treatment	Potatoes	Winter wheat	Spring barley
Control	0	0	0
Sludge 1	330 kg N, 240 kg P	0	0
Sludge 3	990 kg N, 720 kg P	0	0
FYM 1	330 kg N, 70 kg P	0	0
FYM ½	165 kg N, 35 kg P	110 kg N*	55 kg N*
Barley straw + N	120 kg N* + 5 t straw (4 kg P)	140 kg N*	70 kg N*

*nitrogen was added in the mineral fertilizer CAN 27% N

RESULTS AND DISCUSSION

An increasing trend with a higher amount of phosphorus added in organic fertilisers was found when evaluating phosphorus intensity in soil solution estimated with 0.01M $CaCl_2$. The average amount of phosphorus in the soil solution at the control treatment was only 0.09 mg P/l while at the sludge 3 treatment 0.2 mg P/l. Almost the same results as at the control treatment were reached at both treatments fertilised with farmyard manure, where the content ranged about 0.17 mg P/l. Similar results at the treatments fertilised with farmyard manure were reached in the work by Kulhánek et al. (2007), where the concentrations of phosphorus in soil was 1.4 mg P/l, which results in an intensity of 0.14 mg P/l.

For the construction of sorption isotherms the amount of phosphorus bounded on hard phase of soil Q (mg P/kg) was used. It was calculated as a difference between the amount of added phosphorus and the amount of phosphorus measured in the extract. Two basic functions were used for the formation of sorption isotherms. One of them was the root function (1) and the second logarithmic regression (2):

$$\Delta Q = a\sqrt{\Delta I} + b \qquad (1)$$

$$\Delta Q = a\ln(\Delta I) + b \qquad (2)$$

The high coefficients of determination R^2 were reached for both of them. That shows a very close relationship between ΔQ and ΔI. This relationship was described in many works (Fox and Kramparth 1970, Barrow 1983, Okajima et al. 1983). McGechan and Lewis (2002) described usage of the logarithmic function while Schweitzer et al. (2005) used the root function.

Parameters *a* and *b* were calculated from the form of the isotherms. Then, the equilibrium capacity c_{equ} was calculated, when ΔQ reached 0. The c_{equ} value is also the intensity of phosphorus in soil solution, where the equilibrium between soil solution and exchangeable sorbed phosphorus is reached. Then, the phosphorus buffering capacity was calculated. That means the possibility of soil to equalise the changes of phosphorus concentrations in soil solution. BC was calculated as a portion of a/c_{equ}. The results are summarised in Table 3.

From Table 3 is clear that the lowest c_{equ} after harvest of all crops was calculated in the soil at the control treatment. In the soil after the harvest of potatoes the concentration of phosphorus was estimated at 0.16 mg P/l, after wheat 0.11 mg P/l and after barley 0.20 mg P/l. Low contents were also found in the treatment fertilised with barley straw (5 kg P/ha added). Here the values ranged from 0.20 to 0.23 mg P/l. The obtained results suggest that in nonfertilised treatments and treatments fertilised with low amounts of phosphorus at equilibrium, phosphorus concentration in soil solution is low. It means, that only a low amount of phosphorus is plant-available.

Interesting results were obtained at the treatments fertilised with sewage sludge. Great differences were found especially in samples after the harvest of potatoes. In both treatments fertilised with sewage sludge lower values of c_{equ} were obtained, compared to the treatments fertilised with farmyard manure. At the sludge 3 treatment (720 kg P/ha) the c_{equ} was 0.28 mg P/l and only 0.26 mg P/l at the sludge 1 treatment (240 kg P/ha). Thus, at the FYM 1 treatment (70 kg P/ha) the c_{equ} was 0.45 mg P/l and at the FYM ½ treatment (35 kg P/ha) 0.39 mg P/l. Therefore, sewage sludge is a smaller source of readily available phosphorus in comparison with farmyard manure. One of the possible explanations is that with the application of sewage sludge into the soil, high amounts of Fe and Al oxides are added. These oxides are adsorbing phosphorus leading to the non-available form. Soil solution also contained lower amounts of readily available phosphorus. The same results were obtained by McDowell et al. (2003).

The highest phosphorus buffering capacity (BC) was always measured at the control treatments. Therefore, nonfertilised soils have the highest ability to sorb phosphorus added to soil. Phosphorus concentrations reached 133 mg P/kg in soil after potatoes cropping, 132 mg P/kg after wheat and 167 mg P/kg after barley. High values were calculated at the treatments fertilised with barley straw (5 kg P/ha) as well. Here, almost the same results were obtained at all treatments. The values ranged from 110 to 113 mg P/kg.

With increasing equilibrium of phosphorus concentration the BC decreased. The lowest BC was obtained in soils after the harvest of potatoes fertilised with farmyard manure. At the FYM 1 treatment amended with 70 mg P/kg the BC was 61 mg P/kg and at the FYM ½ treatment fertilised with 35 kg P/ha the BC was 65 mg P/kg. Compared to the control treatment, the application of sewage sludge resulted in lower BC values, but compared to the FYM treatments the BC was significantly higher. Comparable results were obtained by Anderson and Wu (2001). They compared the

Table 3. Characteristics of sorption parameters estimated with the logarithmic function

Crop	Treatment	*a*	*b*	R^2	c_{equ} (mg P/l)	BC (mg P/kg)
Potatoes (maize)	control	20.85	38.62	0.96	0.16	133
	sludge 1	24.68	34.22	0.98	0.25	99
	sludge 3	25.05	31.74	0.95	0.28	89
	FYM 1	27.71	22.02	0.97	0.45	61
	FYM ½	25.58	23.84	0.95	0.39	65
	straw + N	22.41	35.63	0.96	0.20	110
Wheat	control	18.69	41.50	0.97	0.11	172
	sludge 1	21.69	39.49	0.97	0.16	134
	sludge 3	22.65	36.77	0.96	0.20	115
	FYM 1	23.14	36.29	0.97	0.21	111
	FYM ½	22.32	35.47	0.96	0.20	109
	straw + N	22.55	35.73	0.96	0.21	110
Barley	control	19.87	42.25	0.97	0.20	167
	sludge 1	20.96	32.10	0.95	0.22	97
	sludge 3	28.29	25.66	0.98	0.40	70
	FYM 1	23.12	33.19	0.97	0.24	97
	FYM ½	21.88	32.47	0.95	0.23	97
	straw + N	22.01	36.03	0.96	0.20	113

c_{equ} – equilibrium concentration; BC – phosphorus buffering capacity

influence of fertilising with pig and cattle slurry on the BC. With increasing ratio of fertilisers they also obtained decreasing BC.

When observing the subsequent influence of organic fertilisers to other crops the differences between the sewage sludge and farmyard manure were almost identical. The lowest differences were obtained after the harvest of wheat where the BC values ranged between 109 and 134 mg P/kg. The low differences were also calculated in the soil after the harvest of barley, where the same BC of 97 mg P/kg at the sludge 1, FYM 1 and FYM ½ treatments were found.

The root function was relatively low and accurate. Lower coefficients of determinance R^2 were calculated; however, the greatest disadvantage of this method was that this function was not accurate especially in the lower parts of the figures. This part is the most important for estimating the equilibrium concentration. The results from the root function are summarised in Table 4.

The trend of evaluating the c_{equ} with the root function was very similar with the logarithmic function. The lowest values were also found at the control treatments. The values calculated were 0.79 mg P/l after the harvest of potatoes, 0.78 mg P/l after wheat and 0.72 mg P/l after barley, which means the values of about 0.5 mg P/l higher then the values computed with the logarithmic function.

At the other treatments the same trend was observed. The highest c_{equ} values were obtained in soils after the harvest of potatoes. Here the c_{equ} reached 1.24 mg P/l at the FYM 1 treatment fertilised with 70 kg P/ha and 1.19 mg P/l at the half ratio of farmyard manure (FYM ½). In comparison with the sludge 1 (240 kg P/ha) and sludge 3 treatments (720 kg P/ha) the values were higher of about 0.31 mg P/l and 0.25 mg P/l, respectively.

A similar value as at the control treatment was observed in the soil after the harvest of wheat at the sludge 1 treatment. Otherwise, at the other treatments almost the same results between 0.84 and 0.85 mg P/l were obtained.

From the results of soil analysis after the harvest of barley it is clear that the highest c_{equ} was at the sludge 3 treatment, where it reached 1.13 mg P/l. There were no significant differences found between other treatments.

Table 4. Characteristics of sorption parameters estimated with the root function

Crop	Treatment	a	b	R^2	c_{equ} (mg P/l)	BC (mg P/kg)
Potatoes (maize)	control	22.35	5.68	0.95	0.78	29
	sludge 1	22.15	2.75	0.95	0.88	25
	sludge 3	22.65	0.20	0.97	0.99	23
	FYM 1	19.80	–4.18	0.98	1.24	16
	FYM ½	19.84	–3.41	0.99	1.19	17
	straw + N	21.12	1.37	0.97	0.94	23
Wheat	control	27.06	6.55	0.96	0.79	34
	sludge 1	23.55	5.40	0.95	0.80	30
	sludge 3	22.72	4.00	0.95	0.84	27
	FYM 1	22.61	3.74	0.96	0.85	27
	FYM ½	21.73	3.88	0.95	0.84	26
	straw + N	22.05	3.81	0.95	0.84	26
Barley	control	23.49	7.66	0.94	0.72	33
	sludge 1	18.99	3.76	0.95	0.82	23
	sludge 3	21.16	–2.59	0.97	1.13	19
	FYM 1	20.05	3.82	0.94	0.83	24
	FYM ½	20.69	2.20	0.97	0.90	23
	straw + N	22.16	3.89	0.96	0.84	26

c_{equ} – equilibrium concentration; BC – phosphorus buffering capacity

Compared to the logarithmic function, the obtained BC was significantly lower. With decreasing c_{equ} BC increased again. Therefore, the highest values were reached at the control treatment where the BC in the soil after the harvest of potatoes reached 29 mg P/kg, after wheat 34 mg P/kg and after barley 33 mg P/kg.

The lowest BC was obtained in the soils after the harvest of potatoes fertlilised with farmyard manure. No significant differences were found between the results after the harvest of wheat. The level of BC in the soils after the harvest of barley was significantly lower only at the treatment sludge 3.

From the results obtained it is clear that using the logarithmic and root function resulted in a similar trend for c_{equ} and BC. Due to the form of the isotherms, the logarithmic function seems more suitable.

Farmyard manure used before sowing of potatoes appeared to be the most effective fertiliser from the point of view of c_{equ} and BC. Despite of this, the lowest values of c_{equ} and the highest values of BC were reached at the treatments fertilised with straw and at the control treatments. In the case of straw, c_{equ} and BC was influenced by the low content of phosphorus that was added to the soil and by biological immobilisation of phosphorus.

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26) Crop influence on mobile sulphur content and arylsulphatase activity in plant rhizosphere

Vliv plodiny na obsah mobilní síry a aktivitu arylsulfatázy v rhizosféře rostlin

Vzhledem ke vzrůstajícímu nedostatku přístupné síry v půdách stoupá význam pochopení mechanismů příjmu tohoto prvku. Jedním z nich je produkce enzymu arylsulfatázy. Na významu také nabývá studium obsahu síry v rhizosféře rostlin, kde může být situace ve srovnání s okolní půdou značně rozdílná. Cílem níže uvedené publikace bylo vyhodnotit vliv různých systémů organického hnojení na distribuci síry a enzymu arylsulfatázy v rhizosféře pšenice ozimé, řepky ozimé a lupiny. Za tímto účelem byly založeny pokusy s rhizoboxy, kde byly hodnoceny půdy dlouhodobě hnojené čistírenskými kaly nebo chlévským hnojem ve srovnání s nehnojenou kontrolou. Testovanou půdou byla kambizem ze stanoviště Lukavec. V rhizosféře (0-6 mm od kořene) sledovaných rostlin byly stanoveny následující parametry: hodnota aktivního pH, aktivita arylsulfatázy, obsah S ve vodném výluhu a obsah organické minerální a celkové S. Obsahy celkové a vodorozpustné síry byly v rhizosféře rostlin vždy vyšší (ve srovnání s okolní půdou), což pro celkovou extrahovatelnou síru potvrzují i výsledky Barber et al. (1995) a Hu et al. (2003). Nejvyšší hodnoty obou uvedených forem S byly naměřeny vždy u lupiny, následované řepkou a pšenicí. Současně byly nejvyšší obsahy těchto forem naměřeny u variant hnojených organickými hnojivy ve srovnání s kontrolou. V případě řepky a pšenice byla zaznamenána zvýšená aktivita arylsulfatázy v rhizosféře, u lupiny byla aktivita arylsulfatázy nejvyšší v okolní půdě. Dále byl potvrzen pozitivní vliv organických hnojiv na aktivitu arylsulfatázy. Obsah organické síry vykazoval negativní korelace s aktivitou arylsulfatázy. To přímo ukazuje na mineralizaci organické S působením arylsulfatázy v půdě. Ukázaly se i značné rozdíly v hodnotách pH jednotlivých vrstev rhizosféry u sledovaných plodin. Zatímco u pšenice hodnota pH se vzdáleností od kořenů klesala, u řepky byl zaznamenán opačný trend. Hodnota aktivního pH v rhizosféře lupiny se se vzdáleností od kořenů prakticky neměnila, avšak byl zde nejpatrnější vliv variant hnojení. To je v souladu s výsledky Youssef et Chino (1989), Hu et al. (2002), Hinsinger et al. (2003), kteří uvádějí, že pokles nebo vzestup pH v rhizosféře je přímo ovlivněn původním pH půdy, druhem rostliny i hnojením dusíkem.

Crop influence on mobile sulphur content and arylsulphatase activity in the plant rhizosphere

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ABSTRACT

The changes of mobile sulphur (S) contents were investigated in the plant rhizosphere using precise model experiments with rhizoboxes. The tested plants were winter wheat (*Triticum aestivum* L.), winter rape (*Brassica napus* L.) and white lupine (*Lupinus albus* L.). In this experiment, a Cambisol from a precise field experiment treated with sewage sludge or manure was used. Total extractable S concentration and mineral S (S-SO_4^{2-}) concentration in the water extract were higher in the plant rhizosphere (< 6 mm from the root) compared to the so-called bulk soil (> 6 mm). The contents of total extractable S^0 decreased in order: lupine (5–35 mg/kg) > rape (4–18 mg/kg) > wheat (1.5–3 mg/kg). The same order was observed for mineral S-SO_4^{2-} where the contents in soil extracts were 1–10 mg/kg, 2–7 mg/kg, and 0.5–3 mg/kg, respectively. The highest total extractable S and mineral S (S-SO_4^{2-}) contents were found in the treatments fertilized with organic fertilizers. In the case of rape and wheat the arylsulphatase (ARS) activity was higher in the rhizosphere compared to the bulk soil; the opposite was recorded for lupine. It was acknowledged that the ARS activity was higher in the treatments fertilized with organic fertilizers (manure or sewage sludge) with all three tested plants. The highest determined ARS activity was found after wheat cultivation, the lowest after the lupine cultivation. The organic sulphur content followed an opposite tendency (lupine > rape > wheat).

Keywords: sewage sludge; manure; rape; wheat; lupine; rhizosphere; sulphur; arylsulphatase

Sulphur (S) concentration in majority of agricultural soils ranges from 50 to 500 mg S/kg. It is mostly organically bound (up to 98% of the total soil S). The main sources of organic S in soil are basically (dead) plant roots, crop residues, and also organic fertilizers (Tabatabai 1982, Freney 1986, Hu et al. 2003, Knauff et al. 2003, Vaněk et al. 2007).

Rhizosphere is the key zone as for the plant nutrients uptake. Physico-chemical processes between soil and roots generally differ from the processes outside the rhizosphere (Darrah 1993). Changes in nutrient dynamics in the plant rhizosphere were intensively studied for phosphorus (Gahoonia and Nielsen 1992, Zoyza et al. 1997, Balík et al. 2007), nitrogen, potassium, calcium and magnesium (Moritsuka et al. 2000). There are however limited data on S fractionation in the rhizosphere (Han and Yoshida 1982, Hu et al. 2002, 2003). Due to the plant-microorganism relationship, rhizosphere is metabolically a very active zone where many important reactions take place. Nutrient availability and nutrient plant uptake is determined especially by soil conditions in the rhizosphere. Root exudates play an important role here; they are a mixture of organic acids, chelants, sugars, vitamins, amino acids, purines, nucleosides, inorganic ions (HCO_3^-, OH^-, H^+), gas molecules (CO_2, H_2) and enzymes (Dakora and Phillips 2002).

The presence of microorganisms which oxidize S is supported mainly by the rape root exudates (Grayston and Germida 1990). Although it is obvious that plant rhizosphere is concerned in the N and S dynamics in the soil and influences their uptake by plants, this aspect was not examined properly. Grayston and Germida (1990) indicate that higher S oxidation takes place in the rhizosphere and there is also a much bigger and diverse group of heterotrophic organisms compared to the bulk soil.

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Hu et al. (2003) studied S fractionation in the plant rhizosphere of rape and rice. They found that it differs depending on crop type. More inorganic S-SO_4^{2-} was present in the rhizosphere of both tested plants compared to bulk soil. Higher concentrations of organic S were found in the rape rhizosphere but, on the other hand, in the rice bulk soil. Sulphur in the sulphate ester bond was found rather outside the rape rhizosphere. Amino acids like cysteine and methionine are the main S compounds directly bound to carbon (Tabatabai 1982, Freney 1986). Paul and Schmidt (1961) observed slightly higher concentrations of cysteine and methionine in the rhizosphere compared to the bulk soil. Sulphur-containing amino acids do not accumulate as free forms in the soil because they are readily degraded in aerobic conditions (Fitzgerald 1978).

Soil enzymes are potential indicators of soil quality because they correlate with soil biology, their activity is easy to determine and they react on changes in the soil management (Bandick and Dick 1999, Trasar-Cepada et al. 2000). Such indicators include physical, chemical and biological characteristics and can be used for monitoring long-term impacts of management on soil fertility.

Enzymes catalyze biochemical reactions and are an integral part of nutrient cycles in soil. They also play a major role within organic compounds decomposition in soil releasing mineral nutrients (Chròst 1991, Sinsabauch 1994). Some enzymes are tightly connected to microorganisms, other are fully extracellular. Sulphatases participate in biochemical mineralization of organic sulphur and especially arylsulphatase was found in most of the soil samples (Tabatabai and Bremner 1970b, 1971, Gupta et al. 1993, Farrell et al. 1994). Arylsulphatase activity can be used as an indicator of biochemical mineralization intensity of organic sulphate esters in the soil. However, detailed information on factors influencing the activity and kinetics of (aryl)sulphatase in soils is still missing. Sulphatases can be found in the soil also as exoenzymes and are closely related to organic matter. Several studies have shown that organic fertilizers, such as compost and manure improve soil organic sulphur availability (Saranthchandra and Perrott 1981, Knauff and Scherer 1998).

The aim of this experiment was to investigate the influence of different systems of organic fertilization on sulphur distribution and arylsulphatase activity in the rhizospheres of winter wheat, winter rape and white lupine.

MATERIAL AND METHODS

Three tested plants (winter wheat – *Triticum aestivum* L., winter rape – *Brassica napus* L. and white lupine – *Lupinus albus* L.) were planted on the soil taken from a precise field experiment with sewage sludge or manure application. The soil is classified as a Cambisol.

The length of each experimental period using rhizoboxes (Wenzel et al. 2001) was 8 weeks (from sowing till harvest). The soil was sieved (2 mm) and dried. First, the root compartment was sowed with tested plants (10 wheat plants, 8 rape plants and 4 lupine plants per rhizobox). After the striking (approximately 7 days) the rhizosphere section was set and the plants were grown for 7 weeks in stable conditions (day: 16 h, 24°C, humidity 95%; night: 8 h, 16°C, 60% humidity). Plants were watered with demineralized water. At the end of the experiment the plants were harvested and the soil was separated to one-millimeter layers.

The pH value was determined using the extraction with demineralized water with the w/v ratio of 1:10. The suspension was shaken for 16 h and left to settle for 1 h. Then the sample was shaken mechanically and the pH_{H_2O} was measured with the glass electrode.

Modified methodology of Tabatabai and Bremner (1970a) was used for determination of arylsulphatase activity. Methodology: after the addition of a *p*-nitrophenylsulphate [4-nitrophenyl sulfate potassium salt (Sigma)] solution, was added to the soil (1 g) and it was incubated at 37°C for 1 h. *p*-nitrophenol (pNP) released by arylsulphatase activity was extracted and colored with NaOH and determined photometrically.

The total extractable and mineral S concentration in the soil was determined in the water extract (ratio 1:10 w/v). The concentration of total extractable S was determined using ICP-OES, Varian VistaPro, Australia. The concentration of mineral S-SO_4^{2-} was measured on Skalar SAN^{plus} SYSTEM. Organic S was assessed from the difference of total extractable sulphur concentration and mineral S-SO_4^{2-}. The total S amount was determined using CNS device LECO.

RESULTS AND DISCUSSION

Main agrochemical characteristics of the Cambisol used in the experiment are summarized in Table 1 (soil prior to the experiment). The soil was taken from the long-term precise field experiment. On

Table 1. Nutrient concentrations (mg/kg) and pH_{CaCl_2} of the tested Cambisol at the beginning of the experiment

Treatment	$pH/CaCl_2$	Available concentration*				S_{tot}
		P	Ca	Mg	K	
Control	5.0	116	1603	78	217	762
Sewage sludge	5.1	180	1664	83	201	807
Manure	5.1	152	1166	87	253	793

*Mehlich III

Table 2. Plants yield (g of dry matter per rhizobox)

Treatment	Lupine		Wheat		Rape	
	aboveground biomass	roots	aboveground biomass	roots	aboveground biomass	roots
Control	4.5	0.28	1.6	0.3	3.7	1.08
Sewage sludge	3.7	0.20	2.2	0.5	4.0	1.05
Manure	4.7	0.27	1.8	0.2	3.8	0.98
F-test	5.94	1.11	11.89	10.03	0.89	1.15
d_{min} ($\alpha = 0.05$)	0.7	n.s.	0.3	0.2	n.s.	n.s.

n.s. – not significant

average, 88.4 kg S/ha was added every three years with the sewage sludge application and 55.8 kg S/ha with manure application. It means there was on average 0.9% S in the dry matter of sewage sludge and 0.3% in the dry matter of manure.

At the end of the rhizobox experiment, the harvested biomass was divided into the aboveground part and roots and the dry matter yield was determined (Table 2). In the case of wheat and rape the lowest yield was found on unfertilized control and the highest yield after the sewage sludge treatment. In the case of lupine, the lowest yield was found after the sewage sludge treatment and the highest yield after the manure treatment. Wheat had the lowest yield from the tested plants, rape and lupine had similar but higher yields.

Table 3 shows total S content in the aboveground biomass of tested plants. The lowest S concentrations were found in all tested plants after the manure treatment and the highest after the sewage sludge treatment. While rape and lupine had the highest S concentration in the aboveground biomass (0.11–0.32%, 0.12–0.24%, respectively), wheat had the lowest (0.07–0.10%). Sulphur uptake by the plants' aboveground biomass is shown in Table 4.

In the rhizosphere of wheat, pH_{H_2O} was the highest close to the roots compared to the bulk soil. Only minor differences were found in the pH_{H_2O} values after different treatments (Figure 1a). Youssef and Chino (1989) measured the pH changes in the barley rhizosphere. Barley was planted in

Table 3. Sulphur concentrations in the aboveground biomass (%)

Treatment	Lupine	Wheat	Rape
Control	0.206	0.070	0.255
Sewage sludge	0.235	0.088	0.324
Manure	0.127	0.100	0.106
F-test	35.84	7.07	80.03
d_{min} ($\alpha = 0.05$)	0.027	0.012	0.037

Table 4. Sulphur uptake by the aboveground biomass (mg per rhizobox)

Treatment	Lupine	Wheat	Rape
Control	9.236	1.099	9.395
Sewage sludge	8.645	1.906	13.079
Manure	5.980	1.772	3.972
F-test	18.11	44.82	23.73
d_{min} (α = 0.05)	1.304	0.207	3.008

the sand culture with a pH value 5. The increase of pH appeared up to 2 units. In our experiments the pH value increased on average by 0.46 in the wheat rhizosphere compared to bulk soil. The pH increase could be caused by the cation accumulation (Ca^{2+}, Mg^{2+}) in the rhizosphere (data not presented here).

In the rhizosphere of rape, pH_{H_2O} was the lowest close to the roots and the highest in the bulk soil. The decrease in pH_{H_2O} value was 0.47. Comparing the treatments, the lowest pH_{H_2O} was in the soil fertilized with manure and the highest after the sewage sludge treatment compared to the unfertilized control (Figure 1b). Chaignon et al. (2002) found that pH increased in the rape and tomato rhizosphere grown on acidic soil and on the contrary decreased in the alkaline soil. Hinsinger et al. (2003) observed that when the pH value was 4.8 in the bulk soil, it was significantly increased in the rape rhizosphere and, on the other hand, rhizosphere acidification occurred when the bulk soil pH value exceeded 7.1.

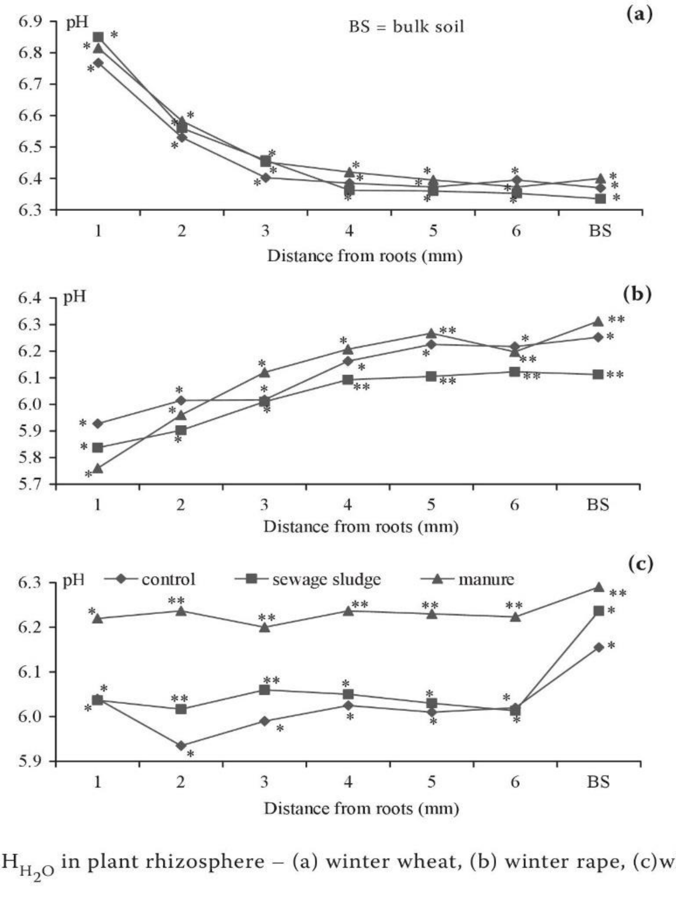

Figure 1. Changes of pH_{H_2O} in plant rhizosphere – (a) winter wheat, (b) winter rape, (c)white lupine

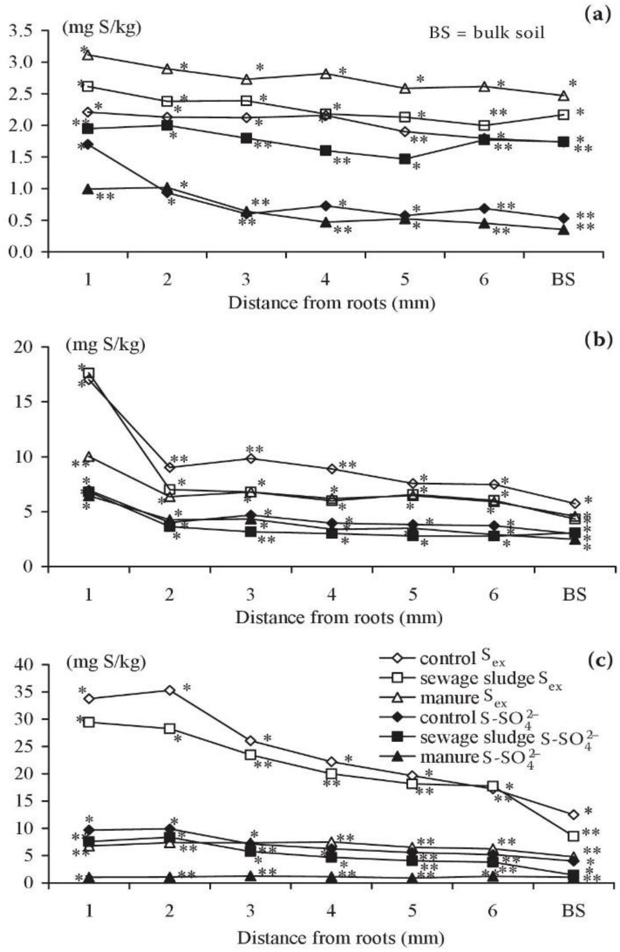

Figure 2. Total extractable sulphur concentrations (S_{ex}) and mineral sulphur concentrations ($S\text{-}SO_4^{2-}$) (water extract) in plant rhizosphere (mg S/kg soil) – (a) winter wheat, (b) winter rape, (c)white lupine

In the lupine rhizosphere acidification also occurred (pH decrease of 0.16) but it was not as pronounced as in the case of rape (Figure 1c). The highest pH_{H_2O} values were found after the manure treatment.

Root-induced pH increase and decrease in the rhizosphere depend mainly on the initial soil pH value, plant species and nitrogen nutrition (Youssef and Chino 1989, Hu et al. 2002, Hinsinger et al. 2003).

Total extractable S content (S_{ex}) in the water extract was the highest in the distance of 1 mm from the roots in the wheat rhizosphere. The S_{ex} value slightly decreased with the distance from the root. The highest S_{ex} was found on the soil fertilized with manure and the lowest one was on the unfertilized control (Figure 2a). The S_{ex} value reached from 1.5 to 3.0 mg/kg. Figure 2a also shows mineral S ($S\text{-}SO_4^{2-}$) concentrations, which follow a similar pattern. These results correspond well with Lasserre et al. (2000) who determined a significantly higher content of S_{ex} in the barley rhizosphere in comparison with the bulk soil.

The S_{ex} values in the water extract were higher in the rape rhizosphere compared to the wheat rhizosphere. S_{ex} reached values from 4 to 18 mg/kg and it was the highest in the distance of 1 mm from the root (Figure 2b). Higher S_{ex} values were measured on the unfertilized control compared to the sewage sludge treatment. It is obvious that mineral S content ($S\text{-}SO_4^{2-}$) accounts approximately for 50% of the total extractable sulphur (S_{ex}).

The S_{ex} values in the lupine rhizosphere determined in the water extract were the highest from

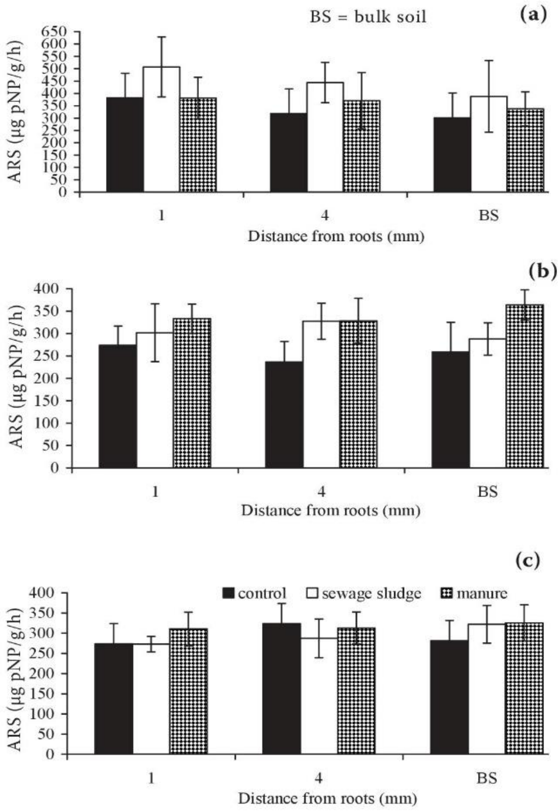

Figure 3. Changes of arylsulphatase (ARS) activity in plant rhizosphere (µg pNP/g/h) – (a) winter wheat, (b) winter rape, (c)white lupine

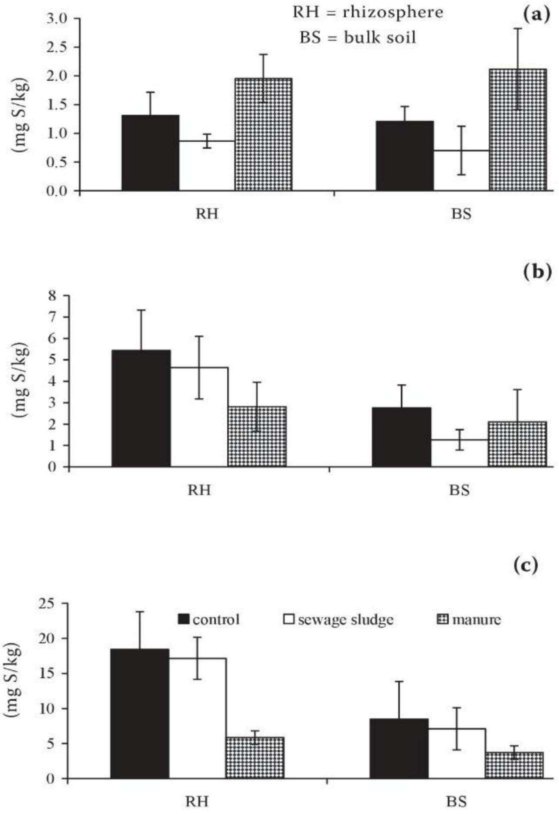

Figure 4. Organic sulphur (S_{org}) concentrations in plant rhizosphere and bulk soil (mg S/kg) – (a) winter wheat, (b) winter rape, (c)white lupine

three tested plants. S_{ex} reached values from 5 to 35 mg/kg and it was the highest in the unfertilized control, slightly lower after the sewage sludge treatment and the lowest in the manure treatment (Figure 2c). The amount of mineral S-SO_4^{2-} ranged from 1 to 10 mg/kg.

The content of total extractable S is on average 6 times higher in the rhizosphere of all tested plants compared to the bulk soil. Similar results were presented by Barber (1995) and Hu et al. (2003) who determined higher concentrations of total extractable S outside the rhizosphere of rape and rice. Results of Morche et al. (2007), who dealt with S distribution in the rape and rye-grass rhizosphere, correspond well with our findings. Authors determined higher S-SO_4^{2-} concentrations in the water extract of the rhizosphere soil with the above-mentioned two plants compared to the bulk soil. They also compared S-SO_4^{2-} in treatments fertilized either with an organic fertilizer (compost) or a mineral fertilizer. They concluded that higher S-SO_4^{2-} concentrations were found in organically fertilized soil (Morche et al. 2007).

When wheat was cultivated on the Cambisol, arylsulphatase (ARS) activity was the highest in the distance of 1 mm from the root and decreased with distance from the root (Figure 3a). This correlates with Knauff et al. (2003) who found higher enzyme activity in the immediate vicinity of roots. Comparing treatments, the highest ARS activity was after the sewage sludge treatments; comparing the tested plants, ARS activity values were the highest in the wheat rhizosphere and reached 300–500 µg *p*-nitrophenol/g/h. However, Knauff et al. (2003) found the highest ARS activity in the rape rhizosphere. Figure 3a shows a significant relationship of ARS activity and the distance from the roots. It decreases with distance from the roots. S_{org} concentrations on the Cambisol ranged from 0.5 to 2 mg/kg and there are no substantial differences among the treatments (Figure 4a). The highest S_{org} content was determined after the manure treatment. Wheat rhizosphere contained the lowest S_{org}.

Figure 3b shows that the highest ARS activity while growing rape was recorded in the treatments with organic fertilizers (sewage sludge and especially manure) compared to the unfertilized control. Similar results were acknowledged by Knauff et al. (2003). The ARS activity value was 240–400 μg *p*-nitrophenol/g/h. As it is further obvious from Figure 3b there was no significant relationship between the ARS activity and the distance from the roots. Knauff et al. (2003) also found out that the distance from the roots has no impact on the ARS activity, except for the significant increase of ARS activity 0.25 mm from the *Sinapis albus* root. We discovered higher S_{org} concentrations in the treatments with organic fertilizers and in the rhizosphere (Figure 4b). Hu et al. (2003) also confirmed more organic S in the rape rhizosphere.

In the case of lupine, the lowest ARS activity was observed in the rhizosphere and it gently increased towards the bulk soil (Figure 3c). It was also acknowledged that the highest ARS activity was in the treatments fertilized with organic fertilizers. Comparing the tested plants, ARS activity values were the lowest and reached 250–350 μg *p*-nitrophenol/g/h. As in the case of rape, there was no significant relationship of the ARS activity and the distance from the roots. In the lupine rhizosphere, the highest S_{org} concentrations were observed (Figure 4c). Again more S_{org} was found in the lupine rhizosphere compared to the bulk soil.

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27) The influence of long-term sewage sludge application on the activity of phosphatases in the rhizosphere of plants

Vliv dlouhodobé aplikace čistírenských kalů na aktivitu fosfatáz v rhizosféře rostlin

Již dříve bylo prokázáno, že fosfor v rhizosféře rostlin se chová jinak ve srovnání s okolní půdou. V důsledku obtížné přístupnosti P pro rostliny dochází v okolí kořenů k vytvoření ochuzené zóny, která vzniká v důsledku odčerpání P rostlinami a zároveň velmi pozvolného doplňování fosforu z okolní půdy. Dosud však nebyl dostatečně prozkoumán vliv aplikace čistírenských kalů na dynamiku P v rhizosféře. Je možné se domnívat, že aplikace vysokých dávek P ve formě čistírenských kalů nemusí zákonitě vést ke zvýšení obsahu přístupného P v rhizosféře, popřípadě k výrazným změnám aktivity fosfatáz. Cílem níže uvedené studie bylo vyhodnotit změny okamžitě přístupného P a aktivitu kyselé a alkalické fosfatázy v rhizosféře rostlin pšenice ozimé a řepky ozimé hnojených chlévským hnojem nebo čistírenskými kaly. Proto byly založeny pokusy s rhizoboxy (Wenzel et al. 2001), kde byl v rhizosféře (0-6 mm) rostlin pšenice a řepky sledován obsah okamžitě přístupného fosforu (stanoveného vodným výluhem) a aktivita kyselé a alkalické fosfatázy (dle Tabatabai a Bremner 1969). Výsledky jasně ukazují na pozitivní vliv organického hnojení na obsah okamžitě přístupného P v rhizosféře rostlin. Aktivita obou fosfatáz byla vždy vyšší v rhizosféře rostlin, přičemž aplikace organických hnojiv vedla vždy k lepším výsledkům ve srovnání s kontrolou. Pravděpodobným důvodem je zvýšená mikrobiální aktivita v důsledku aplikace organických hnojiv (Nannipieri et al. 1978, Spiers et McGill 1979) a/nebo zvýšená tvorba kořenových exsudátů spojená s intenzivnějším růstem rostlin (Marschner et al. 2007). Dick et Kandeler (2005) poukazují rovněž na pozitivní korelace stoupající enzymatické aktivity s vyšší tvorbou kořenových exsudátů, přičemž oba tyto parametry klesají s narůstající vzdáleností od kořenů. Hnojení chlévským hnojem dále vykazovalo vyšší aktivitu obou fosfatáz ve srovnání s čistírenskými kaly, ačkoliv prostřednictvím kalů bylo aplikováno výrazně více P.

The influence of long-term sewage sludge application on the activity of phosphatases in the rhizosphere of plants

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ABSTRACT

Model experiments using rhizoboxes were carried out in order to evaluate the influence of different plants (wheat, rape) on the changes in water extractable contents of P, the pH/H_2O value and the activity of acidic and alkaline phosphatase in soil of plant rhizosphere. For this experiment, a Cambisol with different long-term fertilizing systems was used: (i) control (with no fertilizer application), (ii) sewage sludge, and (iii) manure. A lower content of water-soluble P was observed in close vicinities of root surfaces (up to 2 mm) at all the studied variants. The control (non-treated) variant reflected a significantly lower content of water-soluble P in the rhizosphere compared to the fertilized ones. The activities of the acidic and alkaline phosphatases were significantly higher in the rhizosphere compared to the bulk soil (soil outside the rhizosphere). The long-term application of organic fertilizers significantly increased phosphatase activity; the activity of the acidic phosphatase was significantly higher in the rhizosphere of rape plants compared to wheat. The variant treated with manure exhibited an increased activity of both the acidic and alkaline phosphatases compared to the variant treated with sewage sludge. In the case of the variant treated long-term with sewage sludge, the portion of inorganic P to total soil P content proportionally increased compared to the manure-treated variant. Soil of the rape rhizosphere showed a trend of lower pH/H_2O value of all variants, whereas the wheat rhizosphere showed an opposite pH tendency.

Keywords: sewage sludge; phosphorus; acidic and alkaline phosphatase; wheat; rape; rhizosphere

Total phosphorus concentration in soils varies from 0.01–0.15% according to the soil type, organic matter content and fertilizing intensity. Organic P contents (P_{org}) account generally for 20–85% of the total P content with the largest fraction of organic P in the form of phytin (Dalal 1977). Tarafdar and Claassen (1988) mention that organic compounds (such as phytin, lecithin and glycerolphosphate) are very important in plant nutrition. Phosphorus release from organic compounds is governed by the activity of different enzymes. Phosphatases catalyze the release of P from organically bound P in the form of esters: C-O-P (Eivazi and Tabatabai 1977). Acidic phosphatases are present in the rhizosphere of most plant species. In plants, this enzyme is mainly present in the cell nucleus, cell walls and intracellular spaces, and to a lower extent in amyloplasts, mitochondria and the endoplasmatic reticulum (Chen et al. 1992). Acidic phosphatase from roots is an ectoenzyme released mostly in apical root zones.

The activity of the acidic phosphatase is not only plant-specific (Helal 1990, Yadav and Tarafdar 2001) but it is also dependent on the level of P deficit in plant nutrition (Tarafdar and Jungh 1987, Tadano and Sakai 1991, Mudge et al. 2003, Wasaki et al. 2003). Phosphatase activity produced by roots of rice without sufficient nutrition was twice higher compared to the control (Hirata et al. 1982). A similar effect was observed by Goldstein et al. (1988) in an experiment with tomatoes. Nevertheless, Li et al. (2004) found that the selected plant species play an important role as well. The activity of the acidic phosphatase was 2–3-fold higher in the case of Chinese cabbage compared to corn; corn and Chinese cabbage produced more acidic phos-

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phatase when phytin was added to the soil, compared to the addition of KH_2PO_4 or $Ca(H_2PO_4)_2$. Marschner et al. (2007) showed that the P uptake by plants (*Brassicae* and *Poaceae*) significantly correlated with the activity of acidic phosphatase only at low P levels in soils. Interestingly, the activity of the acidic phosphatase and P uptake by plants correlated well at early stages of plant growth (phase of 6 leaves, blooming phase), but not at later stages of mature plants. Therefore, the authors assume that the mobilization of organic P is more emphasized at earlier growth stages. Similarly, the alkaline phosphatase hydrolyzes P from organic compounds. It is produced by soil fungi and bacteria (Dick and Kandeler 2005). Bhadraray et al. (2002) found a high correlation between the activity of the alkaline and acidic phosphatases in the rhizosphere of plants and the degree of evolution of the rice panicle.

Nannipieri et al. (1978) and Spiers and McGill (1979) state, that organic fertilizers stimulate phosphatase activity in the rhizosphere of cereals due to the increased microbial activity in the soils. At the same time, the activity was inhibited by inorganic soil P. Furthermore, in the experiment of Kizilkaya and Bayrakli (2005) the addition of sewage sludge to soils increased the activity of alkaline phosphatase. On the other hand, Speir et al. (2007) showed that the activity of the acidic phosphatase was partly inhibited at high dosages of applied sewage sludge. A negative correlation was found between the activities of the acidic phosphatase and zinc concentrations in soil. Crecchio et al. (2004) found a positive correlation between compost addition to soil and phosphatase activity. The work of Landi et al. (2000), based on balance procedures, confirmed that the addition of only inorganic P or combined with stable manure, decreases phosphatase activity.

Sewage sludge presents an important source of nutrients and organic matter; however, it can contain increased concentrations of risk elements, organic pollutants and pathogenic microorganisms (Balík et al. 2002). It is thus necessary to evaluate the changes of soil characteristics during intensive long-term sewage sludge application. Studies dealing with the rhizosphere are important to determine the dynamics of nutrient changes in soils (Hinsinger 2001).

The aim of this work was to evaluate the changes in water-soluble P and the activity of acidic and alkaline phosphatase in the rhizosphere of plants in relation to different sources of organic fertilization.

MATERIAL AND METHODS

Rhizobox experiments were carried out in order to evaluate the changes in P contents in the rhizosphere of plants. The type of rhizobox used was described in detail in Wenzel et al. (2001) and Tlustoš et al. (2006). Cambisols from selected long-term field experiments were chosen to represent non-treated variant and variants treated with sewage sludge and manure. The soil was air-dried and the fraction of < 2 mm was used for rhizobox experiments.

Wheat and oilseed rape were selected as experimental plants; they were grown under controlled conditions in a growth chamber (day: 24°C, humidity 95%; night: 16°C, humidity 60%) and were watered using only demineralized water. After the harvest, the rhizosphere was precisely sliced into 1mm layers.

Water-extractable P in the soil was determined using the H_2O extraction with the w/v ratio of 1:10. After 16 hours of shaking, the suspension was filtered and was analyzed for P using optical emission spectrometry with inductively coupled plasma (ICP-OES; Varian VistaPro, Australia).

The pH value was determined using the extraction with demineralized H_2O with the w/v ratio of 1:10. The suspension was shaken for 16 hours and left to settle for 1 hour. Prior to analysis, the sample was mechanically mixed and the pH/H_2O value was determined potentiometrically using a glass electrode.

Phosphatase activity in the soil was determined as follows (Tabatabai and Bremner 1969): the soil was incubated for 2 hours at 25°C in the solution of *p*-nitrophenylphosphate (Fluka) (1:1 w/v) with added buffer [0.1M TRIS = hydroxymethylaminomethan solution (Merck)]. In order to determine the acidic and alkaline phosphatase, the pH of the buffer was adjusted using concentrated HCl and NaOH, respectively. The formed *p*-nitrophenol was determined spectrophotometrically at $\lambda = 400$ nm.

Total concentrations of P in plant samples were determined after dry ashing dissolution using ICP-OES.

RESULTS AND DISCUSSION

Table 1 summarizes the contents of different P forms at tested variants prior to the rhizobox experiment. It is apparent that the control variant exhibited a lower total P content (aqua re-

Table 1. Phosphorus concentration in the Cambisol before the rhizobox experiment (mg P/kg)

Treatment	pH/CaCl$_2$	Mehlich 3	H$_2$O	AEM-PES	Ammonium oxalate			DL-NaOH			P$_{ar}$
					P$_{ox}$	Fe$_{ox}$*	Al$_{ox}$**	P$_{an}$	P$_{tot}$	P$_{org}$	
Control	5.0	105	12.0	51	505	3077	1305	252	855	603	678
Sewage sludge	5.1	216	16.2	79	774	3907	1593	350	1046	696	878
Manure	5.1	174	16.2	60	726	3293	1408	279	907	628	758

*mg Fe/kg, **mg Al/kg; Mehlich 3 – extraction according to Mehlich (1984); H$_2$O – water extraction
AEM-PES – determination of P using anion exchange membranes (Tiessen and Moir 1993)
P$_{ox}$, Fe$_{ox}$, Al$_{ox,}$ – P, Fe, Al determined by ammonium oxalate extraction (Schwertmann 1964)
P$_{an}$, P$_{tot}$, P$_{org}$ – mineral, total and organic P determined by DL-NaOH extraction: [(CH$_3$CHOHHCOO)$_2$Ca (c = 0.01 mol/l) + HCl (c = 0.01 mol/l); pH = 3.6] + subsequent extraction with NaOH (c = 1 mol/l) (Marks 1977)
P$_{ar}$ – total P determined by aqua regia extraction (EN 13346)

gia/DL-NaOH extraction) and a lower content of more mobile P forms. The content of water-extractable P accounted here for 12 mg/kg, while at the variants treated with sewage sludge and manure, the concentration reached 16.2 mg/kg for both variants. Similarly, the values determined using AEM-PES membranes and by the method Mehlich 3 confirmed significantly higher P contents at fertilized variants. It is clear, however, that variants treated with sewage sludge reflected higher total and mobile P concentrations compared to the variants treated with manure, which corresponds well to long-term P application to separate variants. When comparing the values of P$_{org}$ and P$_{tot}$ determined by the DL-NaOH extraction (Marks 1977) it is evident that the variant with manure exhibited a slightly higher organic P fraction (69.2%) compared to the variant treated with sewage sludge (66.5%). This is in accordance with different sorption characteristics of P from different fertilizers. Data obtained from the ammonium oxalate extraction suggest that in the case of sewage sludge variant a larger portion of P is bound to Fe and Al oxides compared to the variant with manure.

Dry biomass yields of the aboveground and root biomass are presented in Table 2, with the lowest values found for the control variant and the highest for the variant with sewage sludge. This is due to the fact that the sludge has higher macro- and micro-nutrients contents (with the exception of N, K) compared to the manure. Phosphorus contents in plants are summarized in Table 3, with the lowest value for the control variant (with the only exception of rape roots). The yield factor and P content in plants corresponds well with total P uptake (Table 4). It is possible to assume that these results reflect well the total and mobile P contents at the control variant as well as at fertilized variants. Furthermore, the aboveground biomass accumulated significantly higher P concentrations compared to roots.

Phosphorus contents in the rhizosphere of plants are summarized in Figure 1. It is important to note that the presented values are not the real concentrations in the soil solution but P concentrations obtained from the water extraction (w/v 1:10). It is widely accepted that the concentrations of salts in the soil solution in the field are 2- to 4-times higher than in the soil extract obtained from the

Table 2. Dry yields of aboveground and root biomass (g/rhizobox)

Treatment	Wheat		Rape	
	aboveground biomass	roots	aboveground biomass	roots
Control	1.6	0.3	3.7	1.1
Sewage sludge	2.2	0.5	4.0	1.1
Manure	1.8	0.2	3.8	1.0
F-test	11.89	10.03	0.89	1.18
d_{min} (α = 0.05)	0.28	0.13	ns	ns

Table 3. Phosphorus concentrations in plants (mg/kg dw)

Treatment	Wheat		Rape	
	aboveground biomass	roots	aboveground biomass	roots
Control	2297	1181	3118	3462
Sewage sludge	2810	2372	3539	3155
Manure	4223	2355	3127	3786
F-test	3.03	7.63	5.47	4.32
d_{min} (α = 0.05)	ns	323	329	486

Table 4. Phosphorus uptake by plants (mg/rhizobox)

Treatment	Wheat			Rape		
	aboveground biomass	roots	total uptake	aboveground biomass	roots	total uptake
Control	3.62	0.52	4.14	11.46	3.72	15.18
Sewage sludge	6.11	1.19	7.30	14.16	3.32	17.48
Manure	7.50	0.47	7.97	11.73	3.71	15.44
F-test	5.18	12.25	45.43	4.74	2.20	2.70
d_{min} (α = 0.05)	2.65	0.38	2.73	2.17	ns	ns

"saturated soil paste" (Marschner 2003). For both wheat and rape, the lowest content was found in the close vicinities of plant roots, which is in accordance with the results of Fusseder and Kraus (1986). Results from the control variant also suggest that low P concentrations were found at 3–6 mm from root surfaces. Interestingly, the sewage sludge variant exhibited lower concentrations compared to the variant with manure even though before the start of the experiment, soil analyses showed that water-extractable P was similar for both variants; still, the contents of available P (obtained from Mehlich 3, AEM-PES) were higher at the variant with sewage sludge. Higher plant uptake can explain only the experiments with rape, not with wheat; it is related to an increased P sorption to Fe and Al oxides and/or a lower mineralization rate of P from organic compounds at the variants with sewage sludge. Furthermore, higher P concentrations were found in the rhizosphere of rape compared to wheat, which confirms a higher ability of rape to mobilize P from less available forms. This ability can be further confirmed by the pH/H_2O values (Figure 2). The results reflect the acidifying effect of rape on the rhizosphere environment. Hypothesis that this acidification was caused by the lack of P

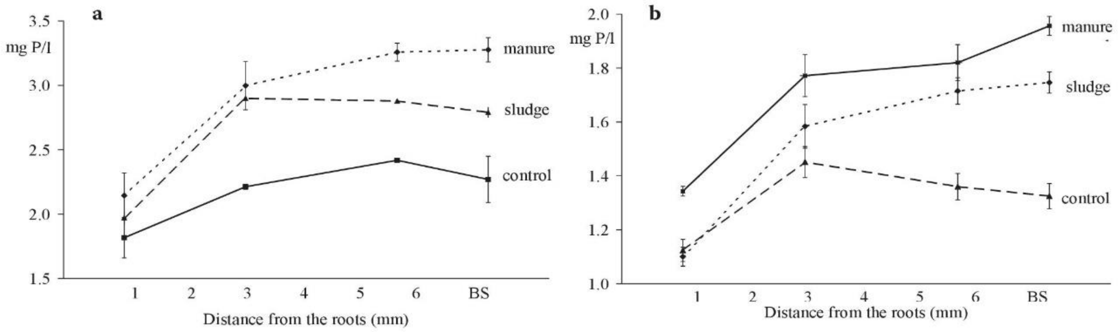

Figure 1. Changes of water extractable P (mg/l) in (a) oilseed rape and (b) wheat rhizosphere

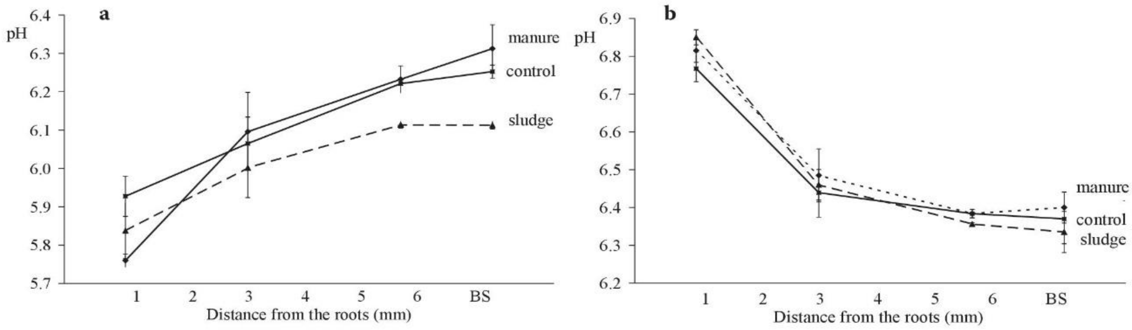

Figure 2. Changes of pH/H_2O in (a) oilseed rape and (b) wheat rhizosphere

cannot be approved because the acidification occurred even at variants fertilized with P. According to the Czech regulations using the Mehlich 3 procedure, very high concentrations of available P are present in the soils. A more probable reason for the pH change is the unbalanced ratio between the cations and anions taken up by the rape plants (Marschner 2003, Balík et al. 2007).

Contrary to the rape, an increase of pH was observed in the close vicinities of wheat roots. This can be caused by the presence of increased concentrations of cations (Ca, Mg) (data not shown). A comparable increase of accumulation of Ca and Mg in the rhizosphere of barley was found by Youssef and Chino (1987).

Due to the fact that direct N fertilizing was not applied, the influence of N forms is not so important as for the acid-base equilibrium and the pH of the rhizosphere. Analyses of N_{min} in soils at all variants showed that the prevailing form was nitrate. Its influence was more emphasized at the control variant compared to the fertilized ones.

The results of phosphatase activity analyses are presented in Figure 3. Significant differences in the acidic phosphatase activity were found in the rhizosphere and in the bulk soil. In our case, it was an average sample of the rhizosphere at 0–4 mm from root surfaces. The chosen distance is in accordance with the work of Nuruzzaman et al. (2006) who found an increased activity at as far as 3–4 mm from root surfaces. Contrary to that, Dick and Kandeler (2005) highlight that the activity of enzymes is the highest at root surfaces and their closest vicinities (0–1.3 mm). This is mostly caused by the accumulation of fast degradable exudates, mass flow and limited diffusion of dissolved organic compounds, which are used by soil microorganisms. Dick and Kandeler (2005) summarize information about positive correlations between enzymatic activities and amounts of exudates; both these factors decrease with increasing distance from root surface. It is thus possible to assume that the values in the close vicinities of roots would be significantly higher. The influence

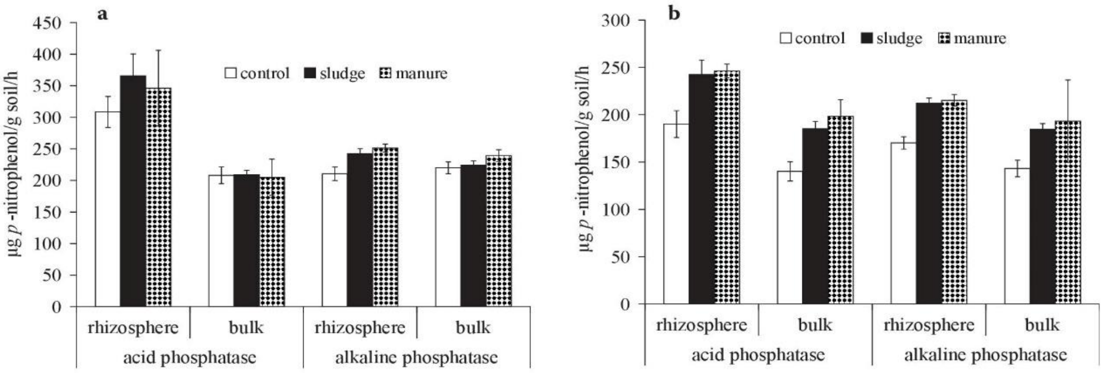

Figure 3. The phosphatase activity in (a) oilseed rape and (b) wheat rhizosphere (µg *p*-nitrophenol per 1 g of soil per hour)

of the plant species on the activity of the acidic phosphatase was also proved, which is in compliance with the works of other authors (Helal 1990, Yadav and Tarafdar 2001, Li et al. 2004). Based on the results obtained from plant analyses and P contents in the rhizosphere it is possible to anticipate that the increased activity of acidic phosphatase at variants with sewage sludge and manure were not caused by insufficient P nutrition. The increased phosphatase activity is probably related to the overall higher microbial activity caused by fertilization (Nannipieri et al. 1978, Spiers and McGill 1979) and/or by an increased production of root exudates as a consequence of more intensive plant growth. Marschner et al. (2007) found that P uptake by plants (*Brassicae* and *Poaceae*) significantly correlated with the activity of the acidic phosphatase only at low P levels in soils. Furthermore, it is important to note that in our experiments, the sampling along the root axis was not performed, but a whole volume of soil was sampled at 0–4 mm distance from the roots. It is possible to expect more significant differences between variants at root tips. As it was stated in the work of Eltrop (1993) hydrolytic enzymes (acidic phosphatase, phytase) are characterized by low mobility in soils and are mostly connected to cell walls and mucilage at the root tips. Similar results were obtained by Dinkelaker and Marschner (1992).

Alkaline phosphatase hydrolyzes P from organic compounds. It is produced by fungi and bacteria in the soil (Dick and Kandeler 2005). Phosphatase activity was higher in the rhizosphere than in the bulk soil, which confirms a higher microbial activity in this zone caused by a higher content of easily degradable root exudates (Figure 3). An increased activity was found at the variant with sewage sludge compared to the control, which is in good agreement with the work of Kizilkaya and Bayrakli (2005). From the figures mentioned above, it is clear that the increased activity was found at the variant with manure compared to the variant with sewage sludge. This is probably caused by the addition of a higher concentration of higher quality organic matter, together with an increase of microbial activity at the variant with manure.

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28) The influence of different intensities of phosphorus fertilizing on available phosphorus contents in soils and uptake by plants

Vliv různé intenzity hnojení fosforem na obsah přístupného P v půdě a jeho odběr rostlinami

Tato publikace byla zaměřena na vztah mezi stupňovaným hnojením fosforem, obsahem přístupného P v půdě a odběrem P rostlinami. Za tímto účelem byly srovnávány převážně jílovité půdy odebrané z šesti stanovišť dlouhodobých přesných polních pokusů ÚKZÚZ, zahrnujících různé půdně klimatické podmínky. Hodnoceny byly varianty se stupňovanými dávkami P: 1) nehnojená kontrola, 2) chlévský hnůj (2 kg P/ha/rok + 6 a 9 kg N a K/ha/rok), 3) N2P0K2 + hnůj (2 kg P/ha/rok), 4) N2P1K2 + hnůj (24 kg P/ha/rok), 5) N2P2K2 + hnůj (36 kg P/ha/rok), 6) N2P3K2 + hnůj (52 kg P/ha/rok), kde N2 a K2 uvedené u některých variant znamená vždy 64 a 68 kg N a K/ha/rok. Hnůj byl aplikován vždy v dávce 40 t/ha k okopaninám v osevním postupu. Obsahy okamžitě přístupného P byly stanoveny vodným výluhem a 0,01 mol/l $CaCl_2$. Potenciálně přístupný fosfor v půdě byl stanoven vždy po sklizni ječmene metodami Mehlich 3 a Olsen. Odběr fosforu rostlinami byl dopočten na základě výnosů hlavních i vedlejších produktů. Všechny pokusy probíhaly v době odběru vzorků více než 25 let. Hnojení fosforem se pozitivně projevilo na obsahu fosforu v půdě stanoveného všemi extrakčními činidly. Odběr fosforu rostlinami se pohyboval v rozmezí 9 – 14 kg/ha, přičemž stoupající tendence byla zaznamenána s narůstající dávkou P hnojiv. Schmidt et al. (1997) dosáhli podobných tendencí v dlouhodobých pokusech s aplikací 0 – 440 kg P/ha. Z korelační analýzy vyplývá nejtěsnější vztah mezi odběrem fosforu rostlinami a obsahem P v půdě stanoveným vodným výluhem (r=0,81). To je v částečně souladu s výsledky Luscombe et al. (1979), kteří potvrdili těsný vztah výsledků vodného výluhu s výnosy rostlin. Výrazně horší, avšak přesto průkazné vztahy vykazoval odběr P s metodami Mehlich 3 a $CaCl_2$ (r=0,40 a 0,36). Neprůkazné (r=0,25) byly naopak vztahy odběru fosforu rostlinami s metodou dle Olsen et al. (1954). Průkazný vztah mezi výsledky vodného výluhu a odběrem P rostlinami potvrdila i regresní analýza (R^2=0,65). Výpočtem jednoduché bilance bylo zjištěno, že samotný chlévský hnůj nestačil k dorovnání obsahu P a roční ztráta na této variantě činila -10 kg P/ha. Ukázal se rovněž negativní vliv nevyrovnané výživy rostlin, kdy varianta N2P0K2 + hnůj vykazovala horší výsledky než samotný hnůj. Naopak roční dávky fosforu 24 kg/ha a vyšší už vedly ke kladné bilanci P.

The influence of different intensities of phosphorus fertilizing on available phosphorus contents in soils and uptake by plants

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ABSTRACT

A gradual increase of phosphorus (P) concentrations in combination with organic and mineral fertilizers was applied to clayey soils at six precise experimental field sites with different climatic conditions within the Czech Republic. Soil samples were collected after the harvest of barley. Readily available P concentrations were determined using water and 0.01M $CaCl_2$ extractions, mobile phosphate contents were determined using the extraction procedures Mehlich 3 and Olsen. Furthermore, P uptake by barley plants was assessed. All selected procedures showed that increasing concentrations of applied P increased the mobile and readily available P contents in soils. Phosphorus uptake by barley plants ranged between 9.00 and 14.5 kg/ha and increased P application resulted in increased P concentrations in plants. Statistical analyses were performed using the Spearman test. The closest relationship was obtained for P uptake by plants and the water extraction (r = 0.81). Significant relationships were also obtained for plant uptake and the Mehlich 3 and $CaCl_2$ methods (r = 0.40 and 0.36, respectively). There was no statistical dependence between P uptake and the Olsen method (r = 0.25). Regression analysis was expressed best as linear regression. The closest relationship was found for water extracts and P uptake by plants (with a determination coefficient of 65%). Determination coefficients for P uptake and other methods used were significantly lower (max. 20%).

Keywords: long-term experiments; mobile forms of P; readily available P; plant uptake of P

Almost all phosphorus (P) fractions in soils converted to phosphate ions are taken up by plants. While $H_2PO_4^-$ is the prevailing P form in acidic soils, HPO_4^{2-} is the predominant form in alkaline soils (Mengel 1991, Schilling 2000, Blume et al. 2002). Marschner (1995) found that the contents of the water-extractable P fraction, closely related to yields, reach only 0.8–8.0 mg P/kg. Another fraction influencing P uptake is partially mobile (i.e. exchangeable sorbed) P, which is easily and rapidly released into soil solution (McGechan and Lewis 2002).

Mobile phosphate contents in non-fertilized soils vary significantly (10–100 mg P/kg). The vast majority of P is bound to less soluble soil compounds (Marschner 1995). Tiessen and Moir (1993) showed that it is indispensable to precisely define the availability of P especially for predicting plant uptake. It is important to note that plant species vary in the ability of taking up P due to different root systems, mycorrhiza and growth speed (Mengel 1991). Another important factor involved in P mobilization are soil microorganisms (Kuono et al. 1995). Due to the fact that available P is consistently supplied by less available P through dissolution, desorption and mineralization of organic P, the level of available P is strongly dependent on specific soil-climatic conditions, the cultivated crop and date of soil sampling (Tiessen and Moir 1993). McGechan and Lewis (2002) found that the determination of P availability is complicated since P applied as mineral fertilizers can be effectively sorbed. At sites fertilized with P, it is possible to observe mobile P in colloid forms and/or bound to soil particles.

There are several methods used for the determination of readily available P, such as the $CaCl_2$

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Table 1. Characteristics of the studied sites

Site	Founded in year	Altitude above sea level (m)	Average		Soil type	pH ($CaCl_2$)
			precipitation (mm)	temperature (°C)		
Jaroměřice	1975	425	535	7.5	luvisol	6.3
Staňkov	1981	370	511	7.8	luvisol	6.0
Vysoká	1983	595	655	7.4	pseudogley	6.3
Sedlec	1972	300	581	8.4	chernozem	7.1
Pusté Jakartice	1979	290	650	8.0	luvisol	5.8
Uherský Ostroh	1972	196	551	9.2	luvisol	6.6

extraction (Houba et al. 1994) and the water extraction (Luscombe et al. 1979). Methods for determining mobile P in soils include, among others, the Mehlich 3 (Mehlich 1984) and Olsen (Olsen et al. 1954) procedure.

MATERIAL AND METHODS

Six sites designed for long-term experiments with different climatic conditions were chosen in the Czech Republic (Table 1). The soils are classified as sandy loam. Separate nutrients applied to soils are summarized in Table 2.

Organic fertilizers (40 t manure/year) were applied regularly within crop rotation to root crops (potatoes, sugar beet). Nitrogen was applied during soil preparation before sowing or planting in the form of ammonium sulfate; further fertilization was in the form of ammonium nitrate with lime. The source of P was superphosphate. Potassium was applied as 60% potassium salt.

Soil samples were collected after the harvest of barley (included in the crop rotation system), air-dried and sieved through a 2-mm sieve. Extracts were analyzed spectrophotometrically using the SKALAR SAN^{PLUS} SYSTEM®.

The standard reference material ISE PER 2003-1, SAMP 1-4 (International Soil-analytical Exchange Programme, University Wageningen, The Netherlands) was used.

In order to determine readily available phosphorus, water extracts (Luscombe et al. 1979) and calcium chloride extracts (Houba et al. 1994) were used.

In order to determine the concentrations of mobile phosphates, the extractions Mehlich 3 (Mehlich 1984) and Olsen (Olsen et al. 1954) were used.

Phosphorus uptake was calculated from plant analyses, main yields and by-products. These data were obtained from the cooperation with CISTA (Central Institute for Supervising and Testing in Agriculture, Brno).

RESULTS AND DISCUSSION

The average yearly P balance was evaluated throughout the whole experiment. Table 3 shows the negative P balance at non-treated variants and

Table 2. Fertilizing system of the precise field experiment (average contents of applied nutrients in kg/ha/year)

Variant number	Fertilizing variant	Nutrients		
		N	P	K
1	control	0	0	0
2	manure (to root crops)	6	2	9
3	$N_2P_0K_2$ (+ manure to root crops)	64	2	68
4	$N_2P_1K_2$ (+ manure to root crops)	64	24	68
5	$N_2P_2K_2$ (+ manure to root crops)	64	36	68
6	$N_2P_3K_2$ (+ manure to root crops)	64	52	68

Table 3. Long-term balance between P input by fertilization and P output by harvest (average values from all sites throughout the whole experiment)

Variant number	Fertilizing variant	Average balance (for one site) (kg/ha)	Average balance (for one site) (kg/ha/year)
1	control	–458	–16
2	manure (to root crops)	–295	–10
3	$N_2P_0K_2$ (+ manure to root crops)	–396	–14
4	$N_2P_1K_2$ (+ manure to root crops)	182	6
5	$N_2P_2K_2$ (+ manure to root crops)	476	17
6	$N_2P_3K_2$ (+ manure to root crops)	904	32

at variants treated with P in a non-mineral form. The addition of mineral P together with manure to root crops (24 kg P/ha year) resulted in a change of the balance to +6 kg P/ha year (var. 4). Increasing concentrations of P resulted in an increase of the positive balance to +32 kg/ha year (var. 6: $N_2P_3K_2$ + manure).

In the case of the control variant, the negative P balance of –458 kg P/ha was observed – the application of manure resulted in an increase to –295 (var. 2) and –395 kg P/ha (var. 3). Only the addition of P in mineral form (24 kg/ha) resulted in a positive balance (+182 kg P/ha). The highest balance value (+904 kg P/ha) since the beginning of the experiment was observed at the variant $N_2P_3K_2$.

Furthermore, changes in contents of different phosphate forms in soils were determined during the experiment. Variants treated with P always resulted in increased P contents in soils. This was the case for both readily available and mobile P. Average contents of readily available P ranged between 3.8 and 9.5 mg P/kg (water extraction) and between 0.2 and 2.8 mg P/kg (0.01M $CaCl_2$ extraction). The highest water extractable P contents were obtained for the variant $N_2P_3K_2$ + manure with an average value of 9.5 mg P/kg. This value is still lower (by 7 mg P/kg) than the value reported by Koopmans et al. (2002) for over-fertilized soils in the Netherlands. On the other hand, the lowest results were obtained for the control, where the average content of readily available P reached only 3.8 mg P/kg. This is in accordance with the values of Marschner (1995) who stated that for untreated soils the concentration of water-extractable P reaches only 0.8–8.0 mg P/kg.

The Mehlich 3 protocol proved to be the strongest extraction procedure for the determination of mobile P, with average values from 45 to 112 mg P/kg. The Olsen extraction procedure resulted in values between 25–55 mg P/kg.

Increasing levels of P fertilizing resulted in increased P uptake by barley plants. The lowest uptake was observed for control variants (9 kg P/ha). The highest values were obtained, as expected, for the $N_2P_3K_2$ + manure variant, where 52 kg P/ha in average was applied to the soil. In this case, the uptake of P by barley was 14.5 kg P/ha. Increased P uptake by plants after soil-applied P to soils was observed by Schmidt et al. (1997) in long-term experiments with P applied in mineral forms (from 0 to 440 kg P/ha).

The main objective of the study was to specify the relationships between P uptake by plants and P contents in soils as determined by different extraction procedures. Due to relatively high differences between individual sites, it was not possible to perform an analysis of variance and thus test evaluating files. The Spearman test with the level of significance set to $\alpha < 0.05$ was used for correlation analysis.

Linear regression proved to be the best method in regression analysis. Significant relationships between P uptake by barley plants and the $CaCl_2$ method, water extraction and Mehlich 3 (Table 4) were found. However, a close relationship ($r^2 = 0.82$) was obtained only in the case of the water extraction, even at the level of significance of $\alpha < 0.0001$. This fact was also proved by Luscombe et al. (1979) who studied relationships between P content in soil and plant yields. Relatively good correlations were obtained between P uptake and the methods Mehlich 3 ($r^2 = 0.37$) and the $CaCl_2$ extraction ($r^2 = 0.34$).

In the case of the Olsen method, the correlation coefficient reached only 0.22, which is not a significant relationship at the set significance level. This method is based on the use of HCO_3^-,

Table 4. Correlations between P uptake and observed methods

	Mehlich 3	Olsen	$CaCl_2$	Water extract
Correlation with P uptake (r^2)	0.371	0.216	0.348	0.818
The level of significance (α)	0.0300	0.2100	0.0400	0.0001

CO_3^{2-} and OH^- ions at pH 8.5 causing thus the precipitation of $CaCO_3$ and a subsequent release of phosphate ions into the solution. Therefore, this method is the most suitable for soils with high carbonate contents. Nevertheless, it is useable even for more acidic soils, where these ions, together with Fe^{2+} and Al^{3+}, form oxyhydroxides (Sims 2000). However, Schoenau and Karamanos (2000) highlight the inaccuracies during the determination of mobile P in these soils using the Olsen method.

The method is further influenced by extraction of an insufficient content of the labile organic fraction, which can play an important role during P uptake by plants (Franzen et al. 1999).

As it was stated before, linear regression was the most suitable form of regression analysis. The relationships between $CaCl_2$, H_2O extractants and P uptake by plants are summarized in Figure 1. It is evident that the water extraction best correlated with P uptake by plants.

The regression equation can be expressed in the form:

$$P_{uptake} = 1.11 \times P_{H_2O} + 5.20 \qquad (1)$$

where: P_{uptake} is uptake of P by plants (kg P/ha) and P_{H_2O} is water extractable P (mg P/kg).

The coefficient of determination R^2 indicates that changes in P contents in the water extract can be expressed in 66% as the change in P uptake by plants, which indicates a close relationship between these two parameters. The $CaCl_2$ method does not correlate well with P uptake. The regression equation can be expressed as:

$$P_{uptake} = 1.44 \times P_{CaCl_2} + 11.10 \qquad (2)$$

where: P_{CaCl_2} is the amount of P measured in 0.01M $CaCl_2$ extract (mg P/kg).

The coefficient of determination however shows that P uptake can be expressed only in 13% as the change of P contents in the $CaCl_2$ extract. This can be caused by the bioavailable phosphate ions forming Ca-P complexes during the extraction that cannot be analyzed (McDowell et al. 2003). This method is thus less accurate. Furthermore, this method is not suitable for conventional agrochemical analyses due to its high time variability (McGechan and Lewis 2002).

Figure 2 shows the relationships between the Mehlich 3/Olsen method and P uptake by plants. Closer relationships were observed between P uptake and contents of mobile phosphates determined by the Mehlich 3 method as it was already

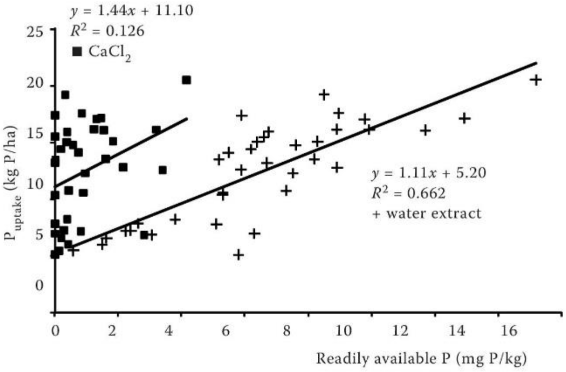

Figure 1. Correlation between P uptake by plants and readily available P in the soil

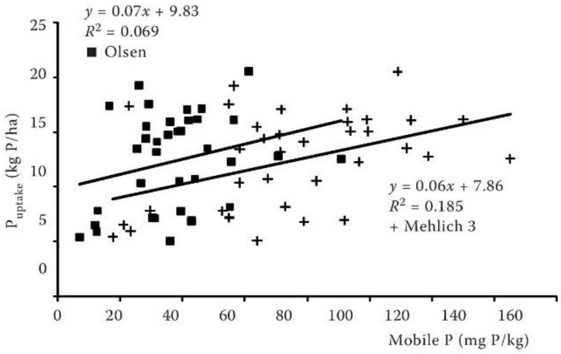

Figure 2. Correlation between P uptake by plants and mobile P in the soil

shown by correlation analyses. The regression equation is as follows:

$$P_{uptake} = 0.06 \times P_{M3} + 7.86 \quad (3)$$

where: P_{M3} is the amount of P measured in Mehlich 3 extract (mg P/kg).

The coefficient of determination R^2 was higher than in the case of $CaCl_2$, but accounted only for 0.2. This indicates that changes in P uptakes by plants are related to P contents determined by the Mehlich 3 method only in 20%. Even a weaker relationship was obtained for P uptake and the Olsen method.

The coefficient of determination $R^2 = 0.08$ showed that there is almost no relationship between the values obtained from the Olsen method and P uptake by plants. Low coefficients of determination are probably caused by the fact that both methods focus on the determination of mobile phosphates, i.e. P fractions not readily bioavailable.

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7. Závěry

Z literatury jasně vyplývá, že zdroje kvalitních fosfátů pro výrobu fosforečných hnojiv jsou na pokraji vyčerpání. Proto jsou intenzivně studovány následující alternativy s potenciálem oddálit hrozící riziko spojené s nedostatkem fosforu ve výživě rostlin:

- Využití odpadních materiálů jako zdrojů fosforu (čistírenské kaly, popel, digestát,…).
- Lokální aplikace fosforečných hnojiv vedoucí k lepšímu využití P rostlinami.
- Šlechtění rostlin zaměřené na vyšší osvojovací schopnost (související s metabolismem a/nebo distribucí kořenů), popř. menší odběr fosforu.
- Použití biostimulantů mobilizujících P z hůře rozpustných forem v půdě.
- Kombinace výše uvedených možností.

Řada možností byla sledována v souboru studií zahrnutých v této habilitační práci. Z výsledků je zřejmé, že výzkum by měl začínat analýzami půd i rostlin. Zde je velmi důležitý správný odběr vzorku, jeho zpracování, analýza, a především správná interpretace dosažených výsledků. Při srovnání metod bylo dosaženo značných rozdílů mezi extraktanty určenými pro stanovení přístupného fosforu. Pořadí metod dle extrakční účinnosti na nekarbonátových půdách bylo následující: 0,01 mol/l $CaCl_2$ (1:10) < vodný výluh (1:10) < Olsen < CAL < AEM – membrány < DL (dříve Egner) < Mehlich 3. U slabých extrakčních činidel (zejména 0,01 mol/l $CaCl_2$ a H_2O) přitom dochází k vyšším sezónním výkyvům a naopak silným extrakčním činidlem Mehlich 3 jsou stanoveny i hůře přístupné formy P.

Dále bylo v rámci práce sledováno působení několika odpadních materiálů jako potenciálních zdrojů fosforu. Nejpodrobněji bylo průběžně hodnoceno působení čistírenských kalů, aplikovaných v dlouhodobých přesných polních pokusech. Byl prokázán jejich pozitivní vliv na obsah přístupného fosforu. Ve srovnání s hnojem (při stejné dávce aplikovaného dusíku) bylo zjištěno, že obsah přístupného P v půdě po aplikaci kalů byl až několikanásobně vyšší. Naopak hnůj se ukázal jako zdroj stabilnějších forem P. Ve screeningových nádobových pokusech byl rovněž sledován vliv dalších odpadních materiálů (slámový a dřevěný popel, torefikované čistírenské kaly, separovaný digestát). Aplikace digestátu se z hlediska fosforu ukázala jako nevýhodná z důvodu nepoměru živin (nadbytek N a K). V případě popele a torefikovaných kalů byla prokázána jen velmi obtížná přístupnost fosforu, a proto byla tato hnojiva testována rovněž spolu s biostimulanty mobilizujícími fosfor. K aplikaci odpadních materiálů je třeba zároveň dodat, že je nutné sledovat další parametry představující různá rizika (aplikace rizikových prvků a organických polutantů, negativní vliv a strukturu půdy aj.).

V rámci evropského rámcového programu BioFector byla hodnocena řada biostimulantů určených pro mobilizaci fosforu, a to buď samotných, nebo v kombinaci s různými zdroji obtížně dostupného fosforu (mletý fosfát, popel, …), a rovněž i s dusíkatými hnojivy s amonnou formou N. I přes rozsáhlou škálu pokusů bylo dosaženo pouze několika průkazně pozitivních výsledků. V nádobových i polních pokusech bylo prokázáno spolupůsobení amonného dusíku a bakterie *Pseudomonas sp.* na růst kořenového vlášení kukuřice. Aplikace *Paenibacillus mucilaginosus* vedla v nádobových pokusech k mobilizaci P z dřevěného popela, avšak rovněž k imobilizaci nitrátového dusíku. Vliv na výnos kukuřice byl tedy ve výsledku negativní. Ukazuje se tak, že působení biostimulantů je díky snadnější publikovatelnosti signifikantních výsledků a tlaku firem spíše nadhodnocováno. Pozitivní efekt v provozních podmínkách je pravděpodobně limitován celou řadou přirozených faktorů (pH, průběh počasí, obsah přístupného dusíku i jiných živin) i agrotechnických zásahů (termín a způsob aplikace biostimulanů aj.). Biostimulanty tak zatím představují větší potenciál v ochraně rostlin (kde jsou zpravidla aplikovány na list), než v jejich výživě.

Podstatná část přiložených prací je rovněž zaměřena na chování síry v prostředí, zejména v zemědělsky využívaných půdách. Zde byl při rozborech vzorků z dlouhodobých pokusů (roky 1981 a 2007) zjištěn výrazný pokles obsahu přístupné síry v půdě, kterému nezabránily ani různé varianty běžného hnojení. Tento pokles je možno přičítat zejména omezení vstupů S z atmosférických depozic. Síra ve výživě rostlin se tak dostává do popředí zájmu.

Hlavní studovanou oblastí se v této práci staly možnosti vyhodnocení různých frakcí síry v půdě a jejich potenciál stát se zdrojem přístupné S pro rostliny. Proto byla použita řada extrakčních činidel od „slabých“ roztoků stanovících přístupné formy S (vodný výluh, NH_4Cl, NaH_2PO_4, $NH_4H_2PO_4$, Mehlich 3), po roztoky stanovící okludovanou (HCl), estersulfátovou (redukce HI) i reziduální a celkovou S (lučavka královská, CNS analyzér). Kromě výrazného poklesu přístupných forem síry v půdě bylo zjištěno, že se obsahy estersulfátové, celkové i reziduální frakce S v průběhu studovaného období výrazně nezměnily. Jejich postupnou mineralizací tak může být určitý podíl S stále uvolňován do půdního roztoku. Dále byly prokázány velmi těsné korelace ($r > 0,85$) mezi výsledky metody Mehlich 3 a metodami určenými ke stanovení přístupných forem S (vodorozpustná a adsorbovaná). Naopak velmi slabé korelace Mehlich 3 s metodami zahrnujícími organické frakce S ukazují na fakt, že tato metoda je pro stanovení přístupné S přinejmenším stejně vhodná jako vodný výluh a 0,032 mol/l NaH_2PO_4. Vzhledem k možnosti využití metody Mehlich 3 ke stanovení téměř všech živin toto zjištění představuje možnost značných úspor při analýzách půd. Vhodnost metody Mehlich 3 pro stanovení přístupné S rovněž potvrzují průkazné korelace výsledků s obsahy síry v nadzemní hmotě rostlin pšenice ozimé a řepky ozimé. Ke zpřesnění výsledků mohou rovněž posloužit doplňující metody, zejména využití tzv. indexu výživy sírou (SNI - sulfur nutrition index). Jedná se o poměr aktuálního obsahu S v nadzemní hmotě ke kritickému obsahu síry. Optimální poměr N:S a současně i kvalitativní parametry zrna (Zelenyho test, obsah bílkovin a mokrého lepku) vykazovaly rostliny s indexem SNI přesahujícím hodnotu 0,80 ve fázi počátku odnožování, 0,70 v pozdní fázi odnožování a 0,60 na počátku metání.

Další část výzkumu představoval monitoring běžně užívaných systémů hnojení z hlediska změn obsahů síry v půdě. Ten probíhal v dlouhodobých pokusech ČZU a ÚKZÚZ i tříletém pokusu zaměřeném na stupňované dávky síry ($CaSO_4$) dodané v N-S hnojivu. Z výsledků dlouhodobých pokusů ČZU založených v roce 1996 vyplývá, že čistírenské kaly jsou zdrojem především vodorozpustné síry, aplikace chlévského hnoje zvyšovala obsahy adsorbované S v půdě a hnojení NPK v minerální podobě (6,5 kg S/ha/rok v trojitém superfosfátu) vedlo výraznému zvýšení obsahu okludované S. V dlouhodobých pokusech ÚKZÚZ byly porovnávány obsahy S v archivních vzorcích půd odebraných v letech 1981 a 2007. Z výsledků zde jasně vyplývá, že ani hnojení hnojem samotným či hnojem s minerálním NPK nezabránilo výraznému poklesu obsahu minerální (zejména vodorozpustné) síry v půdě, a to až o 60 %. Tato tendence byla potvrzena na 10 stanovištích s různými půdně klimatickými podmínkami. V tříletém pokusu zaměřeném na stupňované dávky S k ozimé pšenici a ozimé řepce bylo zjištěno následující: aplikace ledku amonného se sírou (ve formě $CaSO_4$) vedla ke zvýšení obsahů přístupné S v půdě, které pak během vegetace klesaly pravděpodobně z důvodu odběru rostlinami. Hnojení S rovněž zvýšilo výnos zrna pšenice, avšak nebyl jednoznačně potvrzen vyšší odběr síry v hnojených variantách. Velmi podobných tendencí bylo dosaženo i v pokusech s ozimou řepkou.

Předložená práce přináší některé odpovědi v oblasti problematiky výživy rostlin fosforem a sírou. Zároveň však vyvstává řada dalších otázek a námětů k návaznému výzkumu, který by měl být zaměřen především na studium možností mobilizace nepřístupného P z půdy a hnojiv, lokální aplikaci fosforu a šlechtění rostlin ve vztahu k vyšší využitelnosti P. V případě síry se pak jedná o vyhodnocení míry mineralizace S v půdě, sledování účinnosti různých organických a minerálních hnojiv a rovněž další upřesňování kritérií v oblasti analytických metod pro stanovení obsahu S v půdě i v rostlině včetně zahrnutí dalších souvisejících živin. Nemalá pozornost by měla být věnována rovněž chování P i S v rhizosféře rostlin.

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