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Frakcionace síry a uhlíku v půdě při různé intenzitě hnojení organickými a minerálními hnojivy

Doktorská disertační práce

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Ing. Pavel Suran

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Děkuji

1 Literární přehled - síra

1.1 Význam síry

Síra je klíčovým prvkem ve výživě rostlin, kde se vyskytuje při syntéze aminokyselin, glutationu, chlorofylu a (Mehta et al. 2005; Marschner 2012) a její dostatečné zásobení může ovlivnit kvalitu sklizně, zejména výnos (Bender et al. 2012; Steinke et al. 2015), nebo například obsah bílkovin v zrnu obilnin (Flaete et al. 2005). Tato živina se v posledních desetiletích ovšem stává limitujícím prvkem ve výživě rostlin díky snižujícím se obsahům tohoto prvku v půdě (Scherer, 2009).

Lehmann et al. (2008) a Yang et al. (2007) poukazují na tři hlavní faktory, které jsou zodpovědné za snížení obsahů síry v půdě v posledních desetiletích. Za prvé jde o snížení vstupů S do půdy z atmosférických depozic. Jako druhý faktor uvádějí pěstování výkonnějších odrůd rostlin, které se vyznačují vyšším odběrem S. Za třetí se jedná o používání velmi kvalitních hnojiv, pro které je typický velmi nízký obsah balastní síry. Nejdramatičtější změny ve vývoji těchto tří faktorů byly zaznamenány právě ve změnách depozic atmosferické síry. Jejich vývoj v EU popisuje studie Gao et al. (2018), kde se uvádí snížení vstupů S do půdy z tohoto zdroje z hodnot až stovek kilogramů síry.ha^{-1}.rok^{-1} na hodnoty jen několika desítek kilogramů S.ha^{-1}.rok.$^{-1}$ za posledních čtyřicet let. Obdobný trend popisují také Balík et al. (2009) na území ČR. Vliv těchto faktorů může vést k podcenění změn obsahu síry v půdě, jejíž určitý podíl je přijímán rostlinami (Lehmann et al. 2008).

1.2 Koloběh síry

Světový koloběh síry zahrnuje pevninské a vodní prostředí a také atmosféru. Na rozhraní těchto tří prostředí stojí živé organismy, které zprostředkovávají mnohé z fyzikálních a chemických přeměn (Stevenson et Cole 1999). Na obrázku č.: 1 je znázorněn stručně koloběh síry v prostředí. Je zde vidět, že síra se vyskytuje v široké škále sloučenin, ale pro výživu rostlin jsou nejdůležitější právě ty ve formě síranového aniontu v půdě (Marschner 2012). V minulosti byl tento iont dodáván do půdy právě z atmosféry prostřednictvím suché a mokré depozice (Scherer, 2009; Lehmann et al. 2008; Yang et al. 2007). V rámci suché depozice dochází ke spadu oxidu siřičitého, nebo pevných částic nesoucích síru přímo na půdu. Při mokré depozici nejprve dochází k reakci plynných částic s atmosférickou vodou a následnému spadu na půdu v podobě deště, sněhu, nebo mlhy.

Aktuálně se průměrné Evropské depozice pohybují pod 10 kg $S.ha^{-1}.rok^{-1}$ (Marchetto et al. 2021) a v České republice až pod 5 kg $S.ha^{-1}.rok^{-1}$ (CHMI 2019). Historicky se depozice síry se pohybovaly nad hranicí 100 kg $S.ha^{-1}.rok^{-1}$ v ČR a dalších Evropských státech (Krupová et al. 2018). Spad síry v takovém množství vedl například k acidifikaci půd napříč celou Evropou (Schöpp et al. 2003). Snížení depozic bylo dáno společenskými změnami v devadesátých letech, které vedly k tvorbě dohod cílených ke snížení emisí síry do atmosféry úpravou technologií v průmyslu, nebo zemědělství (Engart et al. 2017). Evropská agentura pro životní prostředí uvádí snížení průměrných emisí oxidu siřičitého v Evropě až o 74 % mezi lety 1990 a 2011 (EEA 2015). Obdobný trend je pozorovatelný také v ČR, kde emise SO_2 dosahovaly hodnoty přes 1800 $Kt.rok^{-1}$ v roce 1990 (MŽP 2007) a v roce 2019 se emise tohoto plynu snížil na 80 $Kt.rok^{-1}$ (CHMI 2020) (snížení o více, jak 90 %).

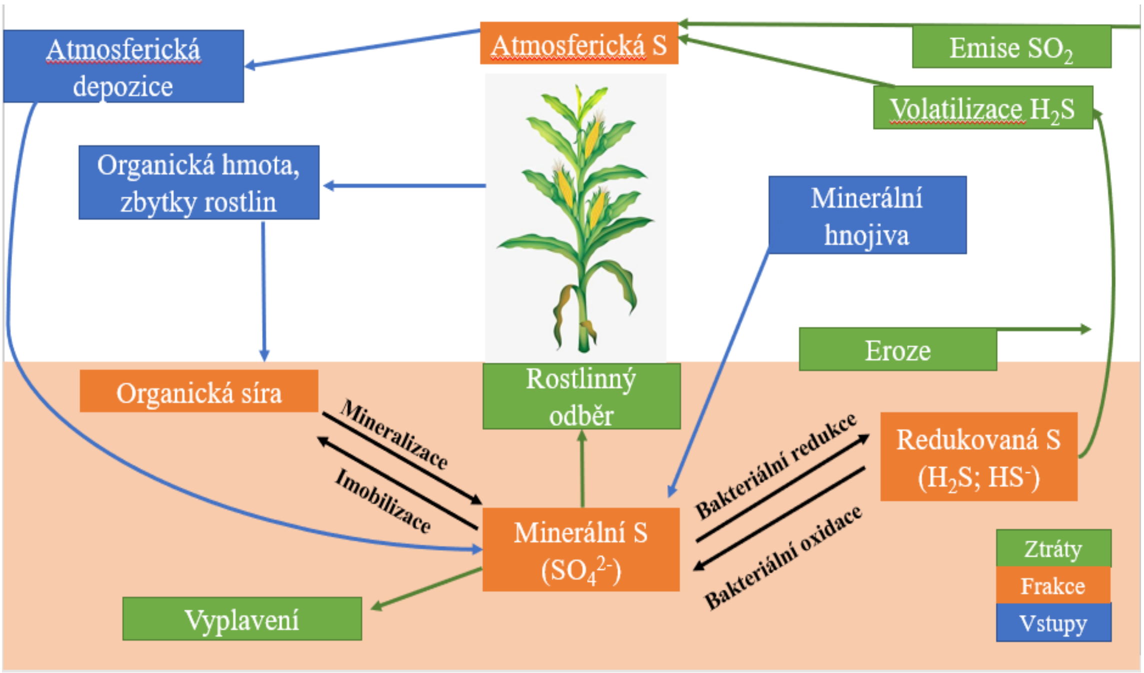
Tyto hodnoty odpovídají pouze emisím z průmyslu. Ovšem vzhledem k tomu, že výměra zemědělské půdy je podle ČUZK (2021) 4 200 204 ha (tedy přes 50 % celkové výměry ČR) je dalším potenciálním zdrojem emisí samotné zemědělství. V půdě probíhají přeměny forem S zprostředkovávané mikroorganismy (Marschner 2012; Vermien et al. 2018) a produkty/meziprodukty těchto reakcí mohou být právě plyny SO_2 a H_2S (Burton et al. 2006; Kinsela et al. 2011). Podle Kinsela et al. (2011) jsou emise oxidu siřičitého více ovlivněny teplotou půdy ($r^2 = 0{,}387$; $p < 0{,}01$) zatímco emise sirovodíku spíše souvisí s vlhkostí půdy ($r^2 = 0{,}124$; $p < 0{,}01$). To je v souladu se studií Mengel et al. (2001), ve které byl zjištěn větší obsah síry v oxidačním stavu S^{2-} na silně podmáčených půdách, kde převládají redukční podmínky. Podle Kinsela et al. (2011) jsou zemědělské půdy schopny emitovat v průměru 3,7 kg $S.ha^{-1}.rok^{-1}$ (součet emisí SO_2 a H_2S). Pokud tento údaj vztáhneme k výměře zemědělské půdy ČR, dochází ke každoroční emisi 15,5 Kt síry z celého zemědělského půdního fondu ČR. Je nutné ovšem podotknout, že hodnoty ve studii Kinsela et al. (2011) byly naměřeny na Australských půdách bohatých na S a jen v relativně krátkých časových intervalech (2 měsíce). Vzhledem k těmto faktům je pravděpodobné, že emise ze zemědělských půd v ČR budou dosahovat mnohem menších hodnot.

V minulosti potřeba hnojení rostlin sírou nebyla pozorována, neboť si rostliny vystačily s přídělem z každoroční depozice. Aktuální vývoj depozic síry ovšem vede k tomu, že rostliny vyčerpávají půdní zásobu této živiny (Hu et al. 2005), protože při svém vývoji spotřebují více, než se jim z půdy dostává (McGrath et al. 2002). To vede k potřebě hospodářské plodiny hnojit. Potřeba hnojení S je u jednotlivých druhů plodin různá. Vliv má samotný druh rostliny, původní zásoba na stanovišti, ale také použitý hybrid v rámci jednoho druhu (Bender et al. 2013). Nedostatek síry v rostlině může mít vliv jednak na velikost výnosu (Grant et al. 2012; Varényiová et al. 2017; Ercoli et al. 2012; Kulhánek et al. 2014), tak na kvalitu sklizené hmoty (Varényiová et al. 2017; Ahmad 2007; Khan et al. 2006; Ercoli et al. 2012).

Mezi jedny z nejnáročnějších plodin patří rostliny z čeledi brukvovitých. Tyto rostliny vyžadují síru, mimo jiné, pro produkci glukosinolátů (Fismes et al. 2000; Grant et al. 2012; Aarabi et al. 2016; Zhang et al. 2020). Jedná se o sekundární metabolity, které mají antibakteriální účinek (Dubuis et al. 2005) a zvyšují odolnost proti hmyzu a herbivorům (Sugiyama et Hirai 2019; Jeschke et al. 2019). McGrath et Zhao (1996) uvádějí, že rostliny řepky ozimé odebírají z půdy až 51 kg S.ha^{-1}. Toto zjištění odpovídá výsledkům ostatních studí. Významný nárůst výnosů při aplikaci hnojiva S v dávce 50 kg S.ha^{-1} o 10 % popsali Zhao et al. (1993), zatímco Fismes et al. (2000) uvádějí nárůst až 20 % při dávce 75 kg S.ha^{-1}. Ovšem Grant et al. (2012) a uvádějí optimální dávku mezi 20 – 60 kg S.ha^{-1}. Zároveň při dávce hnojiva nad 20 kg S.ha^{-1} popisují Ahmad et al. (2007) významné zvýšení olejnatosti semena a obsahu glukosinolátů. Také Sýkora (2006) popisuje nárůst obsahu glukosinolátů až o 30 % při dávce 50 kg S.ha^{-1}.rok^{-1} a uvádí odběr S téměř 90 kg S.ha^{-1}.rok^{-1} při této dávce. Naptori tomu například Varényiová et al. (2017) zmiňují, že při dávkách S hnojiva nad 60 kg S.ha^{-1} již začíná docházet ke snížení výnosů, ale významné zvýšení olejnatosti je patrné již při dávce 15 kg S.ha^{-1}.

Mezi méně náročné plodiny patří například kukuřice, která je schopná poskytnout krmivo pro některá hospodářská zvířata díky vysokému výnosu, dobré stravitelnosti a přijatelnosti živin (Rafael et al. 2006). Odběr této rostliny činí necelé 3 kg S.ha^{-1} pokud není hnojena S (Sakal et al. 2000). Pokud je rostlina hnojena S, dochází k nárůstu odběrů až k téměř 10 kg S.ha^{-1} (Sakal et al. 2000; Dwivedi et al. 2002; Suran et al. 2021). Tohoto zvýšení dosáhli Sakal et al. (2000) při dávce hnojiva 40 kg S.ha^{-1}, Dwivedi et al. (2002) již při dávce 34 kg S.ha^{-1}. Při dávce 60 kg S.ha^{-1} uvádějí Mehta et al. (2005) zvýšení výnosu zrna a biomasy o 29,1 % a 25,1 % na půdách s běžným obsahem síry. Dávku 60 kg S.ha^{-1} doporučují také Khan et al. (2006), který zaznamenává zvýšení výnosu sušiny až o 55 % na půdách, které jsou velmi chudé na S. Vyšší dávky již na rostlinu mohou působit negativně (Khan et al. 2006), případně se k rostlině ani nedostanou a může dojít k jejich vyplavení (Suran et al. 2021; Riley et al. 2002; Bergholm et Majdi 2001).

Obilniny jsou další významnou kategorií plodin díky jejich využití pro výrobu pečiva. Zpracovatelská kvalita je dána obsahem a kvalitou lepku (Schofield 1994). Obsah lepku a jeho kvalita se výrazně zvyšuje při hnojení rostlin sírou (Ercoli et al. 2012). Pšenice ozimá odebírá v průběhu vegetace mezi 10 – 30 kg S.ha^{-1} (Kulhánek et al. 2014), kdy nižší hranice byla pozorována u rostlin které nejsou hnojeny S, zatímco vyšší hranice byla pozorována u rostlin hnojených 40 kg S.ha^{-1}. Ercoli et al. (2012) uvádějí podobné výsledky, ovšem nejvyšší odběr zaznamenal při dávce 60 kg S.ha^{-1} a uvádějí, že vyšší dávka neměla na obsah S v rostlině vliv. Ječmen odebírá v průběhu vegetace 15 – 25 kg S.ha^{-1} (Hřivna et al. 2011) a při hnojení dávkou 20 kg S.ha^{-1} je rostlina zvýšit výnosy cca. o 14 %. To potvrzují také Zhao et al. (2006), kteří při stejné dávce na půdách chudých na S zvýšil výnos až o 27 %. Zpracovatelská kvalita (jako je obsah bílkovin) sklizně se podle Fox et al. (2003) a Zhao et al. (2006) se při této dávce S hnojiva také zvyšuje. Je tedy evidentní, že síra je důležitou živinou a vzhledem k nízkým atmosférickým depozicím a zmíněným nárokům rostlin je nutné věnovat výživě tímto prvkem pozornost, zvlášť, když zmíněné studie ukazují, že rostliny jsou schopné reagovat na hnojení S již v relativně malých dávkách (v mnoha případech již kolem 15 kg S.ha^{-1}).



Obrázek 1: Schéma koloběhu síry

Síranový aniont, který rostliny přijímají je v půdě ovšem velice mobilní. Na potenciální problém je možné ukázat v půdách hnojených sírou, případné v půdách, kde byly dříve vysoké depozice S z atmosféry. Několik studií se zaměřilo na vyplavování síry z těchto půd. Riley et al. (2002) popisují vyplavování S v pokusu s jílkem vytrvalým. Z aplikované dávky hnojiv s různou rozpustností síry se vyplavuje významné množství S. V prvním roku jejich pokusu bylo zjištěno, že došlo ke ztrátám S vyplavením 72, 26 a 7 % z aplikované síry v podobě síranu amonného, elementární síry a směsi bentonitu s elementární sírou z dávky 50 kg S.ha^{-1}. Dále je nutné podotknout, že za celou dobu experimentu došlo k simulovaným depozicím z atmosféry v podobě 22 kg S.ha^{-1} (nejsou zahrnuty ve výpočtu procentických ztrát). Zmíněné ztráty vyplavením se třetím rokem navýšily na 96, 75 a 33 %. Bergholm et Majdi (2001) také uvádějí, že se v jejich šestiletém experimentu vyplavuje významné množství síry (41,2 % z celkové dávky 821 kg S.ha^{-1}.6 let^{-1} let v podobě síranu amonného). Nicméně je nutné podotknout, že jejich práce se zabývala lesními půdami. Suran et al. (2021) popisují vyplavení značného množství S z monokultury kukuřice. Autoři ve své studii zjistili, že z varianty hnojené síranem amonným se každoročně v průměru vyplaví necelých 143 kg S.ha^{-1}.rok^{-1}, což je více, než dodávaná dávka hnojiva 142 kg S.ha^{-1}.rok^{-1} ve formě síranu amonného. Obdobně autor popsal vyplavení 95,3 kg S.ha^{-1}.rok^{-1} na variantě hnojené síranem hořečnatým v dávce 84 kg S.ha^{-1}.rok^{-1}. Je tedy patrné, že dochází k uvolnění síry také z jiných zdrojů než z hnojiva. Tímto zdrojem budou nejspíše pozůstatky z atmosférických depozic předešlých desetiletí. Z práce Riley et al. (2002) je evidentní, že se tyto ztráty vyplavením dají omezit použitím hnojiv s nízkou rozpustností (jako je a směs bentonitu s elementární sírou). Na druhou stranu Santoso et al. (1995) uvádějí, že rychle rostoucí rostliny, jako je například kukuřice mohou mít snížený odběr síry právě vlivem použití hnojiv s nižší rozpustností. I přes rozdílnost výsledků těchto studií je patrné, že dochází k vyplavování síry, čímž dochází k znepřístupnění značného podílu S rostlinám. Nehledě na to, že takto vyplavená síra musí někde končit a jedním z možných míst jsou právě podzemní vody (Sharma et Kumar. 2020), nebo povrchová vodní tělesa (Kopáček et al. 2014). Singh et al. (2004) uvádějí, že meliorovaná krajina umožňuje rychlejší mineralizaci organické síry a urychluje tak její vyplavování. Kopáček et al. (2014) to svým výzkumem potvrzuje. Na Českých zemědělských půdách uvádějí právě největší ztráty síry vyplavením na počátku devadesátých let, zejména na meliorovaných půdách z předchozích 20 let. Konkrétně v hodnotách vyplavené S až 80 kg S.ha^{-1}.rok^{-1} a kdy zároveň autoři pozorovali nejvyšší vstupy do půd. Následný pokles k hodnotám pod 10 kg S.ha^{-1}rok^{-1} již autoři vysvětlují snížením depozic z atmosféry a uvádějí, že vliv mělo také následné sníženy zastoupení meliorovaných půd.

1.3 Síra v půdě

V geosféře je síra hojně se vyskytujícím nekovovým prvkem. V půdách se celkový obsah síry pohybuje v rozmezí 0,01 až 0,1 % (Morche, 2008). Průměrný celkový obsah síry v půdách ČR se může pohybovat kolem 200 mg S.kg^{-1} napříč širokou škálou půd, jak popsal Balík et al. (2009). Nicméně právě v této studii byl popsán také výše zmíněný vliv snižujících se depozic, který vedl na vybraných lokalitách ke snížení průměrného obsahu z 221 mg S.kg^{-1} v roce 1981 na obsah 204 mg S.kg^{-1} v roce 2008. V půdě se síra vyskytuje v minerální a organické formě a neustále se mezi těmito formami transformuje (Tabatabai a Al-Khafaji 1980), nicméně pro rostliny je důležitý obsah právě minerální formy (Marschner 2012).

1.3.1 Minerální síra

Minerální síra je ve většině zemědělských půd zastoupena méně, než organicky vázaná síra. Jedná se o vysoce proměnlivou složkou, která je hlavním přístupným zdrojem tohoto prvku pro rostliny a tvoří většinou pouze 5 % z celkového obsahu síry (Tisdale et al., 1993; Barber 1995). Minerální síra se v půdě nachází ve formě rozpustného anorganického síranu v půdním roztoku, dále v podobě sorbovaného anorganického SO_4^{2-} a části síranů vysrážených pospolu s vápníkem nebo hořčíkem v podobě uhličitanu vápenatého a hořečnatého (Tisdale et al. 1993).

Barber (1995) poukazuje na to, že anorganická síra je v zemědělských půdách přítomna zpravidla ve formě síranů (SO_4^{2-}), ale také se zde vyskytuje v nižších oxidačních stavech sulfidů (S^{2-}), siřičitanů (SO_3^{2-}), thiosíranů ($S_2O_3^{2-}$) a elementární (S^0). V dobře provzdušněných půdách je nejběžnější síranová forma, která je dle Knauff in Kulhánek et al. (2016) ta nejdůležitější forma pro výživu rostlin. Siřičitanová forma se přechodně vyskytuje, jako meziprodukt oxidací a redukcí jiných forem, thiosíranová forma se vyskytuje spíše po průmyslových haváriích, kdy dochází k ovlivnění aktivity půdních mikroorganismů a kumulaci této formy S. Tyto formy nemají podstatně význam pro výživu rostlin (McLaren et Cameron 1996).

V **půdním roztoku** se síra objevuje v rozsahu kolem jednoho procenta z celkového obsahu S (Knauff in Kulhánek et al. 2016), přitom je podle Tabatabai (1982) velmi důležitou složkou pro rostliny. Obsahy této složky se velmi rychle mění v průběhu sezóny a to především díky odběru rostlinami (Scherer 2009), mineralizaci/imobilizaci v rámci organických látek v půdě (Blum et al. 2013), aplikace hnojiv obsahujících S (Eriksen 1996).

Dalším významným faktorem je také vyplavování síranů z půd, které je samo také ovlivněno dávkou S, formou hnojiva a počasím v průběhu sezóny (Riley et al. 2002, Bergholm et Majdi, 2001).

Adsorbovaná síra je podobně, jako síra v půdním roztoku důležitá pro výživu rostlin. Snadno dochází k uvolnění sorbovaného iontu do půdního roztoku. V půdách se může vyskytovat v široké škále obsahu. Například Kulhánek et al. (2018) stanovili v půdách ČR průměrný obsah 6,15 mg $S.kg^{-1}$ Obsah adsorbovaných síranů je významně ovlivněn hodnotou pH. Podle Curtin et Syers (1990) a Xiao et al. (2015) dochází k největší sorbci síranů při pH = 3, ale při rostoucím pH se sírany spíše uvolňují a na hodnotě pH = 6 je již téměř veškerý původně sorbovaný síran uvolněn do vodného roztoku. Při rostoucím pH se zvyšuje množství OH^- aniontů, které jsou v kompetici s SO_4^{2-} o vazebné místo na půdních částicích (Korentajer et al. 1983), což vede k uvolnění síranů. 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), zejména v půdách chudých na vápník (Chen et al. 1997; Xiao et al. 2015). Z výsledků Tisdale et al. (1993) vyplývá, že adsorpce síry je rovněž ovlivněna dalšími anionty, a to v pořadí fosforečnany > dusičnany = chloridy. Fosforečnany se v půdě sorbují mnohem silněji a jsou v kompetici se síranovými anionty (Bohn et al. 1986; Barrow et Debnath, 2015). Nárůst obsahu fosforečnanů a síranů v půdě vede ke snížení adsorpce S (Bohn et al. 1986). Protože jsou sírany vázány výrazně slabší vazbou než fosforečnany, vede hnojení fosforem spolu s vápněním k zvýšení přístupnosti S (Scherer 2009). Dále 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. Nicméně podle Martinez et al. (1998) dochází k nižší adsorbci na jílnatých půdách s vyšší organickou hmotou a to díky kompetici síranů o sorpční místa s organickými anionty, jako jsou zbytky organických kyselin, lipidů a jiné.

Obsah **okludované** síry výborně koreluje s obsahem uhličitanů ($r^2 = 0{,}986$; $p < 0{,}05$), jak popsali Chen et al. (1997). Na to navazuje Hu et al. (2005) a popisují, že s rostoucím obsahem uhličitanů roste i obsah okludované síry (v průměru až 11,7 % z celkové S). Tento podíl je ovšem rostlinám pouze přístupný, pokud je uvolněn do půdního roztoku, nebo se transformuje do výměnně-sorbovaného podílu.

1.3.2 Organická síra

Organická síra se v půdě vyskytuje, jako součást rostlin, makroedafonu a mikroorganismů v živém, nebo mrtvém podílu hmoty. Její obsah v půdě dobře koreluje s obsahem celkové S (Biederbeck, 1978), a jak popisují později například Yang et al. (2007), nebo Kulhánek et al. (2018) z celkové půdní síry tvoří převážný podíl právě organicky vázaná forma. Podle Hu et al. (2005) je podíl organické síry v půdě 77 % z celkového obsahu, ale na obhospodařovaných půdách je tento podíl zpravidla větší a to kolem 95 % (Scherer 2009).

Síra vázaná na organickou hmotu se vyskytuje ve dvou základních, hojně zastoupených skupinách (Freney et al. 1986).

- První skupinou je estericky vázaná síra (C-O-S), která je na uhlík nepřímo vázána přes atom kyslíku. Do této skupiny patří například sulfátové polysacharidy, thiogliceridy, cholinsulfát a sulfátované polysacharidy.
  - Již méně významnou skupinou jsou například sulfamáty, kde je S vázána přes dusík (C-N-S).
- Druhou skupinou je síra vázaná přímo na uhlík (C-S), jako například v aminokyselinách metioninu a cysteinu, merkaptanech, sulfolipidech a disulfidech sulfonových kyselin.

Estericky vázaná síra činí mezi 30 – 70 % z organického podílu S (Neptune et al. 1975). Tato frakce S je více mobilní (Shan et al. 1997) a také v ní nastává zabudování SO_4^{2-} do C-O-S rychleji (Fitzgerald et al. 1982), než C-S. Síra přímo vázaná na uhlík činí z organického podílu 19 – 43 % a vykazuje významně vyšší obsahy v systémech s intenzivním organickým hnojením (Förster et al. 2012). Scherer et al. (2012) také popisují, že se C-S a C-O-S nejvíce kumulují na jemných půdních částicích (zejména < 0,002 mm), nicméně tyto částice se v půdách vyskytují zpravidla v menším množství. Částice větší než 0,002 mm tvoří podstatně větší podíl a budou mít významnější vliv na celkové množství sorbované S.

1.4 Transformace síry v půdě

Jak již bylo zmíněno výše, jednotlivé frakce S se mezi sebou neustále transformují z organické na minerální a naopak. Organicky vázaná síra je rostlinám samostatně nepřístupná, nicméně mineralizace tohoto podílu se významně podílí na doplňování zásoby anorganického síranu v půdě, který rostliny využívají (Blum et al. 2013). Tento mineralizační proces je zprostředkován pomocí enzymů a mikroorganismů (McGill et Cole, 1981). Síra vázaná v mikrobiální mase je schopná rychlé cirkulace a díky tomu má velký význam (Randlett et al. 1992) i přes to, že její obsahy jsou kolem 6,15 mg S/kg v průměru (Chowdhury et al. 1999). Mikroorganismy jsou schopné mineralizovat, nebo také imobilizovat elementární síru, což je důležitý faktor pro hospodaření se sírou (Jaggi et al. 1999). Na rozdíl od Neptune et al. (1975) Ghani et al. (1992) popisují, že většina takto mineralizované síry pochází z C-S. V této frakci je velké množství bílkovin, které se v prvním kroku rozloží na sirné aminokyseliny v procesu aerobní hydrolýzy. Dále se vytvoří sulfan, který se v aerobních podmínkách oxiduje na síranový aniont (Marschner 2012). Pokud nejsou v půdě aerobní podmínky, tak je sulfan oxidován pouze do podoby elementární síry (sulfurifikace) chemotrofními bakteriemi rodu *Thiothrix,* nebo *Thiobacillus*. Tento proces také mohou vykonávat i fotosyntetizující bakterie čeledí *Thiorhodaceae*, Opačným procesem se v půdě označuje desulfurikace a vzniká při něm sulfan z oxidovaných forem S. Jedná se tedy o redukce S za účasti bakterií rodu *Desulfovibrio*. Vysoká přítomnost síry v oxidačním stupni S^{2-} je typická pro půdy, kde převládají redukční procesy, často tedy na podmáčených půdách (Mengel et al. 2001).

Mikrobiální mineralizace C-S je závislá převážně na aktivitě půdních mikroorganismů, na druhou stranu mineralizace sulfatické síry probíhá spíše na základě aktivity enzymů, které jsou schopné hydrolyticky štěpit vazbu C-O-S. Jako první enzym z této skupiny byla popsána arylsulfatáza, ovšem není to jediný příklad (dále: alkyl-, gluko-, chondro-, mykosulfatázy) (Eriksen et al. 1998). Tato frakce S je více mobilní (Shan et al. 1997), je labilnější a podléhá více vlivům, než síra ve vazbě C-S (McLaren et al. 1985). Nicméně je to bohatá skupina látek a Klose et al. (1999) popsali, že v průběhu jejich dvouletého experimentu se obsah estersulfátů významně nelišil, což vede k závěru, že tato skupina S látek je variabilní ve své rozpustnosti. Lou et Warman (2004) uvádějí, že hydrolýza těchto látek je velmi závislá na jejich poloze v molekule látky, ve které se vyskytují. Autoři v této studii popisují, že estersulfáty z molekul s nižší hmotností jsou náchylnější k mineralizaci, než u molekul s větší hmotností. Zároveň zmiňují, že náchylnější jsou také sírany přítomné na okrajích molekul, které jsou lépe dosažitelné pro hydrolytické enzymy, oproti síranům uzavřeným hluboko v molekulách. Na základě toho Eriksen et al. (1998) předpokládají, že C-O-S frakce zaujímá ve své dostupnosti pro rostliny velmi široké spektrum od sloučenin dobře mineralizovatelných, až po pomalu mineralizovatelné. Největší podíl mineralizovaných estersulfátů pochází z hydrolytického rozkladu pomocí mikrobiálních enzymů, které zprostředkovává široká škála různých organismů (Nannipieri et al. 1990). Aktivita těchto enzymů je tedy dána přítomností a aktivitou mikroorganismů (Tabatabai et Fu, 1992). Vyšší aktivita byla také popsána v půdách s vyšším obsahem humusu (Klose et Tabatabai, 1999) a také v menší hloubce (Tabatabai et Bremner, 1970a). Dále je také popsán vliv vlhkosti půdy a teploty (Castellano et Dick, 1991). Zde autoři zmiňují, že aktivita byla vyšší počátkem léta a na osetých plochách, což vysvětlují zvýšenou aktivitou kořenových exsudátů a rozkladem odumřelých kořenů a listů. Běžně se udává, že cca. polovina aktivity tohoto enzymu je mimo buňku a za druhou polovinu je odpovědná mikrobiální biomasa.

Celkově je mikrobiální mineralizace organických sloučenin S ovlivněna různými faktory. Jaggi et al. (1999) zjistili, že nejvyšší míra mineralizace S probíhá při teplotě 36 °C. Matula (2007) poukázal na vliv takovéto mineralizace pro výživu rostlin řepky ozimé v jarním období a zjistil, že čistá mineralizace dosahovala v průměru kolem 4 kg S.ha^{-1} a to jen u úrodných druhů půd. Na neúrodných půdách byl zaznamenán spíše úbytek. To je u náročné plodiny, jako je řepka nedostačující a mineralizace organického podílu nedokáže pokrýt její nároky. Obdobné výsledky prezentují také Eriksen et al. (1995), kteří změřili čistou mineralizaci mezi 3,30 až 6,70 mg S/kg půdy. V laboratorních pokusech není situace na první pohled o moc příznivější. Podle Tabatabai a Al-Khajaci (1980) lze mineralizovat mezi 16 až 86 mg S.kg^{-1} za 26 týdnů. Dle Knights et al. (2001) je rychlost mineralizace pouze 14 až 35 mg S.kg^{-1} po době 28 týdnů, nicméně podle Vermien et al. (2018) předchozí studie nepočítají s remineralizací čerstvě imobilizované síry, jejíž labilita je mnohem vyšší. Právě tito autoři použitím isotopového trasování (^{35}S) popisují mnohonásobně vyšší mineralizaci organické S (včetně remineralizace) a to v hodnotách 30,94 až 96,46 mg S.kg^{-1} po 26 týdnech experimentu. Zároveň má také vliv samotné dusíkaté hnojení, které snižuje mineralizaci S a mnohdy spíše způsobilo dokonce imobilizaci (Matula, 2007).

Nelson (1973) popisuje, že rostliny jsou schopné zužitkovat reziduální síru, která se do půdy vrátí po zapravení posklizňových zbytků rostlin. Pokud se jedná o biomasu bohatou na síru (například nadzemní části řepky ozimé), tak je toto opětovné zužitkování až 85 %. Zajímavým přístupem k určení, zda půda bude spíše imobilizovat, nebo mineralizovat S je využití poměru C:S, které navrhuje Barrow (1960). Autor uvádí, že k mineralizaci dojde spíše pokud je poměr C:S < 200:1 a k imobilizaci bude docházet pokud je poměr C:S > 400:1. Ideální je tedy pohybovat se v rozmezí těchto dvou hodnot, aby nedocházelo k příliš vysokému vyplavování síranů, jako popisuje například Riley et al. (2002), a aby nedocházelo k tak velké imobilizaci S, která by mohla mít negativní vliv na přístupnost S rostlinám. V souvislosti s tím zmiňuje Eriksen (1997), že přídavek jednoduchého zdroje uhlíku způsobí redukci příjmu S, právě díky její imobilizaci do nové organické hmoty, což potvrzuje zjištění Chapman (1997). Tento autor popisuje, že v regionech s deficiencí S může mít zapravení rostlinných zbytků chudých na S za následek zhoršený růst rostlin. Morche (2008) dále uvádí, že transformacím podléhají také minerální frakce. Autor ve své práci uvádí, že vodou extrahovatelná frakce S je v průběhu vegetace doplňována také z ostatních anorganických frakcí. Nicméně odběr „vodorozpustné“ S rostlinou není plně kompenzován přísunem z ostatních podílů v této práci. To dokládá zjištěním, že obsah vodou rozpustné S v průběhu vegetace neustále klesá. Bylo zaznamenáno také snížení obsahů adsorbované S, které se dá vysvětlit právě tím, že dochází k desorpci do půdního roztoku, při vytvoření nové rovnováhy mezi těmito frakcemi (Tisdale et al. 1993, Barber 1995). Dále Morche (2008) zaznamenává snížení obsahu frakce okludované S, což autor vysvětluje mobilizací této frakce do přístupnějších forem.

1.5 Stanovení síry v půdě

Koncentrace rostlinám přístupné minerální síry se během roku mění (Ghani et al. 1992) 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).

1.5.1 Stanovení minerální síry

1.5.1.1 Vodný roztok

Jak již bylo zmíněno výše, síra se v půdě vyskytuje v mnoha frakcích a pro výživu rostlin jsou nejdůležitější ty frakce, které jsou rostlinám přístupné. Běžně se pro stanovení této frakce používá extrakce síry vodným výluhem (Tabatabai, 1982; Kulhánek et al. 2016). Tato extrakce má za cíl stanovit obsah síry v půdním roztoku, která je tou nejmobilnější a tedy nejlépe přijatelnou frakcí. Významnou korelaci ($r^2 = 0{,}878$) mezi sírou v tomto extraktu a obsahem S v rostlině popisuje například Matula (1999). Vzhledem k problémům s disperzitou v případě extrakcí vodou se často dává přednost slabým roztokům solí, jako

$CaCl_2$, NaCl, LiCl, $BaCl_2$, KH_2PO_4 (Tabatabai 1982, Saalbach et Aigner 1987, Ketterings et al. 2011), které neextrahují adsorbovanou frakci (Sahrawat et al. 2009). Matula (1999) 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. Stanovení ve vodném výluhu je tedy stále jedno z těch nejrozšířenějších a nejběžnějších (Balík et al. 2009; Kulhánek et al. 2016; Kulhánek et al. 2018; Suran et al. 2021; Morche 2008; Lavatya et al. 2019 a mnoho dalších).

1.5.1.2 Sekvenční stanovení

Frakcionační stanovení mají za úkol extrahovat síru různými činidly uloženou v půdě. To má umožnit rozlišení jednotlivých frakcí síry, na základě schopnosti jednotlivých extrakčních činidel uvolnit tento prvek. Každé činidlo je typické tím, že uvolní jen část z celkového obsahu. Tento obsah se dá následně srovnat například s obsahem v rostlině a určit, která frakce (nebo součet více frakcí) je rostlinám přístupná. Vzhledem k jejich významu při výživě rostlin je pozornost věnována minerálním frakcím S.

Jedno z prvních frakcionačních stanovení S popsali již Freney et al. (1970), jež mělo za cíl sekvenčně stanovit obsah vodorozpustné a adsorbované S v půdě s následným stanovením organické síry dle Johnson et Nishita (1952). Nicméně v průběhu let se tato metodika postupně upravovala a dnes se využívají postupy, které umožňují stanovit také okludovanou frakci S. Například metoda dle Shan et al. (1997) dále upravená dle Morche (2008) má za cíl stanovit frakci rozpustnou ve vodě (extrakce destilovanou vodou), adsorbovanou na půdních částicích (extrakce pomocí 0,032 M NaH_2PO_4) a okludovanou ve sraženinách uhličitanů (za pomoci 1 M HCl) a jejich součet označuje jako rostlinám přístupnou síru. Obdobně postupují také Lavanya et al. (2019). Na rozdíl od Morche (2008) je při této extrakci použit místo NaH_2PO_4 1 % roztok NaCl, za účelem extrakce velmi lehce rozpustných složek minerální S, která se neuvolnila do vody. Autoři označují jako rostlinám přístupný obsah součet vodou + NaCl extrahovatelné. Použití různých činidel má své důvody. Pro úplnou extrakci adsorbovaného podílu se používají fosforečnanové soli (díky schopnosti fosforečnanů uvolnit sírany z povrchu částic) a soli chloridů se používají na úplné uvolnění pouze vodorozpustné frakce. Tento proces je možné následovat použitím metod pro stanovení organické S a určit tak rozložení S v jednotlivých frakcích v téměř celém obsahu (Morche 2008; Lavanya et al. 2019). Tato analýza je typická tím, že je jeden stejný vzorek sekvenčně extrahován výše zmíněnými činidly.

Tyto analýzy je možné provádět také za účelem dlouhodobého studia změn v půdě. Lavanya et al. (2019) provedli studii, ve které dlouhodobá aplikace hnoje po dobu 30 let vedla ke zvýšení obsahů vodorozpustné, přístupné, okludované, organicky vázané a celkové síry oproti variantám bez aplikace hnoje. Podobně Balík et al. (2009) popisují dlouhodobé změny frakcí S v půdě na široké škále půd ČR, které jsou také hnojeny hnojem v letech 1980 a 2007 s použitím metody dle Morche (2008). Autoři popisují opačný trend, kdy dochází ke snížení obsahů celkové síry. Dále dochází ke snížení vodorozpustné síry až na 35 % (4 – 9 mg $S.kg^{-1}$) z původní zásoby. Obdobně také obsah adsorbované S se snížil na 61 % z původní zásoby. Obdobný trend ve svých pracích na Evropských půdách popisují také Morche (2008), Lehmann (2008), nebo Suran (2021). Jedním z důvodů těchto rozdílů může být fakt, že studie Lavanya et al. (2019) (provedena v letech 1986 a 2016) byla na indických půdách, které jsou stále silně zatíženy v některých regionech depozicí S z atmosféry (Feinberg et al. 2021), zatímco půdy v ČR vykazují dlouhodobě klesající příjmy z depozice (CHMI, 2019). Dále může mít vliv rozdíl průměrné dávky aplikovaného hnoje (Lavanya et al. 2019 – 15 $t.ha^{-1}$- o 50 % více, než Balík et al. 2008), samotná pěstovaná rostlina, nebo další faktory.

Tyto faktory popisují například výsledky studie Chen et al. (1997), kdy autoři zmiňují, že adsorbované množství je ovlivněno množstvím Ca v půdě. Dalším možným jevem je dle Curtin et Syers (1990) zjištění, že v půdách s nízkým pH dochází ke zvýšené adsorpci síranů, což odpovídá i zjištěním Chen et al. (1997) a McLaren et Cameron (1996), podle kterých při snižujícím se pH roste kladný náboj povrchů půdních částic a to vede k vazbě záporně nabitého síranu. V půdách bohatých na uhličitany zjistili Chen et al. (1997) výborné korelace ($r^2 = 0,986$) mezi obsahem uhličitanů a sírou extrahovanou HCl (okludovanou S) a uvádějí, že v půdách bohatých na vápník tvoří okludovaná síra až 39 % celkového obsahu síry na úkor snížení obsahu adsorbované síry. Dalším úskalím této extrakce je schopnost HCl rozpustit také významný podíl organicky vázané S na půdách s nízkým obsahem vápníku. Je tedy nutné při měření obsahu této frakce zvolit metodu, která je schopná rozlišit původ uvolněného síranového aniontu. Proto se jako koncovka této extrakce doporučuje měření užitím iontové chromatografie, nebo kapilární elektroforézy, které jsou oproti ICP-OES schopné rozlišit původ S (Kowalenko et Grimmett, 2007).

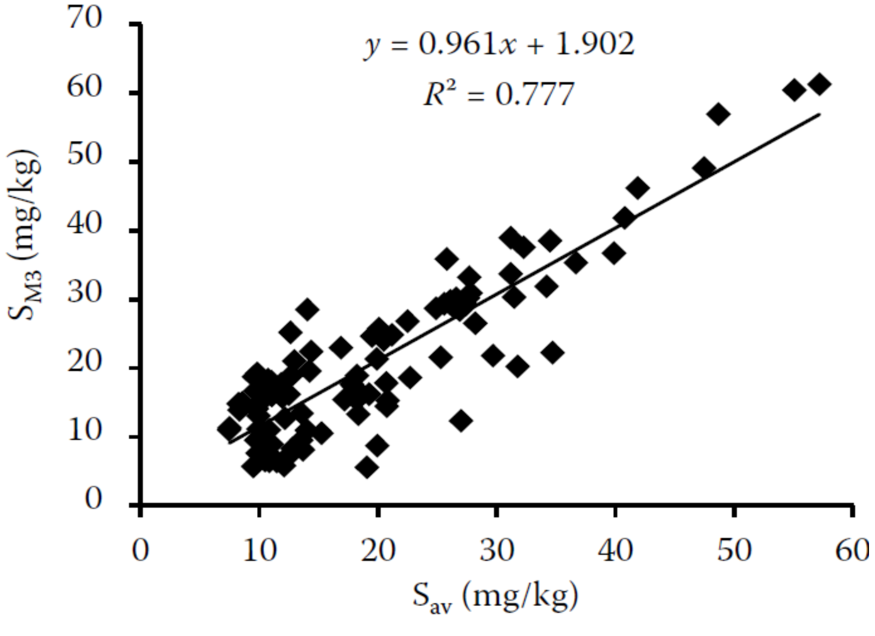
1.5.1.3 Mehlich 3

Jedná se o třetí iteraci extrakčního činidla původně vyvinutého s důrazem na extrakci P, Ca, Mg, K, Na ve své první verzi (Mehlich 1953). Složení tohoto extrakčního činidla je CH_3COOH 0,2 M; NH_4F 0,015 M; HNO_3 0,013 M; NH_4NO_3 0,25 M; EDTA 0,001 M. Při analýze se roztok mísí s vysušenou jemnozemí < 2 mm v poměru 1:10 w/v. V posledních desetiletích tato metoda výrazně nabyla na významu. Podle Ostatek-Boczynski (2012) má metoda Mehlich 3 (Mehlich 1984) jednu velkou výhodu oproti jiným extraktantům - je univerzálně využitelný pro stanovení řady živin, jako fosfor (Vona et al. 2022; McNally 2022; Beure et al. 2022, Tseng et al. 2022), draslík (Tseng et al. 2022; Xu et al. 2022; Mattila et al. 2022), nebo Mg; Ca; S; Mn; Zn; Cu (Zbíral et al. 2016; Zbíral et al. 2018).

Ve světě se objevují studie zaměřující se na schopnost činidla Mehlich 3 extrahovat rostlinám přístupnou síru (Rao et Sharma 1997; Matula 1999; Ketterings et al. 2011; Kowalenko et al. 2014; Kulhánek et al. 2018; zbíral et al. 2018) ovšem v mnohem menší míře.

Jako jedna z prvních studií zaměřujících se na Mehlich 3 a rostlinám přístupnou síru je publikace Rao et Sharma (1997). V této práci autoři popisují významnou korelaci (r^2 = 0,552) mezi obsahem S v roztoku Mehlich 3 a sírou naměřenou v rostlinách čajovníku. Obdobný výsledek se od té doby v literatuře těžko nalézá. Naproti tomu ovšem Matula (1999) uvádí, že toto činidlo není vhodné pro měření rostlinám přístupné síry, díky nízkému korelačnímu koeficientu mezi obsahem S v rostlině a obsahem S v roztoku Mehlich 3 (r^2 = 0,224) a faktu, že tato metoda extrahovala až 31 % z celkového obsahu S v půdě. Tento závěr je odůvodněn tím, že Mehlich 3 musí stanovit i jiné frakce síry (než jen přístupnou), vzhledem k tomu, že organicky vázaná S v půdě tvoří 95 % z celkové zásoby. Je pravda, že organicky vázaná síra tvoří většinu celkové zásoby, ale obsah může být variabilní, například studie Hu et al. (2005) uvádějí 77 %. Ovšem Sedlář et al. (2021) zmiňují významný vztah obsahu S v rostlinách pšenice ozimé a v roztoku Mehlich 3. Konkrétně na půdách, kde je pH > 7,0 byl regresní koeficient r = 0,526 ve fázi sloupkování a 0,408 ve fázi květu. Na půdách s pH < 7,0 byl r = 0,623 a 0,420 ve zmíněných fenofázích. Z těchto výsledků je vidět, že se v průběhu vegetace vztah mění.

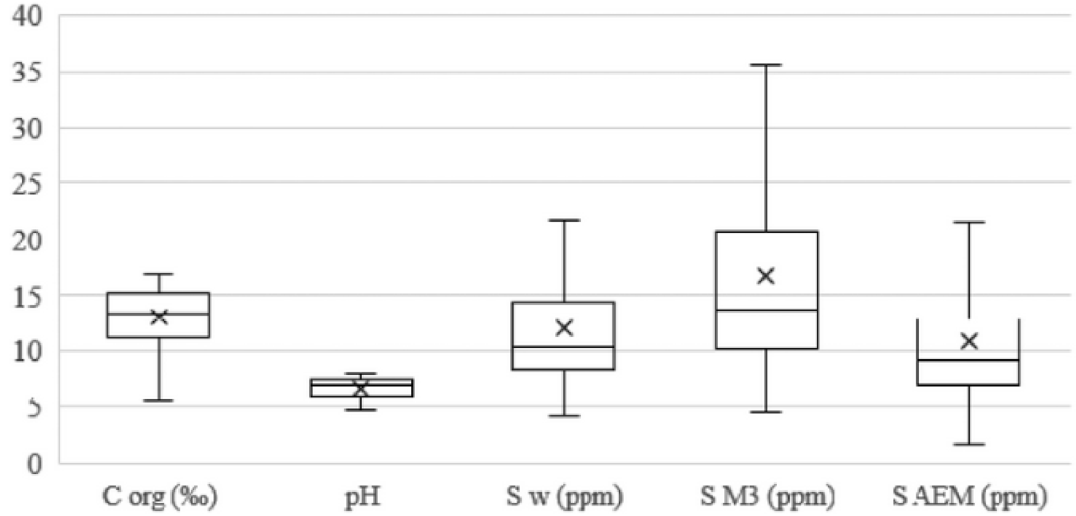
Ve studii Katterings et al. (2011) byla extrakční schopnost Mehlich 3 srovnávána mimo jiné se schopností slabého roztoku $CaCl_2$. Autoři popsali zvýšený obsah S v roztoku Mehlich 3 oproti $CaCl_2$ a domnívá se, že je to část adsorbované frakce, kterou roztok slabé soli není schopný uvolnit. Kowalenko et al. (2014) uvádějí, že vliv na extrakční schopnost roztoku Mehlich 3 má také pH půdy. Na kyselejších vzorcích (pH < 6) popsali vyšší obsah S v roztoku, oproti půdám s vyšší hodnotou pH (> 6).Vzhledem k tomu, že adsorpce aniontů roste s klesajícím pH (Scherer 2009) se Kowalenko et al. (2014) domnívají, že Mehlich 3 uvolňuje z půdy i adsorbovanou frakci. To je ve shodě s výsledky Kulhánek et al. (2018), kde autoři uvádějí korelační koeficient pro obsah S v Mehlichu 3 a rostlinám přístupné S měřené sekvenční extrakcí dle Morche (2008) ($r^2 = 0,882$; $p < 0,001$). Zároveň autoři v této studii poukazuje na to, že obsah těchto dvou frakcí se pohyboval kolem hodnoty 18 mg $S.kg^{-1}$ bez statisticky významného rozdílu. Dále autoři uvádějí slabý korelační koeficient pro organicky vázanou S ($r^2 = 0,349$; $p < 0,01$). Kulhánek et al. (2018) tedy poukazuje na to, že Mehlich 3 je schopen určit rostlinám přístupný podíl S. Zbíral et al. (2018) popisují, že toto extrakční činidlo je možné použít také ke sledování dlouhodobých změn stavu S v půdě. Ve své studii autor zjistil snížení obsahu S extrahovatelné Mehlichem 3 na široké škále zemědělských půd v ČR z průměrných 26 mg $S.kg^{-1}$ v roce 1995 na 17 mg $S.kg^{-1}$ v roce 2013. Tento výsledek odpovídá zjištění Balík et al. (2009), kteří použili standardní metody. Vzhledem k těmto faktům je toto činidlo zajímavou alternativou pro standardní metody, založené na extrakci vodou a/nebo solí fosforečnanů a chloridů.



Obrázek 2: Regrese vztahu mezi rostlinám přístupnou sírou (S_{av}) a Mehlich 3 extrahovatelnou (S_{M3}). Převzato z Kulhánek et al. (2018)

1.5.1.4 Aniontově výměnné membrány (AEM)

Další možnou metodou je použití aniontově výměnných membrán. Dle Turrión et al. (1999) je klíčovým prvkem v použití aniontově výměnných membrán (AEM) jejich selektivita pro určité ionty, v tomto případě anionty. Při stanovení P se při třepání fosforečnany desorbují z půdních částic a sorbují se na membrány, ovšem bez membrány by došlo ke zpětné adsorpci na půdní částice a toto použití je již běžné rozšířeno (Goloran et al. 2014; Bertrand et al. 2003). Obdobně jako P je v půdách sorbován i síranový anion, jak uvádějí Chen et al. (1997). Podle Qian et Schoenau (1995) jsou AEM také například používány k měření obsahů přístupného nitrátového dusíku. Turrión et al. (1999) ve své studii zjistili, že obsahy nitrátu naměřené pomocí AEM ve vodném výluhu významně korelovaly s běžně používanými metodami. Qian et Schoenau (1995) vložili membránu na 2 týdny do půdy a při srovnání obsahů NO_3^- extrahovaných pomocí AEM a celkovým obsahem N v rostlině získali průkazné korelační koeficienty. Rostliny musí mít pro správný vývoj i poměr obsahu dusíku a síry v rozmezí určitých hodnot (11,5 – 17,0:1 v závislosti na dané plodině) (Randall et al., 1981, Ercoli et al., 2012). Na základě tohoto faktu Qian et Schoenau (2007) provedli studii, kde se zaměřili na porovnání poměru poměru N:S stanoveného AEM v půdě a poměrem N:S v rostlině. Zjistili, že tyto poměry jsou si velmi blízké a uvádějí, že je možné rychlotest AEM (inkubace 1 h) aplikovat přímo v půdě i pro stanovení správného poměru těchto živin pro následnou výživu. Dle Uchoa et al. (2003) je možné síru pomocí AEM extrahovat stejně spolehlivě jako N a P. Autoři zjistili, že jsou po 48 hodinách třepání vodného výluhu AEM z půd schopni extrahovat okamžitě dostupnou síru, a to na jednom pruhu membrány (20 x 50 mm) po dobu inkubace 48 h. Schoenau et al. (1993) AEM použili ke stanovení přístupné síry aplikací přímo do půdy. Výsledky pak srovnávali s běžnou extrakcí 0,01 mol/l $CaCl_2$ a dospěli k závěrům, že jsou patrné těsné vzájemné korelace mezi oběma použitými metodami. Uvádějí 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. Suran (2018 nepublikováno) provedl podobné stanovení, ale laboratorní formou, a objevil těsné korelace ($r^2 = 0,810$; $p < 0,001$) mezi obsahem S naměřených touto metodou a rostlinám dostupnou sírou. Sedlář et al. (2021) použili AEM pro stanovení rostlinám přístupné síry v polním pokusu. Korelace mezi obsahem S v rostlinách pšenice a extrahované pomocí AEM autoři uvádějí regresní koeficient $^2 = 0,059$ ve fázi sloupkování a 0,110 ve fázi květu na půdách s pH > 7,0 a r = 0,133 ve fázi sloupkování a 0,428 ve fázi květu na půdách s pH < 7,0. Kromě posledního zmíněného koeficientu nejsou žádné z těchto korelací statisticky významné. Je zde evidentní vývoj vztahu po dobu vegetace rostliny a také vliv pH, což nahrává myšlence, že AEM jsou schopné extrahovat sorbovanou síru. Na druhou stranu na základě grafu 3, je vidět, že obsah S uvolněný z půdy se blíží spíše obsahu extrahovatelného vodou. Tyto nesrovnalosti nechávají dostatek místa pro další výzkum.



Obrázek 3: Obsah síry v půdě uvolněný různými metodami. C_{org} – půdní organický uhlík; S_w – vodorozpustná S; S_{M3} – síra uvolněná Mehlich 3; S_{AEM} – síra uvolněná AEM. Převzato z Sedlář et al. (2021).

1.5.1.5 Stanovení organické síry

Již bylo zmíněno, že organicky vázaná síra je v půdě důležitým činitelem, jak pro mikroorganismy, tak pro rostliny. Ovšem při analýzách půd je na tuto frakci kladen menší důraz. Běžné rozšířené metody se zaměřují na redukci estersulfátové frakce S v půdě. Jednu z prvních metod použitých pro tato stanovení popisují Johnson et Nishita (1952). Cílem této analýzy bylo redukovat organicky vázanou síru pomocí redukčního činidla obsahující červený fosfor, kyseliny iodovodíkovou a kyselinu mravenčí při teplotě 115 °C. Do této analýzy také vstupovaly další činidla, jako směsice octanu zinečnatého a sodného za účelem absorbce vzniklých sulfidů, případně plynný N_2, jako hnací plyn a další. Moderní úpravy této metody stále využívají stejný aparát. Nicméně dochází k úpravě a zjednodušení z hlediska přípravy jednotlivých reagencií. Shan et Chen (1995) používají místo červeného fosforu kyselinou fosfornou. Redukční činidlo vytváří smícháním 300 ml HI, 75 ml H_3PO_2 (50 %), a 150 ml 88 % kyseliny mravenčí. K zachycení H_2S, který je hnán pomocí plynného N_2 používají 10 ml 0,05 M NaOH. Následně je sulfan oxidován pomocí H_2O_2 do podoby síranu. Při použití této metody nedochází k uvolnění síry z cysteinu, methioninu (tedy frakce C-S), ale za to dochází k redukci síry z montmorillonitu a illitu. Zároveň je tato metoda ale schopná plně redukovat estericky vázanou síru (C-O-S). Morche (2008) tuto metodu upravil tak, že nejprve vzorek podrobí frakcionaci minerální S a tím odstraní minerální podíl S ze vzorku a až poté tento vzorek podrobí redukci HI, čímž se ve výsledném roztoku objeví pouze C-O-S frakce. Po odečtení této frakce a minerálně extrahované síry od celkového obsahu S je možné dopočítat obsah síry přímo vázané na C.

Pirela et Tabatabai (1988) se pokusili využít aparát Johnsona et Nishity pro stanovení pouze síry vázané přímo na uhlík. Jejich reakční činidlo byla směsice cínu a dehydratované kyseliny fosforečné a předpokládali redukci síry z C-S do podoby anorganického sulfidu. Dehydratovanou H_3PO_4 připravili zahřátím 250 ml této látky na 360 °C. Později je tato kyselina smíchána s $SnCl_2.2H_2O$ za vzniku reakčního činidla ($Sn\text{-}H_3PO_4$). Tato metoda bohužel nebyla specifická a redukovala širokou škálu látek mimo své původní zaměření. Scherer (2009) zmiňuje, že právě nespecifičnost podobných metod je důvodem postupu, že se běžně využívá dopočet C-S frakce po extrakci ostatních frakcí, jak je zmíněno již výše.

1.5.1.6 Nedestruktivní metody

Běžné stanovení C-S organické síry dle Johnson et Nishita (1952) trpí podle Prietzel et al. (2001) nepřesností. Ta je dána způsobem výpočtu tohoto čísla (od celkové síry se odečítá C-O-S a minerální S). Podle autora dochází k podhodnocení C-N frakce. Proto zaměřili svůj výzkum na testování metody XANES (X-ray Absorption Near Edge Spectroscopy, tedy využití rentgenové absorpce v blízkosti absorpční hrany) – nedestruktivní stanovení na suché cestě. Jedná se o metodu, kdy jsou částice půdy ozařovány rentgenovým zářením a následně je pozorována vlnová délka a intenzita záření, které tyto částice poté vyzáří a podle kterých se určí specie prvku a její množství.

Pomocí této metody Xu et al. (2016) zjišťují, že při porovnání výsledků této metody s metodou Johnson a Nishita (1952) je v extraktu metodou na mokré cestě větší množství S, než u XANES, což vysvětlují hydrolýzou určité části organických forem síry do podoby síranů v průběhu analýzy na mokré cestě.

Zatímco tradiční extrakční postupy umožňují stanovení pouze přibližného 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. Nicméně podle Prietzel et al. (2003) tato metoda není schopná rozlišit mezi htestericky vázaným síranem a anorganickým SO_4^{2-}, díky stejným oxidačním stavům. Tito autoři dále zjistili, že XANES není schopna rozlišit specie síry u některých organických sloučenin (thioly, sulfidy a polysulfidy, thiofeny), díky blízkosti oxidačních stavů S (0 až +1). Nevýhodou při těchto stanoveních je také podle Prietzel et al. (2007) nutnost využití synchrotronu, což prakticky znemožňuje širší využití této metody při rutinním stanovení S.

1.6 Příjem síry rostlinami

1.6.1 Příjem síry z půdy

Podle Eriksen (1997) je úloha síry v rostlinách klíčová při syntéze aminokyselin cysteinu

a metioninu, koenzymu A, biotinu, thiaminu, glutationu a chlorofylu. Zároveň se podílí na syntéze glukosinolátů a fixaci N leguminosami. Rostliny přijímají S ve formě SO_4^{2-} pomocí kořenů. Rostliny jsou schopné při deficitu S v půdě využít také atmosferický oxid siřičitý, ale nejdůležitějším zdrojem pro kvalitní výživu rostlin bude u tohoto prvku vždy půdní zásoba (Marschner 2012).

Podle Hawkesford et De Kok (2006) rostliny regulují odběr síry:

- Tvorbou zásob, když je S nedostatek;
- Redistribucí S ze zásob, nebo starších vegetačních orgánů společně s N, vzhledem k tomu, že S a N jsou součástí bílkovin, nebo vlivem nedostatku S v půdě, kdy je rostlina donucená využít S ve svém těle, například v době zakládání klasů;
- Omezením příjmu S při nadbytku prvku, kvůli energetickým požadavkům při metabolismu tohoto prvku, ale může se stát, že obsah síranů v prostředí je tak velký, že se rovnováha vnitřního prostředí rozruší a dojde k příjmu S.

Různé membránové přenašeče pomáhají rostlině s odběrem síry z půdy a její distribuci v buňkách organismu. Mechanismus symportu zahrnuje vstup H+ společně s SO_4^{2-}.

Samotný příjem tohoto aniontu rostlinou z půdy a přenos přes membrány je katalyzován symportéry protonů (Hawkesford et al. 1993). Symportéry jsou podle Kotyk (2003) zprostředkovateli sekundárního aktivního transportu živin, využívající gradient H^+, Na^+ a výjimečně K^+ rostlinných buněk bez využívání energie chemických vazeb. Sírany využívají symport typu H^+/SO_4^{2-} (Leusteck et Saito, 1999), kdy společně s vodíkovými ionty dochází zároveň ke vstupu síranového iontu. Tento proces probíhá díky gradientu protonů, který vyvolává adenosin trifosfatáza. K přenosu síranu přes tonoplast dochází díky elektrickému gradientu mezi cytoplasmou buňky a vakuolou. (Li et al. 2020b; Agrawal, 2003).

Výše zmíněný proces popisuje přenos síranů na velmi krátkých vzdálenostech, jako je například zmiňovaných přechod přes tonoplast do vakuoly v buňce. Samotný síranový aniont je potřeba přepravit i na relativně větší vzdálenosti, jako příklad se dá použít transport od kořene až po list rostliny. Za účelem přenosu síranů na krátké, ale i dlouhé vzdálenosti si rostliny vyvinuly „síranový transportní systém“ (sulfate transporters – SULTR). Tento systém je možné rozdělit na pět skupin dle jejich fyziologických a biochemických funkcí (Narayan et al. 2022). Tento transportní systém je popsán relativně komplexně u rostlin rodu *Arabidopsis*:

- Transportéry první skupiny SULTR1.1 a SULTR1.2 se vyskytují převážně v kořenové epidermis, primární kůře a tenkém kořenovém vlášení. Přičítá se jim odběr síry z půdy (Yoshimoto et al. 2002). Další zástupce z této skupiny (SULTR1.3) se nachází ve floému a je zodpovědný za přenos S mezi zdrojem a místem užitku. Místo původu tohoto přenašeče je v kořeni (Yoshimoto et al. 2003).
- Přenašeči z druhé skupiny (zejména SULTR2.1) se vyskytují ve floému a xylému a jsou zodpovědní za přenos síranů mezi pletivy. Zprostředkovávají například přenos S z kořene do výhonku. Dále umožňují přenos síranů do parenchymu xylému a podílejí se na zvýšení koncentrace S v buněčné stěně. (Maruyama-Nakashita, et al. 2015). Zástupci této skupiny se také účastní přenosu S do semen (Awazuhara et el. 2005).
- K funkci třetí skupiny zatím není mnoho informací. Nicméně výsledky Kataoka et al. (2004) poukazují na fakt, že zástupci této skupiny (SULTR3.5) asistují druhé skupině při přenosu síranů z kořene do výhonku.
- Čtvrtá skupina přenašečů (SULTR4.1 a SULTR4.2) se nachází v membráně vakuoly a je zodpovědná za uvolnění síranů a pokrytí požadavků na tento iont v rostlině (Kataoka et al. 2004).
- Pátá skupina se významně liší od těch předešlých. V této skupině byl transportér SULTR5.2 identifikován, jako přenašeč molybdenu v rostlině (Tomatsu et al. 2007).

Přesný proces samotného přenosu síranů do místa asimilace (v plastidech) zatím nebyl popsán. Všechny zmíněné skupiny reagují zvýšením své aktivity v podmínkách, kdy je síra deficitním prvkem pro rostlinu (Narayan et al. 2022).

1.6.2 Příjem síry z atmosféry

Příjem S z atmosféry je podle Vaněk et al. (2012) schopný pokrýt jen cca. 30 % z celkové potřeby S rostlinou. Síra se v atmosféře vyskytuje ve formě oxidu siřičitého, který se při foliárním příjmu dostává do rostliny převážně skrze průduchy (Marschner 2021). V mezofylu těsně pod průduchem dochází k redukci oxidu siřičitého (Aghajanzadeh et al. 2016). Touto cestou se v listech může ukládat až 90 % oxidu siřičitého, který je sorbován na povrchu listu (Garsed, 1985). Po vstupu do rostliny je oxid siřičitý distribuován po rostlině a je zabudován do různých sloučenin. Příjem touto cestou se odehrává i v noci, ale díky vysoké rozpustnosti oxidu siřičitého ve vodě převažuje přes den (Marschner, 2012).

Podobně také atmosferický sulfan a karbonylsulfid (COS) je rostlina schopna přijmout. Sulfan je v mezofylu asimilován O-acetylserin (thiol)lyázou (OASTL) pro syntézu cysteinu (Fuentes et al. 2019). Rostliny jsou schopné také přijímat sulfan z atmosféry a to zejména přes den při otevření průduchů (Marschner 2012). Nicméně tuto sloučeninu rostliny spíše vylučují, v rámci detoxifikace po kontaktu s vysokou koncentrací oxidu siřičitého v ovzduší. COS se po příjmu v rostlině metabolizuje na CO_2 a H_2S pomocí karbonanhydrázy (CA) (Stimler et al. 2010).

1.6.3 Deficit síry

Z hlediska zemědělské produkce může výrazný nedostatek síry vést ke sníženým výnosům a kvalitě sklizně (Etienne et al. 2018; Ercoli et al. 2012), zatímco mírný nedostatek může mít vliv pouze na kvalitu, a nikoliv na výnos (Hawkesford et al. 2016). Sklizňová kvalita ovšem není z hlediska výživy S dána pouze obsahem bílkovin ve sklízené části rostliny, ale i její formou. Hnojení sírou vede k významně zvýšené kvalitě lepku u rostlin pšenice ozimé (Ercoli et al. 2012; Lerner et al. 2006; Yu et al. 2021). U řepky ozimé dochází také ke zvýšení obsahu bílkovin (Grant et al. 2012); olejnatosti a obsahu glukosinolátů (Ahmad et al. 2007) v semenu. Zároveň tato rostlina velmi dobře reaguje na hnojení sírou zvýšením výnosů (Grant et al. 2012) vzhledem k tomu, že je na tuto živinu velmi náročná. Obdobně také rostliny kukuřice ukládají více bílkovin v zrnu při aplikaci hnojiva se sírou (Chaudhary et al. 2013).

Z hlediska fyziologie rostliny je nedostatek S ve výživě rostliny vede až ke snížené syntéze enzymu Rubisco, který ovlivňuje asimilační rychlost oxidu uhličitého. To může mít za následek zhoršenou syntézu uhlovodíků, která nakonec vyústí v chlorózy na listech (Hawkesford et al. 2016; Yu et al. 2021). Deficit tohoto prvku může také snížit využití N v rostlině. Dochází ke sníženému příjmu dusičnanů a také ke snížení aktivity nitrátreduktázy (Prosser et al. 2001) a zvýšení obsahu rozpustného dusíku v rostlině v podobě amidů a dusičnanů, což vede ke zvýšení poměru N:S a vytvoření nerovnováhy těchto prvků (Carciochi et al. 2017). Překročení určité hodnoty tohoto poměru je jedním z faktorů, podle kterých se dá deficit S určit. Dobře živený porost pšenice ozimé se pohybuje v hodnotách N:S kolem 15:1 a při překročení 17:1 se již dá zaznamenat snížení výnosu (Flaete et al. 2005). U kukuřice by měl činit tento poměr 11,5:1 (Weil et Mughogho 2000).

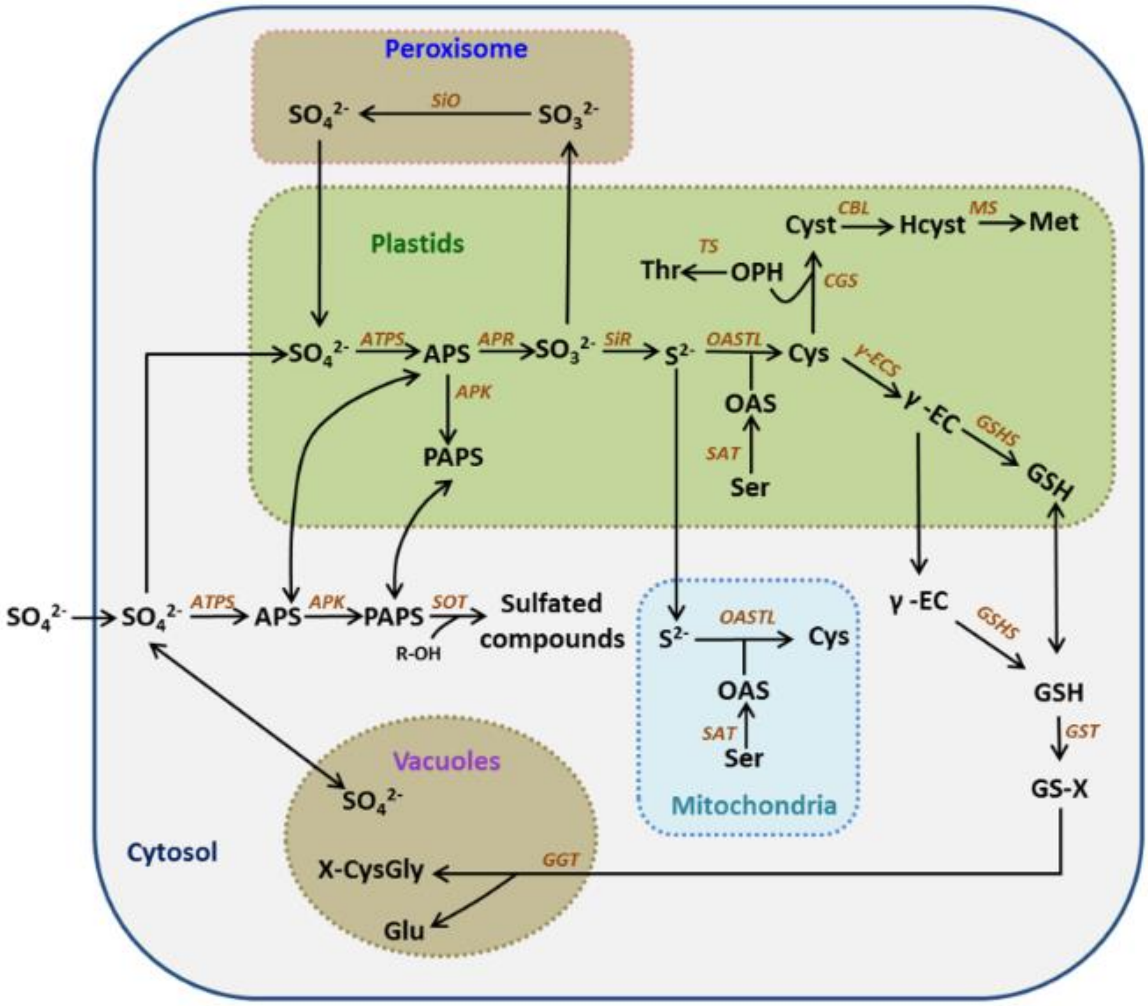
Byla také popsána korelace mezi metabolismem síry a fosforu (Allaham et al. 2020). V rostlinách dochází ke zvýšené tvorbě sulfolipidů při nedostatku P a zvýšené produkci fosfolipidů při nedostatku S (Sugimo et al. 2007). Přenašeče S a P mají podobnou strukturu a regulační mechanismy (Smith et al. 2000). Například se nabízí cytokiny, které regulují příjem a transport S a P (Franco-Zorilla et al. 2005), Při nedostatku P také dochází ke zvýšení aktivity SULTR1.3, který transportuje S z kořene k výhonkům (Hsieh et al. 2009).

1.7 Asimilace síry v rostlině

1.7.1 Cystein

Rostlina není schopná využít síranové ionty okamžitě. Nejprve musí být tento iont aktivován do podoby adenosin 5´-fosfosulfát (APS) a 3´-fosfoadenosin-5´-fosfosulfát (PAPS) (Kaufmann et al. 2019). Adenosin-5-trifosfát sulfuryláza (ATPS) katalyzuje SO_4^{2-} do formy APS, následně je APS fosforylován do podoby PAPS pomocí APS kinázy (APK).

Redukce síranů probíhá výlučně v plastidech ve dvou krocích. V prvním kroku adenosine 5´-fosfosulfát reduktáza (APR) katalyzuje tvorbu siřičitanu z APS. Poté siřičitan reduktáza (SIR) katalyzuje siřičitan do dvoumocného sulfidu (S^{2-}) za účasti ferredoxinu, jako redukčního činidla. Dále je sulfid s asistencí serin acetyltransferázy (SAT) a O-acetylserin (thiol)lyázy (OASTL) integrován do uhlíkového řetězce O-acetylserin (OAS). To vede k tvorbě cysteinu. Samotná syntéza cysteinu již může probíhat nejen v plastidech, ale také v mitochondriích a cytoplasmě buněk a je důležitým krokem, který umožňuje tvorbu dalších metabolitů (Chan et al. 2019).



Obrázek 6: Schéma metabolismu S. Enzymy jsou zvýrazněny oranžově. Zkratky metabolitů: APS - adenosine 5´-fosfosulfát; PAPS - 3´-fosfoadenosine 5´fosfosulfát; R-OH - hydroxylový prekurzor; Ser - serin; OAS - O-acetylserin; Cys - cystein; OPH - O-fosfomonoserin; Thr - threonin; Cyst - cystathionin; Hcyst - homocystein; Met - methionin; γ-EC - γ-glutamylcystein; GSH - glutation; GS-X - glutation konjugovaný; Glu - glutamát; X-cysGly - cysteinylglicin konjugovaný; Zkratky enzymů: ATPS - adenosin-5-trifosfát sulfuryláza; APK - APS kináza; SOT: sulfotransferáza; APR - APS reduktáza; SiO - siřičitan oxidáza; SiR - siřičitan reduktáza; SAT - serin acetyltransferáza; OASTL - O-acetylserin (thiol)lyázy; CGS - cystathionin γ-syntetáza; TS - threonin syntetáza; CBL - cystathion β-lyáza; MS - methionin syntetáza; γ-ECS - γ-glutamylcystein syntetáza; GSHS - glutation syntetáza; GST - glutation-S-transferáza; GGT - γ-glutamyltransferáza. Převzato z: Li et al. (2020b).

1.7.2 Glutation

Glutation (GSH) je hlavní zásobárna a přenašeč organické síry v rostlině. Ke svému vzniku potřebuje cystein. Za účasti γ-glutamylcystein syntetázy (γ-ECS) vzniká γ-glutamylcystein (γ-EC) z cysteinu a glutamátu. Následně γ-EC reaguje s glycinem, nebo alaninem a vzniká GSH za účasti glutation syntetázy (Hendricks et al. 2020; Jez 2019). GSH hraje významnou roli v odolnosti rostliny vůči stresu a je to také prekurzorem pro výrobu fytochelatinů, které hrají významnou roli v odolnosti rostliny vůči vlivu rizikových prvků (Chan et al. 2019; Maruyama et Ohkama. 2017).

1.7.3 Metionin

Tato aminokyselina obsahující S má významnou roli v primárním a sekundárním metabolismu v rostlinách a vzniká z cysteinu. Z metioninu za účasti S-adenosylmetionin synthetázy (SAMS) vzniká S-adenosyl metionin (SAM). SAM je prekurzor pro syntézu etylenu, polyamínů, vitamínů, koenzymů a nicotinaminu (NA). Etylen je důležitým rostliným hormonem. NA je klíčový při absorpci a transportu železa v rostlině (Aarabi et al. 2020; Wawrzynska et al. 2015).

1.7.4 Glukosinoláty a PAPS

Glukosinoláty jsou sekundární metabolity, které se vyskytují převážně v brukvovitých rostlinách (Aarabi et al. 2016; Zhang et al. 2020). Jejich syntéza vyžaduje účast PAPS, cysteinu a sulfotransferázy (SOT). Tyto látky samy o sobě nejsou biologicky aktivní, ale poškození rostliny nastartuje procesy, při kterých se z glukosinolátů vytváří nitrily a thiokyanatany, které již biologicky aktivní jsou. Mezi funkce těchto metabolitů patří například tvorba nepříjemného zápachu, inhibice mikrobiálního růstu, odolnost proti herbivorům a hmyzu (Sugiyama et Hirai 2019; Jeschke et al. 2019). Samotné PAPS je zásobní formou APS a vstupuje do řady procesů, jako substrát (Günal et al. 2019).

2 Literární přehled – půdní organická hmota

Půdní organická hmota (z anglického soil organic matter - SOM) je největší aktivní zásobárnou uhlíku na planetě (Kleber et al. 2021). Její zásoba representuje dlouhodobou rovnováhu mezi vstupy rostlinných zbytků a rozkladem těchto zbytků půdními mikroorganismy. Zemědělství tuto rovnováhu narušuje tím, že zvyšuje rychlost rozkladu organické hmoty (Magdoff et Weil 2004). Část půdní organické hmoty je schopna z rozkladných procesů alespoň na určitou dobu uniknout stabilizací na povrchu půdních částic, které jsou součástí půdních agregátů (Lehmann et al. 2020). Půdní organická hmota je svou funkcí jeden z největších reaktivních povrchů v půdě, který ovlivňuje retenci živin, rizikových prvků a organických polutantů, čímž snižuje jejich mobilitu a potenciál pro vyplavení do povrchových a podzemních vod. SOM tvoří cca. 1 – 6 % hmotnosti zemědělských půd. Méně úrodné půdy, zpravidla písčité, dosahují i pod 1 %. Půdy hlinité dosahují 2 až 3 % a jílovité půdy i přes 5 % SOM (Magdoff et Van Es 2021).

Půdní organická hmota je velký reservoár uhlíku, který je esenciální komponent udržitelnosti půdní úrodnosti a stability ekosystémů (Kwiatkowska-Malina, 2018; Sokol, et al. 2019; Wiesmeier et al. 2019; Xue et An 2018). Obsah půdní organické hmoty je ovlivněn množstvím a původem rostlinných zbytků a organického hnojení (Cotrufo et al. 2019). Například Gregorich et al. (2003), Song et al. (2022) a Wang et al. (2015) uvádějí nárůst SOM při použití různých organických hnojiv (například hnůj a sláma) v porovnání s minerálními hnojivy.

Významným komponentem SOM je půdní organický uhlík (C_{SOM}). Množství uhlíku v půdě je cca. trojnásobné v porovnání s množstvím uhlíku v atmosféře (Stockmann et al. 2013), a zároveň je větší, než součet uhlíku vegetace a atmosféry (Lehmann et Kleber 2015). Tato skutečnost dělá z půdního uhlíku zajímavou cestu pro snížení obsahu CO_2 v atmosféře (Lorenz et Lal 2014). To je jeden z důvodů, které vedly k velkému rozmachu studií zaměřených na obsah a kvalitu SOM a C_{SOM}.

2.1 Půdní organický uhlík

Obsah a kvalita půdního organického uhlíku jsou významnými parametry při určování půdní úrodnosti. Půdní organický uhlík je zejména ovlivněn širokou škálou půdně-klimatických podmínek (Meier et Leuschner 2010, Koven et al. 2017), způsobem využití půdy (Viscara-Rossel et al. 2014; Poeplau et al. 2011), hnojením (Balík et al. 2020), agrotechnikou (Luo et al. 2010, Powlson et al. 2014).

V globálním měřítku teplota a srážky výrazně ovlivňují obsah C_{SOM} (Wiesmeier et al. 2019). Obsah C_{SOM} je vyšší ve vlhčím prostředí (Meier et Leuschner 2010) díky zrychlenému zvětrávání matečních hornin vedoucímu ke zvýšené produkci sekundárních hlinito-křemičitanů, které mohou na svém povrchu vázat C_{SOM} (Doetterl et al. 2015). Orné půdy v teplejších klimatických podmínkách jsou typické snížením C_{SOM} (Koven et al. 2017), díky zvýšené mineralizaci dané vyšší mikrobiální aktivitou (Conant et al. 2011).

Způsob využití půdy je jedním z nejvýznamnějších faktorů ovlivňující obsah C_{SOM} (Viscara-Rossel et al. 2014; Poeplau et al. 2011). Obecně platí, že obsah C_{SOM} se zvyšuje v následujícím pořadí orná půda < lesní půda < trvalé travní porosty (Martin et al. 2011). Obsah C_{SOM} se může snížit o 30 až 80 % pokud dojde ke konverzi využití půdy z lesních nebo trvalých travních porostů na ornou půdu (Poeplau et al. 2011, Wei et al. 2014). Tato ztráta C_{SOM} se dá přisuzovat zvýšené erozi na orné půdě, snížené stabilizaci C_{SOM} kvůli degradaci půdních agregátů a následnému nárůstu mineralizace díky zvýšení teploty a provzdušnění půdy (Hamza et Anderson 2005). Byl pozorován nárůst C_{SOM}, když se orná půda převádí na lesní půdu, nebo trvalé travní porosty (Schulp et Veldkamp 2008). Na orné půdě s monokulturami byl prokázán nižší obsah C_{SOM}, v porovnání s rotací plodin (Jarecki et Lal 2003). Různorodější rotace plodin hrají významnou roli ve zvyšování obsahu C_{SOM} díky zvýšeným vstupům C do kořenů, zvýšené diverzitě mikroorganismů a vyšší stabilitě půdních agregátů (Tiemann et al. 2015), zejména při použití plodin s bohatým a hlubokým kořenovým systémem (Kätterer et al. 2011). Víceleté pícniny podporují kumulaci C_{SOM} (Poeplau et Don 2015).

Dlouhodobé hnojení organickými hnojivy je běžně asociováno se zvýšením obsahu C_{SOM} (Qaswar et al. 2020, Ashraf et al. 2021), zatímco minerální hnojiva poskytují místy protichůdné výsledky (Shah et al. 2020, Waqas et al. 2020), což může být způsobeno různými dávkami a typy použitých hnojiv (Shah et al. 2020). Organická hnojiva zvyšují obsah C_{SOM} dvěma způsoby. Za prvé se jedná o přímý vstup organického C, jehož významnými zdroji jsou klasická organická hnojiva. Dále vstup organických hnojiv vede ke zlepšení fyzikálních vlastností půdy, což vede k nárůstu biomasy a dále ke zvýšeným vstupům organické hmoty skrze posklizňové zbytky (He et al. 2018, Mustafa et al. 2022). Kombinace minerálního NPK hnojení a hnoje vede k významnému nárůstu C_{SOM} (obsah 3,5 %) oproti nehnojené kontrole (1,5 %) a samotnému minerálnímu NPK (1,8 %) (Mustafa et al. 2022). Na méně úrodných půdách je tento efekt více zřetelný. V pokusu Dai et al. (2013) byl pozorován vyšší nárůst obsahu C_{SOM} na variantě hnojené chlévským hnojem (1,1 %) oproti kontrole (0,44 %) a NPK (0,63 %). Obdobné výsledky popisují také například Kuśmierz et al. (2023). Aplikace slámy může vést k významnému nárůstu C_{SOM} (Meng et al. 2024). Kombinace organických a minerálních hnojiv je dalším zajímavým přístupem v péči o obsah C_{SOM}. Například Jaćimović et al. (2023) uvádějí, že při kombinaci aplikace slámy a minerálního N zaznamenali významný nárůst C_{SOM} (1,47 %) v porovnání s nehnojenou kontrolou (1,27 %). Aplikace čistého N bez slámy nevedla v jejich pokusu k významnému nárůstu C_{SOM}. Vliv má také dávka organického hnojiva. Wang et al. (2021) stanovili nárůst C_{SOM} do dávky cca 7 t slámy.ha^{-1}. Při vyšších hodnotách již nedocházelo k nárůstu obsahu C_{SOM}.

Průměrné obsahy C_{SOM} se pohybují v rozmezí 0,7 – 4,0 % (Viscarra-Rossel et al. 2014). Ne všechen uhlík v organické frakci si je ovšem roven a rozdělení C_{SOM} do jednotlivých forem je využíváno pro posouzení schopnosti půd odolávat degradaci (Piccolo 2012, Olk et al. 2019, Savarese et al. 2021). Tyto formy uhlíku je možné považovat za kvalitativní parametry C_{SOM}. Půdní organický uhlík lze rozdělit, pro účely této práce, na:

a) labilní - frakce je reprezentována mineralizovatelným uhlíkem (C_{HWE}), nebo rozpuštěným organickým uhlíkem (C_{DOC}) a jedná se o přechodnou frakci na pomezí čerstvě rozložených rostlinných zbytků a stabilizované organické hmoty. Díky rychlosti jejich obratu jsou dobrým indikátorem změn kvality půdní organické hmoty (Dumale et al. 2009). Půdní mikroorganismy jsou hlavními hráči ovlivňující obsahy labilních forem, a tyto formy se proto používají pro určení mikrobiální aktivity (Kallenbach et al. 2016).

b) stabilní - frakce reprezentována humusem, který se dá rozdělit na huminové kyseliny (HK), fulvokyseliny (FK) a huminy (HU) (Kwiatkowska-Malina 2018). Jejich obsah a potažmo obsah uhlíku v těchto frakcích je velmi důležitým indikátorem kvality půdy a její odolnosti vůči degradaci (Menšík et al. 2018). Za specifickou část této frakce se dá považovat určitá část skupiny glykoproteinů, souhrnným označením „glomalin“, která v půdě může zůstávat téměř pět dekád (Harner et al. 2004).

Je nutné také zmínit, že se část půdní organické hmoty nachází ve frakci primární organické hmoty. Jedná se o původní organickou hmotu, která se dostává do půdy (sacharidy, organické kyseliny, aminokyseliny, bílkoviny, tuky, vosky, celulóza aj.) a nachází se v různém stupni rozkladu. Rychlost rozkladu těchto rostlinných a živočišných zbytků je do jisté míry dána látkovým složením a půdními podmínkami. Obsah primární organické hmoty je cca. 5 – 15 % z celkového obsahu organických látek v půdě (Balík et al. 2019). Zavarzina et al. (2021) uvádějí 2 – 30 %.

Pro určení kvality C_{SOM} se tedy aktuálně používá široká škála metod. Jedním z požadavků pro zemědělské vědy a praxi je vytvoření metody, která je „user-friendly“. Pro takovou metodu jsou požadovány následující vlastnosti: spolehlivé výsledky, jednoduchá procedura, jednoduché vyhodnocení dat, nízká cena, rychlost a nízká spotřeba chemikálií. Z těchto důvodů byla věnována pozornost stanovení obsahu glomalinu (GRSP) jako kvalitativního parametru půdní úrodnosti (Bedini et al. 2013), případně také indexu smáčivosti (Ellerbrock et al. 2005), nebo indexu aromaticity (Cunha et al. 2009).

K určení obsahu C_{SOM} se tradičně přistupuje dvěma způsoby. Určení obsahu C_{SOM} na suché cestě, nebo na mokré cestě (Davis et al. 2018). Na mokré cestě se jedná zpravidla o Walkley-Black metodu (Walkley et Black 1934), v České republice modifikovanou dle Nováka-Pelíška (Jandák et al. 2010). Jedná se o oxidaci C_{SOM} pomocí $K_2Cr_2O_7$ v prostředí H_2SO_4. Obsah oxidovaného uhlíku se stanoví z objemu vyprodukovaného CO_2 nebo z nespotřebovaného objemu oxidačního činidla titrací Mohrovou solí (Bisutti et al. 2004, Davis et al. 2018). Postup na mokré cestě byl původně kritizován díky faktu, že nedokáže oxidovat veškerý C_{SOM} (Tivet et al. 2012). Proto se objevují modifikace, kdy se oxidace uhlíku podpoří zahřátím na teploty kolem 120 °C (Jandák et al. 2010), ale ani takové úpravy nezaručují vždy 100 % oxidaci (Rheinmer et al. 2008). Na suché cestě se dá obsah C_{SOM} určit oxidací půdního organického uhlíku spálením vzorku při teplotách v rozsahu 1000 – 1600 °C (Davis et al. 2018). Tato cesta je problematická díky faktu, že se oxiduje také půdní minerální uhlík a vede k nadhodnocení výsledků. Minerální uhlík se však dá před analýzou odstranit kyselinou chlorovodíkovou (Brodie et al. 2011), případně u půd mající pH < 6 není přítomno významné množství uhličitanů a není potřeba minerální uhlík odstraňovat vůbec (Mikha et Marake 2023) a obsah C_{SOM} je téměř roven obsahu celkového uhlíku (Périé et Ouimet, 2008). Oběma způsoby je možné získat obsah C_{SOM}. Z této hodnoty se dá následně dopočítat obsah půdní organické hmoty dle následující rovnice:

$$\text{SOM}\ (\%) = C_{SOM}\ (\%) * 1{,}724.$$

Hodnota 1,724 je známa pod označením „van Bemmelen faktor“ a po přepočtu bude vyházet SOM cca. dvojnásobně vyšší než C_{SOM}. První zmínky o tomto faktoru lze datovat až do roku 1826 (Sprengel 1826) a vychází z faktu, že v původní práci byl stanoven obsah uhlíku v huminových kyselinách na 58 % a byl předpoklad, že podobně bude uhlík zastoupen ve všech sloučeninách půdní organické hmoty. Tedy, že organická hmota je tvořena z 58 % uhlíkem. Moderní výzkum ovšem uvádí, že v průměru C_{SOM} tvoří kolem 52,6 % z půdní organické hmoty, a tedy faktor 1,9 by byl při přepočtu vhodnější (Pribyl 2010). Je však jasné, že se jedná o průměr ze široké škály půd a v podstatě se jedná pouze o orientační hodnotu.

2.2 Labilní frakce půdního organického uhlíku

Tyto formy se proto používají pro určení mikrobiální aktivity (Kallenbach et al. 2016), neboť reprezentují živý komponent půdní organické hmoty. Tyto frakce labilního uhlíku se považují za prvotní indikátory změn kvality půdního organického uhlíku (Ghani et al. 2003, Kalisz et al. 2017).

2.2.1 Uhlík extrahovatelný horkou vodou (C_{HWE})

Stanovení obsahu lehce mineralizovatelného půdního uhlíku je možné s použitím extrakce horkou vodou (z anglického hot-water extractable carbon - C_{HWE}). Představuje na orných půdách zpravidla 1 – 5 % z organického půdního uhlíku (Jiang et al. 2009), což řádově odpovídá stovkám mg.kg^{-1} půdy (Šimon et al. 2012; Singh et Kumar 2021, Kalisz et al. 2017). C_{HWE} zároveň velmi dobře koreluje s obsahem uhlíku v mikrobiální biomase stanoveným pomocí fumigace půdního vzorku chloroformem (Ghani et al. 2003). Použití extrakce horkou vodou je preferováno, protože je časově méně náročné a bezpečnější (odpadá práce s chloroformem). C_{HWE} obsahuje až 60 % uhlovodíků a amidů pocházejících z biomasy mikroorganismů, enzymů a kořenových exudátů (Ghani et al. 2003). Indikuje tedy především uhlík mikrobiální biomasy. Metodika dle Ghani et al. (2003) je základem pro tyto extrakce.

Je možné použít zjednodušenou modifikaci dle Körschens et al. (2013). Vysušený půdní vzorek (< 2 mm) je zalit demineralizovanou vodou v poměru 1:5 (w/v). Tato suspense se vaří po dobu jedné hodiny. Po vychladnutí se půda oddělí od roztoku většinou odstředěním. Obsah uvolněného uhlíku je možné měřit ve výsledném filtrátu po převedení do formy CO_2 digescí za účasti UV-záření a persíranu, vedoucí k oxidaci uhlíku. Minerální uhlík je odstraněn z roztoku acidifikací kyselinou sírovou. Jako detektor CO_2 se využívá nedisperzního infračerveného (NDIR) analyzátoru díky schopnosti tohoto plynu dobře absorbovat světlo v infračerveném pásmu.

V případě C_{HWE} byl popsán, jak vliv agrotechnických opatření, tak sezónní. Singh et Kumar (2021) zjistili, že obsah C_{HWE} je vyšší při použití krycích plodin o 34 % před setím kukuřice proti variantě se samotným výsevem kukuřice. Toto snížení může být přisouzeno kombinaci rychlých period jarních mrazů a náhlého oteplení společně s nadměrnou vlhkostí (Lipson et al. 1999). Lipson et al. (2000) zmiňují, rychlé že střídání mrazu a oteplení nastartuje mikrobiální aktivitu a vysoká vlhkost v době oteplení vede k tání zmrzlé vody a následnému vyplavení jednoduchých organických sloučenin do nižších horizontů. Singh et al. (2021) dále zmiňují, že vliv krycí plodiny na obsah C_{HWE} byl větší, než jiná agrotechnická opatření (minimalizace zpracování půdy, rotace plodin). Schopnost mikrobiální biomasy reagovat na zásahy do půdy byla demonstrována ve studii Fiedler et al. (2015), kde bylo zjištěno, že se obsah C_{HWE} v průměru zdvojnásobil den po orbě v porovnání s předchozím dnem a další den se prudce snížil pod původní úroveň, což poukazuje na omezenou zásobu labilních frakcí uhlíku v půdě (Uchida et al. 2012). Efekt dlouhodobého hnojení chlévským hnojem, minerálním NPK a jejich kombinace popsal Šimon (2008). Byl pozorován významný nárůst C_{HWE} při kombinaci NPK a hnoje (336 $mg.kg^{-1}$) oproti kontrole (252 $mg.kg^{-1}$). Významný nárůst (303 $mg.kg^{-1}$) byl pozorován také na variantě se samotným hnojem. Obsah C_{HWE} na variantě s minerálním NPK byl srovnatelný s kontrolou. Autor zároveň pozoroval významnou korelaci mezi C_{HWE} a C_{SOM}. Kalisz et al. (2017) pozorovali nárůst C_{HWE} v průměru o 22 – 23 % po aplikaci čistírenského kalu a kompostu v porovnání s kontrolou. Větší nárůst byl ovšem pozorován na variantách s kompostem, což indikuje lepší přístupnost substrátu pro mikroorganismy v tomto hnojivu (Kalisz et al. 2017). Liang et al. (2012) popisují významné korelace mezi C_{SOM} a C_{HWE} (r^2 = 0,950; 0,01). Dále je popsán vliv aplikace chlévského hnoje na významné zvýšení obsahu C_{HWE} na téměř dvojnásobek kontroly (kontrola cca. 220 $mg.kg^{-1}$), zatímco minerální hnojivo bylo srovnatelné s kontrolou.

2.2.2 Rozpuštěný organický uhlík (C_{DOC})

Rozpuštěný organický uhlík (z anglického dissolved organic carbon – C_{DOC}). Jsou malé (< 0,45 μm) rozpustné částice půdního organického uhlíku tvořené mikrobiálními a rostlinnými exudáty a produkty hydrolytických rozkladů organické hmoty, schopné sorbovat se na povrchy jílových minerálů (Leinemann et al. 2018). Zpravidla se hodnoty C_{DOC} pohybují řádově v desítkách $mg.kg^{-1}$ půdy (Bongiorno et al. 2019) a dosahují tak nižších hodnot, než C_{HWE} (Ghani et al. 2003). Obecně vyšší teplota a vlhkost půdy vedou ke zvýšení rychlosti rozkladu půdní organické hmoty a nárůstu obsahu C_{DOC} (Zhou et al. 2015). Velké změny ve využití půdy (konverze lesní půdy na ornou) a zavedení orby zvyšuje mikrobiální aktivitu a také obsah C_{DOC} (van Gaelen et al. 2014). Ovšem jedná se o relativně krátkodobý efekt (Leinweber et al. 2008).

Laboratorní stanovení je možné provést z čerstvého vzorku (< 5 mm) v poměru 1:10 (w/v) roztoku 0,01 mol. L^{-1} $CaCl_2$ (Houba et al. 2000). Směsice vzorku s $CaCl_2$ je dvě hodiny třepána a dochází k uvolnění C_{DOC}. Následné se odstředí extrakt od půdního vzorku. Obsah uvolněného uhlíku je možné měřit ve výsledném supernatantu po převedení do formy CO_2 digescí za účasti UV-záření a persíranu vedoucí k oxidaci uhlíku. Minerální uhlík je odstraněn z roztoku acidifikací kyselinou sírovou. Jako detektor CO_2 se využívá NDIR analyzátoru, díky schopnosti tohoto plynu dobře absorbovat světlo v infračerveném pásmu.

Bongiorno et al. (2019) uvádějí, že vstupy organické hmoty ovlivňují obsahy C_{DOC} (cca. 27,6 mg.kg^{-1} oproti systému bez vstupu organiky 24,2 mg.$kg^{-1;}$ $p < 0,05$), zatímco zpracování půdy (orba versus minimalizace) a interakce těchto faktorů nemá statistický význam. Hodnoty C_{DOC} jsou totiž mnohem více ovlivněné vlastnostmi stanoviště (teplota a vlhkost), než zpracováním půdy (Federici et al. 2017, Mouloubou et al. 2016). Na jaře je obsah C_{DOC} nejnižší z celého roku (Bongiorno et al. 2019), což je pozorováno také ve studii Schiedung et al. (2017). Vliv aplikace zeleného hnojení popsali Li et al. (2024). Aplikace zeleného hnojení v kombinaci s minerálním hnojením výrazně zvýšila obsah C_{DOC} (cca. o 20 %), zatímco samotná aplikace zeleného hnojení neměla výrazný efekt.

Objevují se také studie, ve kterých není pozorován vliv hnojení na obsah C_{DOC} (Margenot et al. 2017, Ladoni et al. 2015). To může být dáno vlastnostmi půdy. Půdní typ může ovlivnit míru vlivu zpracování půdy na kvalitu půdního organického uhlíku. Zároveň může mít v lehčích půdách přídavek organické hmoty vyšší vliv na labilní frakce uhlíku, než při minimalizačním zpracování půdy (Chievenge et al. 2007).

2.3 Humusové látky

Humus je významnou část půdní organické hmoty. Jedná se o široké spektrum molekul v různém stupni rozkladu a syntézy (Baveye et Wander 2019). Nejvýznamnější skupinou látek tvořící humus (z 85 – 90 %) jsou huminové látky (HS), které jsou popisovány jako skupina látek relativně vyšších molekulových hmotností, resistentních mikrobiálnímu rozkladu, o neznámé struktuře molekuly (Zavarzina et al. 2021), vzniklé rozkladem organických látek v půdě (McCarthy et al. 2001). Zbylých 15 – 10 % jsou nehuminové látky, tedy látky jako polysacharidy, aminokyseliny, nízkomolekulární organické sloučeniny a některé cukry (Schnitzer 1972). Huminové látky jsou na základě své rozpustnosti v alkáliích rozdělné do tří kategorií. Huminové kyseliny (HA), které se sráží při pH < 2, fulvokyseliny (FA) rozpustné v celém rozsahu pH a huminy (HU), jako nerozpustné reziduum (Stevenson 1994). Původní tvrzení, že huminy, fulvokyseliny a huminové kyseliny jsou stabilní makromolekuly s vysokou molekulovou hmotností, jsou již značnou dobu konfrontována s novějšími koncepty. Huminové látky je možné popsat spíše jako supramolekulární asociace, než polymery makromolekul (Piccolo et al. 2002). Tedy velké kongregace molekul spojené Van der Walsovými silami, vodíkovými můstky, nebo $\pi - \pi$ vazbou. Článek v periodiku *Nature* dokonce odmítá existenci huminových látek, jako samostatné kategorie přirozených sloučenin (Lehmann et Kleber 2015). Autoři příspěvku navrhují model půdního kontinua (z anglického „soil continuum model“ - SCM). Argumentují tím, že půdní organická hmota je kontinuum biomolekul v různých stádiích rozkladu vedoucí ke snížení molární hmotnosti a zvýšení rozpustnosti. Tradiční rozdělení totiž vychází z metodiky extrakce huminových látek (obrázek 7). Další argument (Lehmann et Kleber 2015) je ten, že toto rozdělení nemá analog v živých organismech a při extrakci dochází k tvorbě artefaktů - při extrakci se uvolňují také frakce humusu nepatřící mezi huminové látky. Průměrné prvkové složení huminových látek je v průměru popsáno v tabulce 1.

Tabulka 1. Prvkové složení huminových látek

Prvek/ Frakce	C	O	H	N	S
			%		
FK [a)]	40 – 50	44 – 50	4 – 6	1 – 3	0 - 2
HK [a)]	50 - 60	30 - 35	4 – 6	2 – 6	0 - 2
HU [b)]	48 – 62	29– 45	7 – 14	3 – 6	0 – 1

[a)] převzato z: Sible et al. (2021)

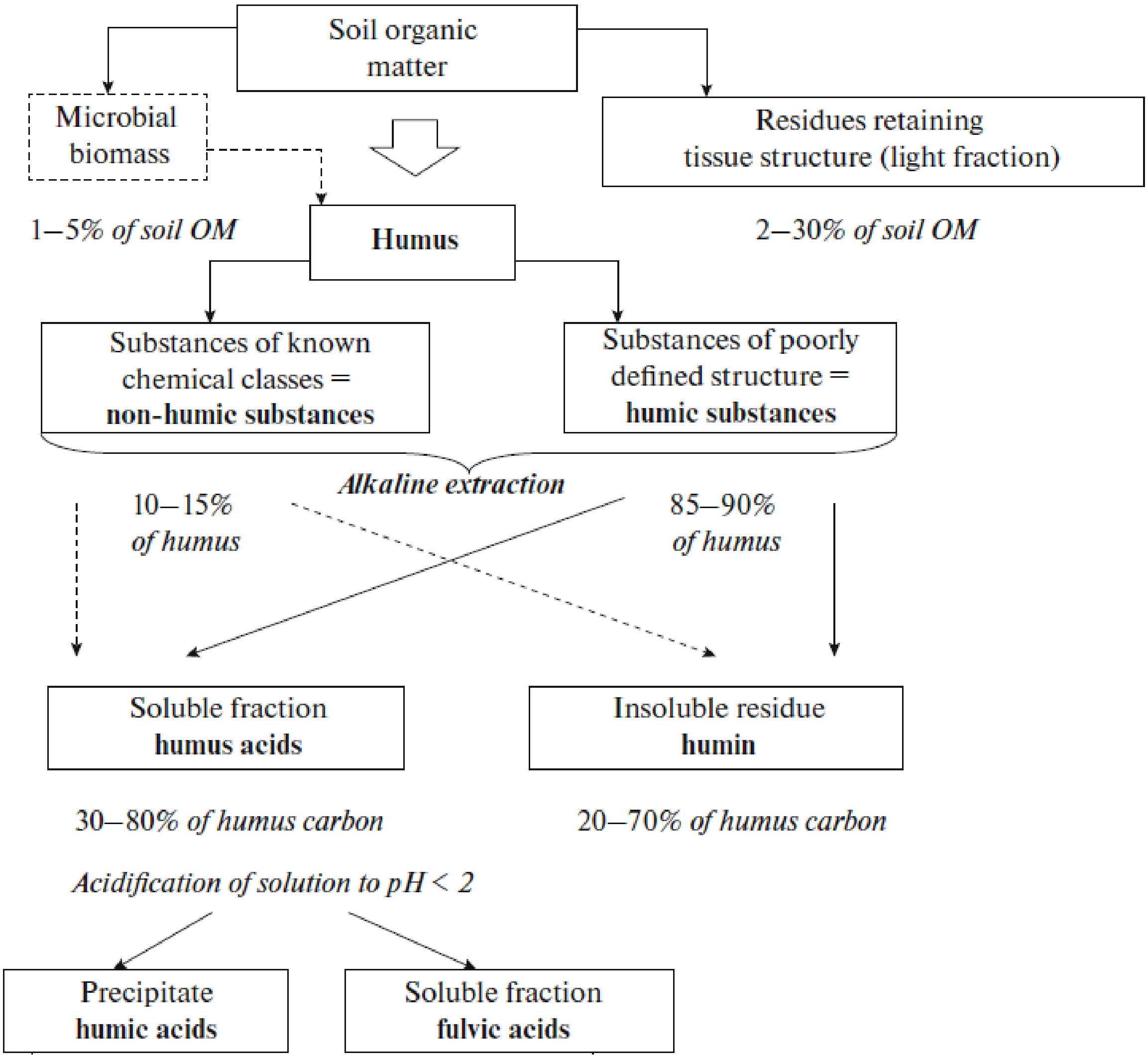
[b)] převzato z: Rice et McCarthy (1991)

I přes tuto kritiku se extrakce huminových látek významně využívá pro posouzení kvality půdního organického uhlíku (Jien et al. 2011, Kukujs et al. 2019, Ampong et al. 2022). Stanovení těchto frakcí uhlíku vychází z úprav původní metodiky dle Kononova (1966). Nejprve je půdní vzorek (5 g) extrahován v poměru 1:20 (w/v) pomocí směsice 0,1 mol.L^{-1} NaOH a 0,1 mol.L^{-1} $Na_4P_2O_7$ po dobu 24 hodin. Tímto dojde k oddělení nerozpustných huminů od fulvokyselin a huminových kyselin, které se převedou do roztoku. Roztok je filtrován. Část filtrátu se odebere a uschová pro stanovení uhlíku v huminových látkách (C_{HS}). Ve zbylém filtrátu se sníží pH na hodnoty 1,0 až 1,5 pomocí koncentrované H_2SO_4 a je zahříván po dobu 30 minut. Po 24 hodinách se oddělí vysrážené huminové kyseliny od fulvokyselin filtrací za účasti 0,05 mol.L^{-1} H_2SO_4 a je získán čistý roztok fulvokyselin. Dále je nutné převést do roztoku vysrážené huminové kyseliny pomocí horkého 0,05 mol.L^{-1} NaOH. Takto získáme roztoky pro stanovení uhlíku v HA (C_{HA}) a FA (C_{FA}). Všechny tři získané roztoky se následně odpaří do sucha. Získané suché vzorky se následně zalijí směsí $K_2Cr_2O_7$ a H_2SO_4 a zahřejí. Dochází k oxidaci uhlíku pomocí $K_2Cr_2O_7$. Zbytek nezreagovaného $K_2Cr_2O_7$ se titruje pomocí KI do bodu ekvivalence.

Svou strukturou jsou huminové kyseliny a fulvokyseliny velice variabilní. Obsah a složení funkčních skupin těchto látek se může lišit dle původu organického materiálu, ze kterého vznikly, jejich stáří, ale také na základě podmínek stanoviště (De Melo et al. 2016). Svou strukturou jsou fulvokyseliny obecně aromatický řetězec uhlíku se třemi až pěti substitenty. Jedná se o hydroxylové funkční a éterické funkční skupiny, alifatické řetězce obsahující ketonické, nebo aldehydické funkční skupiny, navázané na další aromatické řetězce. Dále mohou obsahovat karboxylové funkční skupiny, cukry a aminokyseliny (Schaumann et al. 2006, Tan et al. 2014). Huminové kyseliny mají oproti fulvokyselinám více uhlíku v aromatických sloučeninách (Zhou et al. 2019). Huminové kyseliny také obsahují hydroxylové a karboxylové funkční skupiny, ale zároveň se v jejich struktuře objevuje více chinonů (De Melo et al. 2016). Fulvokyseliny jsou bohatší na C-N vazby, karboxylové a hydroxylové fukněční skupiny, zatímco huminové kyseliny ve své struktuře nesou více $(CH_2)n$, NH_2, a nenasycené C=C vazby (Mu et al. 2024).

Výzkum se zaměřuje převážně na studium FA a HA, neboť mohou zvýšit kvalitu C_{SOM} a půdní úrodnost v relativně kratších časových intervalech (Ampong et al. 2022). Při procesu humifikace dochází k tvorbě huminových látek. Obecně se udává, že fulvokyseliny jsou méně vyzrálé a huminové kyseliny jsou zralejší a stabilnější (McCarthy et al. 2001). Proto při výpočtu poměru obsahu huminových kyselin k fulvokyselinám vyšší hodnoty popisují kvalitnější organickou hmotu, zatímco převaha FA a snížené hodnoty poměru indikují nižší kvalitu a stabilitu humusových látek (Mockeviciene et al. 2021). Huminové kyseliny pozitivně ovlivňují fyzikální vlastnosti půdy a biochemickou aktivitu tím, že zlepšují půdní strukturu a zvyšují retenci vody, mikrobiální aktivitu (Nardi et al. 2017, Fuentes et al. 2018, Shah et al. 2018), a dále také kationtovou výměnnou kapacitu (Yang et al. 2021). Fulvokyseliny zvyšují stabilitu půdních agregátů díky tvorbě komplexních sloučenin s oxidy železa a hliníku (Zhang et al. 2022). Huminové kyseliny a fulvokyseliny obsahují rostlinné živiny a při své mineralizaci je uvolňují (Laskovsky et al. 2020). Jedná se zejména o dusík (Sootahar et al. 2020, Boguta et al. 2017). Dále mohou imobilizovat rizikové prvky a snížit jejich příjem rostlinami (Wu et al. 2017). Některé studie uvádějí, že díky obsahu hormonů, jako auxin a cytokinin v HA, pomáhají svou přítomností k resistenci vůči stresu a stimulaci růstu nadzemní a podzemní biomasy (Canellas et al. 2020, van Tol de Castro et al. 2021, Nardi et al. 2021). Ve studiích zaměřených na přídavky fulvokyselin do ornice byla také popsána schopnost těchto látek ovlivňovat pH půdy. Bylo popsáno jak snížení (Sootahar et al. 2020), tak zvýšení hodnot pH (Cunha et al. 2009). Hlavním faktorem ovlivňujícím chování huminových látek v půdě jsou huminové kyseliny, jejichž složení je ovlivněno typem hnojiva (Kawasaku et al. 2015).

Obsah HA a FA v půdě je ovlivněn primárně půdním typem, ale určitý podíl mají také použitá hnojiva. Obecně platí, že aplikace organických hnojiv zvyšuje obsah HA a FA v půdě. Pozitivní vliv organického hnojení na obsah HA a FA byl popsán v mnoha studiích (Sadej et Żolnovsky 2019, Tang et al. 2018, Lapa et al. 2011). Použití slámy a kombinace minerálního hnojiva (NPK) s chlévským hnojem v průměru zvýšila obsahy C_{HA} na hodnoty 4,02 až 5,34 g.kg^{-1} půdy v porovnání s kontrolou 2,64 g.kg^{-1}. Pro obsah C_{FA} byl nárůst také významný 4,43 až 5,18 g.kg^{-1} v porovnání s kontrolou 3,52 g.kg^{-1}. Obdobné závěry popisují také Sadej et Żolnovsky (2019) ve své studii zaměřené na chlévský hnůj a kejdu v kombinaci s minerálním PK hnojením. Zde byl sledován také vliv vápnění. Bylo zjištěno, že vápněné varianty mají v průměru o 25 % více C_{HA} než C_{FA}. Poměr C_{HA}/C_{FA} byl tedy vyšší (v průměru 1,32 – 1,87 oproti nevápněným variantám 0,75 – 0,97). Na druhou stranu čistě minerální hnojení má pouze slabý vliv na obsah humusových látek v půdě (Banger et al. 2009). Například ve studii Tang et al. (2018) byl obsah C_{HA} a C_{FA} po aplikaci samotného NPK stejný jako u kontroly. Obdobné výsledky je možné najít také u Kawasaki et al. (2008). Naproti tomu Sadej et Żolnovsky (2019) popsali nárůst obsahu C_{FA} při použití samotného NPK (2,20 g.kg^{-1}) oproti kontrole (1,78 g.kg^{-1}) a snížení C_{HA} (1,98 g.kg^{-1}) oproti kontrole (2,29 g.kg^{-1}) na nevápněných variantách a snížení obsahu C_{FA} na vápněné variantě. Nárůst obsahu FA na nevápněných půdách popisují také Mockeviciene et al. (2022).



Obrázek 7: Tradiční schéma rozdělení huminových látek. Převzato z: Zavarzina et al. (2021).

2.4 Glomalin-related soil protein

Velká pozornost byla věnována v posledních desetiletích obsahu glomalinu jako jednoho z mnoha indikátorů kvality půdní organické hmoty (Bedini et al. 2013). Glomalin je produkován na stěnách arbuskulární mykorrhizy (AM). Tato sloučenina byla jako první popsána autory Wright et Upadhyaya (1996). Glomalin je glykoprotein, produkovaný houbou *Glomeromycota*. Přesná molekulární struktura zatím ovšem nebyla popsána (Singh et al. 2016), protože při procesu extrakce glomalinu není možné získat čistý glomalin (Schindler et al. 2007). Proto se ve vědeckých publikacích používá termín „glomalin-related soil protein" (GRSP) (Rillig 2004). V češtině nemá tato skupina půdních glykoproteinů ekvivalent a používá se pouze termín glomalin nebo přesněji GRSP, neboť není možné při extrakci získat čistou molekulu glomalinu. Z těchto důvodů bude dále, v česky psané části práce, používáno rozdělení konsistentní s anglofonní literaturou. Ve své původní práci definují Wright et Upadhyaya (1996) dvě frakce. Jednak lehce extrahovatelný GRSP (EE-GRSP) a celkový GRSP (T-GRSP). Novější literatura ovšem také zmiňuje obtížně extrahovatelný GRSP (DE-GRSP) (Liu et al. 2021; Singh et al., 2016). Obsah DE-GRSP je dopočítán z obsahů T-GRSP a EE-GRSP dle následující rovnice:

$$\text{DE-GRSP} = \text{T-GRSP} - \text{EE-GRSP}$$

Samotná extrakce půdního GRSP splňuje požadavky na uživatelskou přívětivost, ekologičnost, jednoduchost procedury a interpretace výsledků, nízkou cenu a nízkou spotřebu chemikálií (Bedini et al. 2013). Metodika stanovení dle Wright et Upadhyaya (1996) zůstává v podstatě neměnná i po téměř třech dekádách výzkumu. Půdní vzorek je v poměru 1:8 (w/v) extrahován 20 mmol.L^{-1} citronanem sodným (pH = 7,0) po dobu 30 minut při teplotě 121 °C v autoklávu. Extrakt EE-GRSP je následně oddělen od půdního vzorku pomocí centrifugy. Pro stanovení T-GRSP je postup obdobný. Vzorek je také v poměru 1:8 (w/v) extrahován citronanem sodným 50 mmol.l^{-1}, ale při pH 8,0 a po dobu 60 minut (121 °C). Supernatant s extraktem je oddělen od vzorku. Nicméně na rozdíl od EE-GRSP se extrakce na stejném vzorku opakuje, dokud není veškerý T-GRSP uvolněný a supernatanty se postupně slévají dohromady. Postup je u konce v okamžiku, kdy je výsledný extrakt čirý a nabývá světle žluté barvy. Bylo prokázáno, že při opakované extrakci půdního vzorku citronanem sodným je do extraktu uvolněna směsice různých proteinů a dalších molekul, jako například huminové kyseliny (Nichols 2003, Gillespie et al. 2011). Extrakční postup navíc zřejmě neuvolní veškerý T-GRSP přítomný v půdě (Irving et al. 2021). Na rozdíl od T-GRSP, kde dochází k opakované extrakci stejného vzorku, při extrakci EE-GRSP stačí pouze jeden cyklus k uvolnění cílové frakce. Měření koncentrace GRSP se provádí z hodnot absorbance při 595 nm po obarvení extraktu Bradfordovým činidlem. Toto činidlo, ovšem také reaguje s huminovými kyselinami, sacharidy a tuky (Banik et al. 2009). Tento fakt vede k nadhodnocení výsledných obsahů (Jorge-Araújo et al. 2014).

Při výčtu vlastností GRSP literatura zpravidla uvádí, že se jedná o hydrofobní a teplotně stabilní směs glykoproteinů, která je významně odolná vůči rozkladu v půdním prostředí (Rillig 2004, Gadkar et Rillig 2006). Obsah obou frakcí je přímo úměrný zvýšené kolonizaci AM (Holátko et al. 2021, Agnihotri et al. 2018, Agrnihotri et al. 2021). EE-GRSP je považován za relativně mladší produkt aktivity AM, který je labilnější. DE-GRSP je považován za starší, vyzrálejší a méně aktivní frakci, vzniklou přeměnou EE-GRSP (Koide et Peoples 2013, Wu et al. 2014). Produkce GRSP sezónně kolísá zdánlivě podle aktivity AM s vrcholem v letních měsících (Emran et al. 2012). Významná část GRSP v půdě ovšem zůstává i přes 40 let, čímž se podílí na sekvestraci uhlíku (Rillig et al. 2001).

V zemědělsky využívaných půdách se obsahy EE-GRSP pohybují řádově ve stovkách mg.kg^{-1} půdy (Dai et al., 2013, Turgay et al. 2015, Sandeep et al. 2016), ovšem na velmi úrodných půdách, jako jsou mollisoly, mohou dosahovat také přes 3000 mg.kg^{-1} (Curaqueo et al. 2011). T-GRSP dosahuje výrazně vyšších hodnot, běžně v intervalech od 750 do 2500 mg.kg^{-1} (Dai et al., 2013, Turgay et al. 2015, Sandeep et al. 2016). Na orných půdách ČR se obsahy T-GRSP pohybují v průměru v rozmezí 1 900 až 4 200 mg.kg^{-1} půdy (Zbíral et al. 2017). Na velmi úrodných půdách dochází k nárůstu hodnot až k 9000 mg.kg^{-1} půdy (Curaqueo et al. 2011). Jedná se o široké rozmezí, nicméně hodnoty frakcí GRSP odpovídají několikanásobně vyšším hodnotám C_{SOM} (21 050 mg C.kg^{-1}) půdy (Curaqueo et al. 2011), než například ve studii Dai et al. (2013) (6 471 mg C.kg^{-1}). Obsah T-GRSP v půdě významně koreluje s obsahem půdní organické hmoty (Li et al. 2020c). Všechny tři frakce GRSP také korelují s obsahem C_{SOM} (Singh et al. 2016). V půdách ČR byly obdobně popsány významné korelace mezi obsahem C_{SOM} a T-GRSP. Zajímavé je, že ve studii Li et al. (2020c) nebyl popsán významný vztah mezi EE-GRSP a C_{SOM}. To je pravděpodobně dáno tím, že EE-GRSP je více ovlivněný aktivitou AM (Koide et Peoples 2013, Wu et al. 2014). Díky jeho pozitivní korelaci s obsahem půdního organického uhlíku je GRSP považován za indikátor změn půdní úrodnosti (Wright et Upadhyaya 1998, Xie et al. 2015, Dai et al. 2013, Wright et Upadhyaya 1996). Byly také popsány silné pozitivní korelace mezi obsahem GRSP a dalšími indikátory půdní úrodnosti (například obsah půdního N a P) (Singh et al. 2016, Singh et al. 2020), rostlinám přístupným P, celkovým N a K (Šarapatka et al. 2019). Na druhou stranu je prokázán také negativní vztah mezi obsahem GRSP a obsahem půdní organické hmoty (Galazka et al. 2017).

Obecně se dá říci, že obsah GRSP je vyšší při použití minerálních a organických hnojiv v porovnání s nehnojenými systémy (Curaqueo et al. 2011, Dai et al. 2013, Turgay et al. 2015, Sandeep et al. 2016). Dlouhodobá aplikace hnoje (Bertagnoli et al. 2020, Zhang et al. 2014, Valarini et al. 2009, Turgay et al. 2015, Dai et al. 2013), kompostu (Zhang et al. 2014, Řezáčová et al. 2021, Valarini et al. 2009, Turgay et al. 2015), čistírenského kalu (Sandeep et al. 2016) a slámy (Nie et al. 2007, Liang et al. 2017) také vedou ke zvýšení obsahu GRSP. Pozitivní vliv kombinace hnojiv na obsah GRSP byl také prokázán při kombinaci minerálního hnojení a slámy (Nie et al. 2007) a při aplikaci podestýlky hospodářských zvířat

(Zhang et al. 2014, Valarini et al. 2009). Nižší obsahy GRSP byly zaznamenány na orné půdě oproti trvalým travním porostům, což je pravděpodobně dáno vyšší mírou provzdušnění orné půdy vedoucí k intenzivnější mineralizaci půdní organické hmoty (Smatanová et Komprsová 2019). Zároveň byl prokázán vliv agrotechnických opatření na obsah GRSP. Na půdách v bezorebném systému obdělávání půdy byl popsán vyšší obsah v porovnání s konvenčními systémy (Curaqueo et al. 2011). Dále byl popsán vliv pěstované plodiny. V dlouhodobých pokusech v Polsku byl pozorován nižší obsah T-GRSP na monokultuře žita v porovnání s rotací brambor a žita (Wojewódzki et Cieścińska 2012).

I přes to, že molekula glomalinu není známa, jsou uváděna prvková složení GRSP, která jsou dle literatury následovná 3–5 % N, 36–59 % C, 4–6 % H, 33–49 % O, 0,03–0,1 % P, 0,8–8,8 % Fe, a další kationty (K, Ca, Si, Cu, a Mg) (Rillig et al., 2001, Lovelock et al., 2004, Nichols 2008, Zhang et al. 2017). Zastoupení N v glomalinu z N_T v půdě je 5 % (Lovelock et al. 2004).

Jedna z klíčových rolí GRSP je účast této skupiny proteinů při tvorbě a stabilizaci půdních agregátů. GRSP účinkuje jako lepivý, hydrofobní/nerozpustný biofilm vázající minerály, jíly, organickou hmotu a mikroorganismy (Irving et al. 2021, Rillig et al. 2002, Xu et al. 2014). GRSP po dlouhou dobu poutá minerální a organické částice půdy do agregátů, jelikož obsahuje až 85 % polysacharidů, které jsou resistentní k mikrobiálnímu rozkladu (Gunina et Kuzyakov 2015). Podle Rillig (2004) a Harner et al. (2004) je doba degradace GRSP v půdě mezi 6 – 42 lety. Tímto dochází k významnému nárůstu stability půdní organické hmoty (Wright et Upadhyaya 1998, Rillig 2004, Driver et al. 2005, Emran et al. 2012, Galazka et al. 2017). Stabilnější půdní agregáty zpomalují rozklad půdní organické hmoty (Jastrow 1996, Six et al. 2000). Vyšší obsah GRSP byl pozorován u větších půdních agregátů (2,0 – 5,6 mm) v porovnání s obsahem u menších částic (0,25 – 2,00 mm) (Gispert et al. 2013). Zvýšení obsahu GRSP také vede ke zvýšení retence vody, zpomalení eroze, dále podporuje růst kořenů, ovlivňuje aktivitu půdních enzymů a stimuluje růst rostlin (Wang et al. 2015). Snížené obsahy GRSP se dají nalézt na stanovištích s narušenou půdní strukturou (například orbou, nebo suchem) (Wright et Anderson 2000). Obsah GRSP se mění také s hloubkou půdního profilu.

2.5 Index smáčivost a index aromaticity

Infračervená spektroskopie v rozsahu ve střední infračervené oblasti (1,5 – 50 μm, vlnočet 4000 – 200 cm^{-1}) se dá využít ke studiu struktury půdní organické hmoty bez extrakce z půdy. I přes své limity dané komplexností půdního prostředí je tato metoda schopná detekovat některé funkční skupiny půdní organické hmoty a popsat jejich strukturu napříč různými půdními typy (Pavlů et al. 2023).

Metoda infračervené spektroskopie s Fourierovou transformací (z anglického diffuse reflectance infrared Fourier transform spectrometry – DRIFTS) je tedy jedna z možných metod pro určení kvality půdní organické hmoty. Obyčejně se používá ke studiu hydrofobních a hydrofilních funkčních skupin půdní organické hmoty při vlnových délkách 3000 – 2800 cm^{-1} a 1740 cm^{-1}, 1640 – 1600 cm^{-1} (Dai et al. 2013, Sekgota 2018, Ellerbrock et al. 2005). Půdní organická hmota je také charakterizována poměrem C-H (A) a C=O (B) vazeb, označovaných jako index smáčivost (z anglického pottential wettability index – PWI), měřitelný právě pomocí metody DRIFTS. C-H vazby se považuji za hydrofobní (měřené v pásmech 2 948 – 2 920 a 2 864 – 2 849 cm^{-1}) a C=O vazby (měřené v pásmech 1 710 a 1 640 – 1 600 cm^{-1}) se považují za hydrofilní. Tuto metodu použili Demyan et al. (2012) pro studium kvality půdní organické hmoty, kde pozorovali vliv hnojení na hodnotu PWI a její významné korelace s obsahem půdního organického uhlíku. Zvýšené hodnoty indexu smáčivosti poukazují na sníženou smáčivost půdních agregátů (Haas et al. 2018).

$$PWI = A/B$$

Půdní organická hmota je také charakterizována poměrem alifatických a aromatických sloučenin a jejich poměrem označovaným jako index aromaticity (z anglického aromaticity index – iAR) (Cunha et al. 2009). Tento index se počítá z odraznosti alifatických pásem (AL) v rozsahu 3000 – 2800 cm^{-1} a aromatických pásem (AR) při 1520 cm^{-1} molekul. Měření tohoto indexu se dá provést současně s měřením indexu smáčivosti.

$$iAR = AL/(AL+AR)$$

Vyšší hodnoty indexu aromaticity znamenají relativně nižší podíl aromatických součástí, respektive vyšší podíl alifatických složek organické hmoty ve stabilních agregátech než v agregátech nestabilních (Thai et al. 2023). Například Mustafa et al. (2022) zjistili, že stabilnější organická hmota se nachází na variantách orné půdy po aplikace chlévského hnoje. Obeng et al. (2023) uvádějí, že aromaticita je nižší na trvalých travních porostech (index je tedy vyšší), než v lesích. Je to díky zvýšené rychlosti rozkladu ligninu a fenolických sloučenin. Dále byla popsána vyšší stabilita půdního uhlíku po změně využití půdy z orné půdy na lesní (Ovsepyan et al. 2020). Nárůst mineralizace organické hmoty a tvorba alifatických sloučenin v agregátech může vést k nárůstu hodnot indexu aromaticity (Cunha et al. 2009).

3 Cíle práce a hypotézy

3.1 Cíle práce

Pomocí frakcionace síry určit minerálně a organicky vázaný podíl síry a následně z minerálního podílu určit rostlinám skutečně přístupný podíl síry a dále vyhodnotit bilanci síry v dlouhodobých přesných polních pokusech s různými systémy organického a minerálního hnojení.

Prověřit vhodnost nových metod (rychlejších a šetrnějších k životnímu prostředí) ke stanovení různých forem půdní organické hmoty (POH) (obsah glomalinu, index smáčivosti a index aromaticity) jako indikátorů kvality půdní organické hmoty.

Kvantifikovat vztah mezi jednotlivými frakcemi síry v půdě a vybranými kvalitativními indikátory půdní organické hmoty.

3.2 Dílčí hypotézy

Z předchozích výzkumů je zřejmé, že metoda Mehlich 3 je vhodná pro stanovení přístupných obsahů živin. Obsahy síry získané metodou Mehlich 3 v půdě:

- nebudou významně odlišné od obsahu rostlinám přístupné síry.
- budou v dobré korelaci s obsahem síry v rostlině.

Je pravděpodobné, že běžné systémy hnojení založené především na doplnění dusíku mohou vést k nevhodné bilanci ostatních živin včetně síry.

- lze předpokládat vliv různých systémů hnojení na bilanci síry.

K určení kvality POH je možné využít data o obsahu glomalinu, a hodnotách indexu smáčivosti a indexu aromaticity. Tyto tři parametry budou:

- ve vztahu k obsahu uhlíku půdní organické hmoty v půdě.
- ve vztahu k běžně používaným indikátorům kvality půdní organické hmoty (C_{HA}, C_{FA}).
- budou ovlivněny použitým systémem hnojení

Je pravděpodobné, že obsah glomalinu a humusových látek budou ve vztahu k různým frakcím síry, především však k obsahu organické síry.

4 Publikované práce

Tato práce předkládaná formou svázaných vědeckých článků vznikla na základě sedmi, níže uvedených prací. Jedná se o publikovaná díla v časopisech databáze Web of Knowledge s Impact Factorem. Práce publikované mimo impaktovaná periodika, jsou vypsány na konci disertační práce v podkapitole „ostatní publikace“. Zároveň jsou ve stejné podkapitole sepsány publikace mimo rozsah této práce.

4.1 Evaluation of Soil S Pools under 23 Years of Maize Monoculture

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Evaluation of Soil S Pools under 23 Years of Maize Monoculture

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Abstract: Sulfur nutrition is a critical part of proper crop growth and development. In our study, biomass yields (BY) and S uptake were investigated on long-term maize monoculture on haplic luvisol soil during the 23 years of this trial, as well as changes in water extractable (S_w), adsorbed (S_{ads}), mineral (S_{av}), and pseudo-total S (S_t) fractions. Treatments used in this study are: (1) Control (Cont); (2) ammonium sulfate (AS); (3) urea and ammonium nitrate (UAN); (4) UAN + phosphorus and potassium (UAN + PK); (5) UAN + phosphorus, magnesium, sulfur (UAN + PMgS); and (6) Fallow. Recently, the Mehlich 3 method started to be used in the Czech Republic to determine content of plant available S. Using this method, it was found that the content of S extracted by Mehlich 3 (S_{M3}) closely correlates to S_{av} in both topsoil and subsoil ($r = 0.958$ in 1997 and 0.990 in 2019, both at $p < 0.001$). We also found that, on average, during the entire experiment, all treatments had increased yields over Cont (135–147%) and increased S uptake (291, 192, 180, and 246% of Cont for AS, UAN, UAN + PK, and UAN + PMgS, respectively). Examining the changes from 1997 to 2019 in topsoil (0–30 cm depth), we discovered a decrease of S content in S_w, S_{ads}, S_{av}, and S_t fractions on all treatments to an average of 34.6%, 65.8%, 42.2%, and 78.6% of their initial values. The exception was AS treatment, which doubled its initial content in mineral fractions and maintained the same levels of S_t, and which we attribute to the very high dose of S on this treatment (142 kg ha^{-1} year^{-1}). Using the simple balance method, AS and UAN + PMgS treatments lost 142.2 and 95.3 kg S ha^{-1} year^{-1} to other sinks, except plant uptake, from the entire soil profile (0–60 cm) during 23 years of experiment. Other treatments also show significant losses with the exception of Fallow. Given these results, it is clear that content of sulfur in soil is generally decreasing and attention should be paid mainly towards minimizing of its losses.

Keywords: corn; mineral fertilizing; soil sulfur fraction; sulfur balance; Mehlich 3

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1. Introduction

Sulfur is a key nutrient in plant nutrition, where it appears in the synthesis of amino acids, glutathione, chlorophyl, and other processes [1], influencing yields, crop quality, harvest quality, and other parameters [2,3].

Sulfur deficiencies in plant nutrition in European countries were described by Lehmann et al. [4] and Yang et al. [5], who mention that, in recent decades, the content of S in soils is being reduced due to desulfurization technologies in industry, the use of high analysis fertilizers with low amounts of S [6], and N only fertilization, which increases yields and creates greater demands for plant available nutrients [7]. More recent research states, that amount of wet + dry S depositions are roughly around 18 kg ha^{-1} year^{-1} for Europe [8] and 15 to 30 kg S ha^{-1} year^{-1} in the Czech Republic [9], but currently, in 2019, the total depositions of S in the Czech Republic average 4.2 kg ha^{-1} [10], while in the 1980s, depositions reached 100 kg S ha^{-1} year^{-1} [11], which could have been a sufficient supply for crop production or, even in some cases, toxic for some crops. Because of the above-mentioned facts, S fertilizing is often underestimated [4,12].

The content of total sulfur is divided between organic and inorganic S fractions. The most important for plant nutrition is inorganic SO_4^{2-}. Inorganic SO_4^{2-} comprises about 5–10% of the total content [12,13]. The mineral form of S is present in soil in three groups–readily available sulfates in soil solution, which represents usually only about 1% of total S; adsorbed sulfates on soil particles that can be released into soil solution and directly influence the preservation of the sulfate balance with the soil solution [14,15]; and sulfates occluded in calcium and magnesium carbonate precipitates, unavailable to plants. The majority of total S is organic bound. This fraction can play a role in resupplying mineral sulfates throughout the vegetation [16,17], which is a biologically mediated process [18].

The total sulfur in soils at 10 experimental stations in the Czech Republic averaged 221 mg kg^{-1} in 1981, and slightly decreased to 204 mg kg^{-1} in 2007. A much bigger decrease (by 50%) was discovered in plant available S content, mainly because of lower S inputs from atmospheric deposition [9]. Kulhánek et al. [19] investigated the influence of long-term fertilization in stationary field experiments under crop rotation (from 1996 to 2014), and they describe a decrease of mineral S fraction in soil by up to almost 40% on unfertilized treatment on two different stations. On the other hand, it was found that the lowest decrease was observed on farmyard manure and sewage sludge treatments.

Inorganic sulfates are very mobile in soil solution and are susceptible to leaching [20]. Due to higher atmospheric emissions in the past, sulfur cycles in soil were altered by high S inputs and leaching of sulfates from soils was observed [4]. With lowering depositions from the atmosphere, a recovery of S cycles was noted, but it lags behind the decrease of S depositions [21]. On top of that, there is evidence that a significant part of the sulfates present in streams is microbiologically cycled [4] and this mineralization of organic S is responsible for the delayed reduction of sulfates in streams [22].

Sulfates can be supplied either with organic fertilizers, (e.g., farmyard manure, slurry, and others), or mineral fertilizers, e.g., commonly used S fertilizers ammonium sulfate (AS) or magnesium sulfate. AS is water soluble [6] and quite mobile in soil [23–25]. Dijksterhuis and Oenema [23] noticed that a leachate in their pot experiment fertilized with AS had a higher concentration of SO_4^{2-} anion with higher losses on sandy soil in comparison to clayey soil. Riley et al. [24] set up an experiment with soil of natural structure in a monolithic lysimeter fertilized by AS with a dose of 50 kg S ha^{-1}. They found that 72% of S was lost due to leaching. Bergholm and Majdi [25] described a similar problem in field trials, where 684 kg S ha^{-1} supplied as the sum of atmospheric depositions and S fertilizer only showed a loss of 339 kg S ha^{-1} by leaching. Kieserite ($MgSO_4$) is also water soluble and shows similar tendencies to AS. Ponette et al. [26] noticed significant vertical transport of SO_4^{2-} anions in soil after application of kieserite. In the trials of Frank and Stuanes [27], 21% of S added in 35 kg ha^{-1} of kieserite-S was leached below 80 cm soil depth. Generally, these authors mention that drier conditions result in less leaching than wet conditions.

Maize (*Zea mays* L.) shows a good yield response to sulfur fertilization for doses around 25 kg S ha^{-1} [28] and up to 60 kg S ha^{-1} [29]. Requirements for S fertilization can be influenced by soil organic matter content (C_{SOM}) [28], from which S can be released to supply plants [16,17]; the availability of other nutrients that can increase biomass production and, therefore, S uptake [30,31], water availability [32], and genetic gains [7].

Changes of water extractable, adsorbed, and plant available S were previously investigated by Kulhánek et al. [19]. Gourav et al. [33] conducted a similar experiment with the inclusion of organic and total sulfur. Both of these experiments were conducted under crop rotation.

The aim and contribution of this study is to investigate the changes of S fractions and S leaching under somewhat "extreme" conditions of 27 years of continuous maize monoculture. This study aims to assess the effects of mineral fertilization on long-term stationary trials, focusing on: sulfur content in soil, balance of total sulfur inputs and outputs in soil, and influence of fertilizers on biomass yields and S uptake.

2. Materials and Methods

2.1. Setup and Background

A long-term stationary experiment with maize monoculture was established in 1993 in Červený Újezd in the Czech Republic, as part of the Czech University of Life Sciences' experimental fields. Characteristics of the experimental site can be found in Table 1. The field experiment was conducted in a randomized complete block design with an area of 170 m^2 plot size. All variants were replicated four times. The maize plants were planted on each plot, except fallow. The maize hybrids DK 205 (1993–1996), Torena (1997 and 1998), DK 254 (1999), Compact (2000), Etendard (2001–2003), Rivaldo (2004–2011), RGT Indexx (2012–2014), and RGT Sixxtus (2015–2019) were planted on each plot at a density of 80,000 plants per ha.

Table 1. Characteristics of the Červený Újezd experimental site.

GPS coordinates	50°4′22″ N 14°10′19″ E
Altitude (meters above sea level)	410
Mean annual precipitation (mm)	493
Mean annual temperature (°C)	7.7
Soil type	Haplic luvisol
Soil texture	Loam
pH ($CaCl_2$)	6.5
Clay (%) (<0.002 mm)	5.4
Silt (%) (0.002–0.05 mm)	68.1
Sand (%) (0.05–2 mm)	26.5
Bulk density topsoil (g cm^{-3})	1.47
Bulk density subsoil (g cm^{-3})	1.50
C_{SOM} (%)	1.26
Cation exchange capacity ($mmol_{(+)}$/kg)	118

2.2. Treatments

For the purpose of this study, several treatments were selected, namely: (1) unfertilized control with crop production (Cont); (2) ammonium sulfate (AS); (3) urea and ammonium nitrate (UAN); (4) UAN + phosphorus and potassium (UAN + PK); (5) UAN + phosphorus, magnesium, sulfur (UAN + PMgS); and (6) unfertilized fallow without crop production (Fallow). Annual nutrient inputs from fertilizers are described in Table 2. N fertilizers were applied in spring before sowing. P, K, Mg, and S fertilizers were applied in autumn. Each of the fertilizers was applied in a single dose. No additional amendments were added to individual treatments, and only stubble from previous year was incorporated to the soil. No analysis of S content in stubble was performed. The entire harvest of above-ground biomass was removed from the trial fields and all treatments were later ploughed (including Fallow). Total sulfur inputs including annual sulfur deposition (dry + wet) are described in Table 3. Precipitation (as an only source of irrigation) was measured directly at the experimental site; however, there is no equipment for detecting S depositions. Therefore, we used data provided from the meteorological station Prague Ruzyně, which is the nearest professional station measuring S depositions (the distance is about 10 km by air; GPS: 50°6′0.6″ N, 14°15′19.8″ E).

Table 2. Experimental design and annual nutrient dose.

Treatment	kg ha^{-1} Year^{-1}				
	N	**P**	**K**	**Mg**	**S**
Cont	0	0	0	0	0
AS	120	0	0	0	142
UAN	120	0	0	0	0
UAN + PK	120	30	150	0	0
UAN + PMgS	120	30	0	60	84
Fallow	0	0	0	0	0

AS—ammonium sulfate (21% N; 24% S), UAN—urea ammonium nitrate solution (30% N), PK—triple superphosphate (21% P) + potassium chloride (50% K), MgS—kieserite ($MgSO_4$; 15% Mg; 21% S).

Table 3. Total sulfur inputs (deposition + fertilizer) during experiment.

Input Period	Cont	AS	UAN	UAN + PK	UAN + PMgS	Fallow
			kg S ha^{-1}			
1993–1996	80	648	80	80	416	80
1997–2001	61	771	61	61	481	61
2002–2007	48	900	48	48	552	48
2008–2013	37	889	37	37	541	37
2014–2019	29	881	29	29	533	29
Sum 1993–2019	254	4088	254	254	2522	254
Sum 1997–2019	175	3441	175	175	2107	175

Numbers at Cont, UAN, UAN + PK, and fallow represent the net wet + dry S deposition. Data were provided by meteorological station Prague Ruzyně belonging to the Czech Hydrometeorological Institute.

2.3. Plant Analyses

Two rows of maize aboveground biomass (20 m^2 per plot) were harvested at silage maturity (roughly 65% biomass moisture content, BBCH 75) to obtain the dry aboveground biomass yield (BY). Representative subsamples were harvested and chopped using mechanical chopper weight and subsequently dried in a forced-air oven to constant weight at 40 °C for at least 72 h and were then fine milled (Retsch SM100, Haan, Germany). Briefly, the aliquote 0.25 g of milled sample was weighed and immersed in nitric acid (7 mL of 65% HNO_3) and hydrogen peroxide (2 mL of 30% H_2O_2). Samples were then digested in a microwave-assisted high-pressure environment. The whole procedure is further described in Tlustoš et al. [34].

2.4. Soil Analyses

Archived soil samples of topsoil (0–30 cm depth) and subsoil (30–60 cm depth) sampled after the crop harvest were air-dried and sieved (<2 mm). For available sulfur fractions, a sequential extraction method by Morche [35] and modified by Kulhánek et al. [13] was selected. Briefly, samples were extracted with demineralized water (1/10 *w*/*v*) to extract the readily available S (S_w) fraction and, subsequently, with 0.032 mol/L NaH_2PO_4 to extract the adsorbed sulfur (S_{ads}) fraction. The sum of these fractions is then the bioavailable sulfur (S_{av}). Usually, extraction by 1 mol/L HCl follows extraction of S_{ads}, but this fraction is usually measured using ion chromatography (IC), since optical emission spectroscopy with inductively coupled plasma (ICP-OES) tends to be less accurate as it measures a significant part of organic S in the HCl extract, especially on non-calcareous soils, like the soil used in this study. Therefore, HCl extraction was omitted.

The pseudo-total sulfur (S_t) concentration in the soil was determined by modified ISO: 11,466 1995 [36] method using *Aqua regia*. The modification was a microwave assisted high pressure digestion and evaporation of samples using a heating plate (150 °C) and, subsequently, the quantitative transfer with distilled water was allocated to a final volume of a 25 mL glass tube, topped up by deionized water, and kept at laboratory temperature until measurements were taken. Mehlich 3 extraction was also performed following Mehlich [37] in order to also evaluate the S_{M3} fraction.

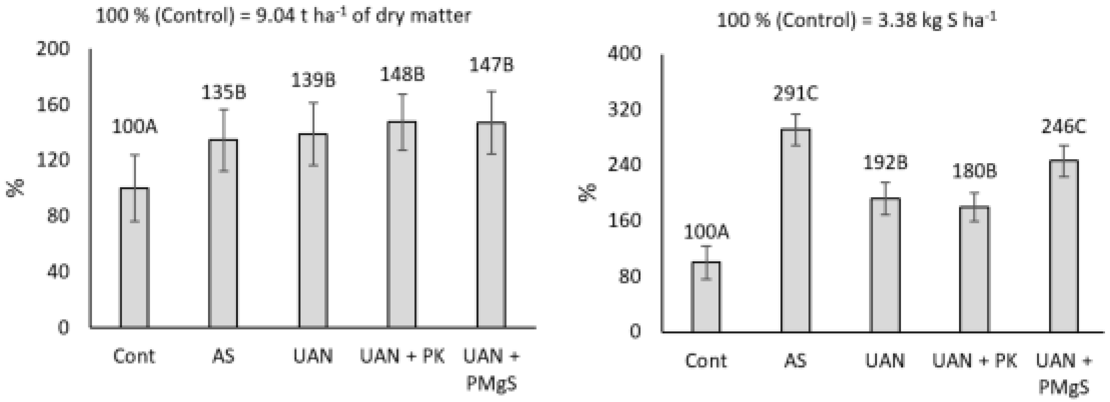
Sulfur concentrations in all digests and extracts were determined using the optical emission spectroscopy with inductively coupled plasma (ICP-OES) and with axial plasma configuration, Varian, VistaPro, equipped with autosampler SPS-5 (Mulgrave, Australia). The operating measurement wavelength for ICP-OES was 180.7 nm for S.

Statistical analyses were performed using one-way or two-way analysis of variance (ANOVA; Tukey HSD post-hoc test $p < 0.05$) using the SAS® system, (Cary, CA, USA). Even though trials started in 1993, we evaluated the period of 1997–2019 since a more representative dataset is available.

3. Results

3.1. Biomass Yield and S Uptake

Statistical analysis of average yield in the period 1993–2019 (Figure 1a) shows significant differences between the unfertilized Cont treatment and fertilized treatments, which do not show any differences between each other.



(a) (b)

Figure 1. Relative average yields (**a**) and sulfur uptake (**b**) at harvest in the years 1993–2019; the capital letters describe statistically significant differences between treatments. Tukey HSD test ($p < 0.05$).

Average sulfur uptake in the same period showed more differences. Fertilized treatments produced significantly higher uptake over the Cont. This time, however, significant differences were present even among the fertilized treatments (as described in Figure 1b), where AS and UAN + PMgS fertilized treatments were comparable to each other, but were higher than UAN and UAN + PK.

A more detailed comparison of S uptake is provided in Table 4. In the period 1993–1996 (judging by the capital letters), differences were already present between the Cont treatment and AS and UAN + PMgS treatments, while UAN and UAN + PK were comparable with Cont. In later periods, UAN and UAN + PK always showed significant increase over Cont. The AS treatment achieved the highest values of S uptake and was always different from other three treatments mentioned. UAN + PMgS was different from UAN and UAN + PK treatments in 1997–2001 and UAN + PK in the 2002–2007 period. AS and UAN + PMgS were not different in any period.

Table 4. Detailed plant uptake of sulfur during the experiment (kg S ha^{-1}).

Uptake Period	Cont	AS	UAN	UAN + PK	UAN + PMgS
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
1993–1996	15.7 ± 0.54 A	39.6 ± 1.39 C	25.3 ± 1.10 AB	22.6 ± 0.97 AB	31.6 ± 0.56 BC
1997–2001	24.8 ± 0.67 A	63.5 ± 1.23 C	42.7 ± 0.65 B	40.1 ± 0.70 B	55.4 ± 0.83 C
2002–2007	22.2 ± 0.49 A b	65.9 ± 1.66 D c	43.2 ± 1.38 BC b	40.1 ± 1.14 B b	56.7 ± 1.90 CD b
2008–2013	17.6 ± 0.56 A ab	58.9 ± 3.04 C b	39.8 ± 1.74 BC b	36.8 ± 1.05 B ab	50.5 ± 1.89 BC ab
2014–2019	16.1 ± 0.55 A a	47.8 ± 1.57 C a	32.3 ± 1.23 B a	30.3 ± 1.20 B a	37.6 ± 1.07 BC a
Sum 1993–2019	96.3	275.6	183.3	169.9	231.7
Sum 1997–2019	80.6	236.1	158	147.3	200.1

The capital letters describe statistically significant differences among treatments. The lowercase letters describe statistically significant differences among years. Tukey HSD test ($p < 0.05$). Only periods of 2002–2007, 2008–2013, and 2014–2019 were compared as these periods last the same number of years.

In order to estimate what fraction was the most important for plant nutrition, a Spearman´s correlation analysis was performed comparing the S biomass content of maize with individual S fractions in topsoil (Table 5). It was possible to compare only the latest values from the year 2019 (n = 20). In case of this study, S fertilizers were applied for several years before the first evaluations were performed, causing a shift from a purely linear relationship to a monotonic.

Table 5. Spearman's correlation coefficients comparing the relationship between individual S fractions in topsoil and plant S content.

	S_W	S_{ads}	S_{M3}	S_{av}	S_t	df
Plant S content	0.674 **	0.747 **	0.579 **	0.725 **	0.288	18

Values marked with ** are significant at $p < 0.01$. Degrees of freedom (df) were the same for each fraction ($n = 20$).

3.2. *Topsoil Sulfur Content*

In topsoil, a two-way ANOVA was performed to analyze the effect of six treatments and three time periods on S content in individual fractions. Results of this analysis (Table 6) showed that both treatment and year had significant effects on content of S in individual fractions. Significant interaction of (year × treatment) was also present for all fractions except S_t. To evaluate pairwise differences, a Tukey HSD post-hoc test at $p < 0.05$ was used. This test revealed that interaction of (AS treatment × year 2019) within any of the S_w, S_{ads}, S_{M3}, and S_{av} fractions was significantly different from any other (treatment × year pair) of the same fraction, with the exception being S_t, where the interaction was insignificant. Interactions of other pairs were omitted because they do not appear significant nearly as often. Additionally, given the number of possible pairs, this report would become unbearable in volume at 6 treatments and 3 years per each of 5 fractions.

Table 6. Results for two-way ANOVA comparing the effects of treatment, year, and their interaction on S content in individual S fractions in topsoil.

S Fraction	Treatment		Year		Treatment × Year	
	F-Value	**df**	**F-Value**	**df**	**F-Value**	**df**
S_W	28.07 *	5	19.68 *	2	16.63 *	10
S_{ads}	27.08 *	5	3.88 *	2	11.27 *	10
S_{M3}	36.17 *	5	4.72 *	2	16.27 *	10
S_{av}	30.35 *	5	17.72 *	2	16.94 *	10
S_t	3.926 *	5	23.77 *	2	2.08	10

Values marked with asterisk (*) describe a significant effect at $p < 0.05$.

Influence of individual effects was described using one-way ANOVA. In samples, S_w, S_{ads}, S_{av}, and S_t content were measured (Figures 2 and 3) in the years 1997, 2008, and 2019, as well as S_{M3} (Figure 4). It is evident that the content of all S fractions was significantly higher in 1997 than in 2019. There are two exceptions, however. Firstly, the content of S_t in 2019 and 1997 on the Fallow treatment (Figure 3b) is not significantly different. Secondly, the content of S_t in 1997 and 2019 on the AS treatment is not significantly different.

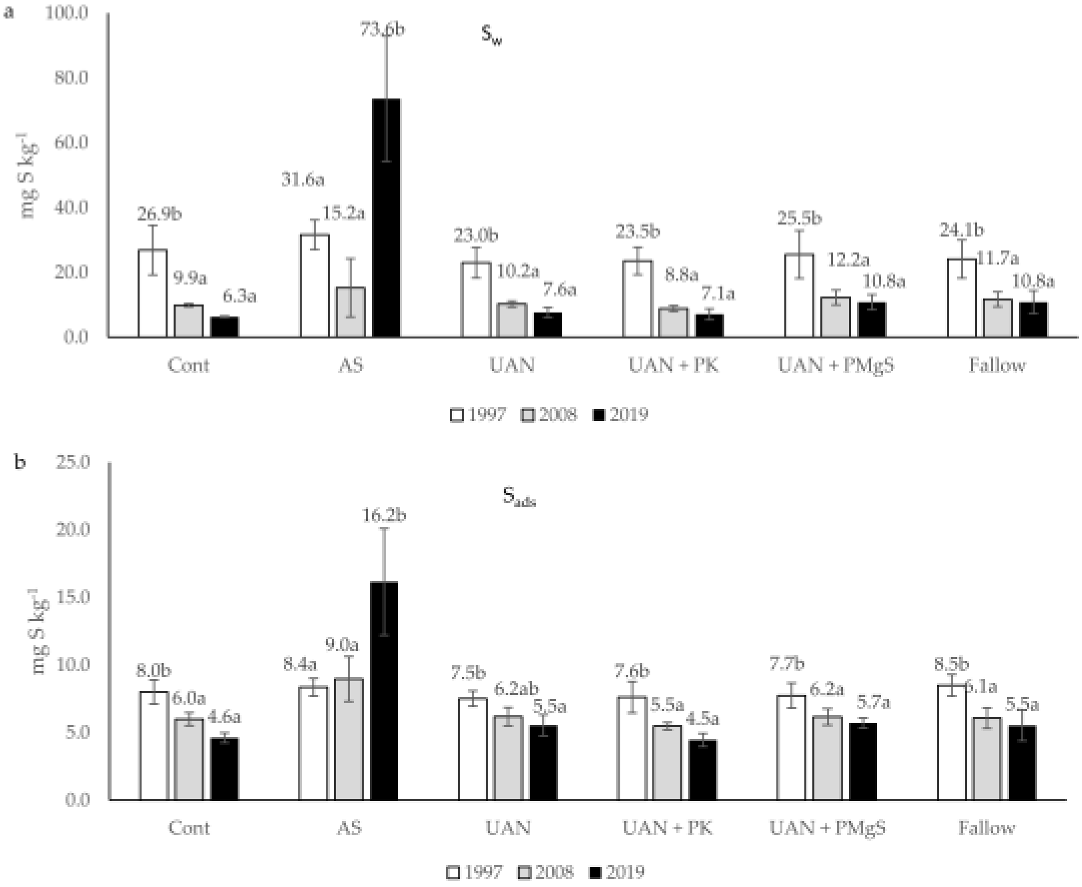


Figure 2. Content of sulfur fractions in topsoil (mg S kg^{-1}): S_w (**a**); S_{ads} (**b**). Comparison of the effect of year within treatment; the lowercase letters describe statistically significant differences between years. Tukey HSD test ($p < 0.05$).

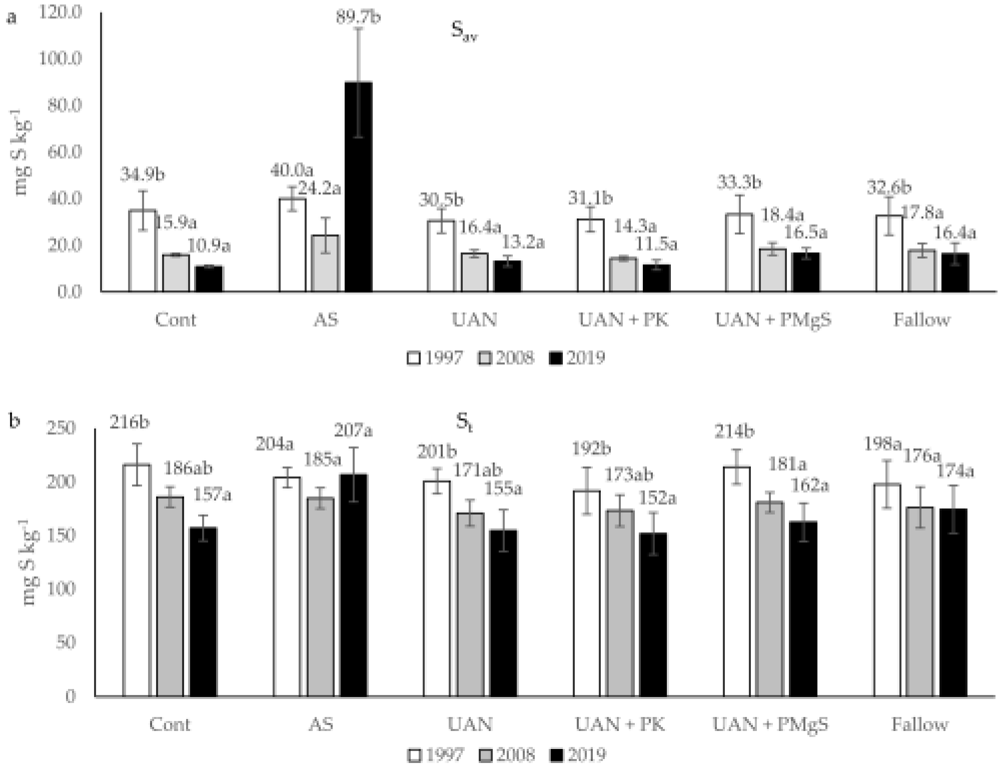


Figure 3. Content of sulfur fractions in topsoil (mg S kg^{-1}): S$_{av}$ (**a**); S$_t$ (**b**). Comparison of the effect of year within treatment. The lowercase letters describe statistically significant differences between years. Tukey HSD test ($p < 0.05$).

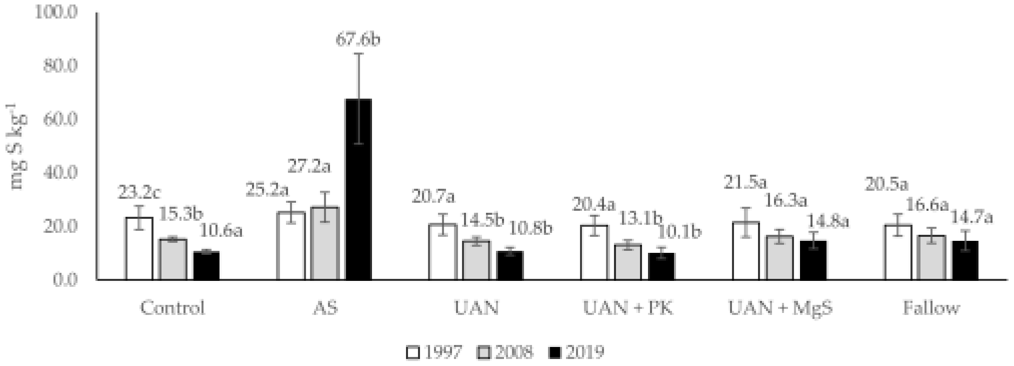


Figure 4. Content of Mehlich 3 extractable sulfur (mg S kg^{-1}) in topsoil; the lowercase letters describe statistically significant differences between years. Tukey HSD test ($p < 0.05$).

Since S$_{av}$ is calculated as S$_w$ + S$_{ads}$, the contribution of each fraction to S$_{av}$ was calculated. For Cont, UAN, UAN + PK, UAN + PMgS, and Fallow, the contribution of S$_w$ to S$_{av}$ was 76% on average in 1997, and 62% in 2019. The only exception was the AS treatment in all fractions that accumulated S, where the increase ranged from 79% in 1997 to 82% in 2019.

The S_{av} fraction of the Cont treatment amounted to 16.2, 8.5, and 6.9% of S_t in 1997, 2008, and 2019, respectively. S_{av} for the Fallow treatment made up 16.5, 10.1, and 9.4% in 1997, 2008, and 2019, respectively. UAN, UAN + PK, and UAN + PMgS behaved very similarly to Cont.

Interestingly, it seems that the S_t content on the Fallow treatment seems to have stabilized in 2008 and almost identical values are measured in 2019, while the Cont treatment shows a lowering content.

Extraction with Mehlich 3 (Figure 4) produced results resembling those in Figures 2a,b and 3a, with UAN + PMgS and Fallow treatments showing no significant differences in S content. Pearson correlation was calculated between S_{av} and S_{M3} fractions resulting in a correlation coefficient of 0.958 and 0.990 (both at $p < 0.001$) in 1997 and 2019, respectively. Including all values for S_{av} (Figure 3a) and S_{M3} (Figure 4), it is possible to calculate the average content in each fraction at 26.0 and 20.0 mg S kg^{-1} for S_{av} and S_{M3}. These numbers are not significantly different according to the Tukey HSD test at $p < 0.05$. It can be argued that mainly the content of S on the AS treatment in both fractions in 2019 distorts this comparison by increasing the standard deviation. After removing all outlying values from both fractions, we obtain averages of 20.9 and 16.0 mg S kg^{-1} for S_{av} and S_{M3}. Even these results produce no significant difference under the Tukey HSD test at $p < 0.05$. This statistical similarity and close correlations among S_{M3} and S_{av} allow us to use S_{M3} values for estimating the S content.

Table 7 presents the relative S contents measured in different soil S fractions comparing 2019 S levels with 1997. It is obvious that (aside from AS) all treatments suffered a decrease of S in time. In 2019, the remaining S_w, S_{ads}, S_{av}, and S_t pool of Cont was 23, 58, 31, and 73%, respectively. UAN, UAN + PK, and UAN + PMgS show a similar decrease of S fractions. On the other hand, AS showed an increase in S_w, S_{ads}, S_{av}, and S_t fractions to 233, 193, 224, and 101% in comparison to 1997.

Table 7. Relative S content (%) in individual fractions comparing actual S content in 2019 with 1997 (S content in 1997 = 100%).

Treatment	S_w	S_{ads}	S_{av}	S_t
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Cont	23 ± 8.00	58 ± 9.40	31 ± 9.44	73 ± 5.05
AS	233 ± 62.60	193 ± 47.73	224 ± 58.54	101 ± 13.37
UAN	33 ± 10.12	73 ± 11.40	43 ± 10.65	77 ± 10.50
UAN + PK	30 ± 5.06	59 ± 10.55	37 ± 6.06	79 ± 2.94
UAN + PMgS	42 ± 5.51	74 ± 11.84	50 ± 7.10	76 ± 5.73
Fallow	45 ± 9.51	65 ± 8.12	50 ± 9.41	88 ± 4.98

3.3. *Subsoil Sulfur Content*

Subsoil S content was also evaluated for S_w, S_{ads}, S_{av}, and S_{M3} fractions (Figure 5) and S_t (Figure 6). Comparison of different treatments was performed only for the year 2019. Content of S_w, S_{ads}, and S_{av} fractions shows the same tendencies. Cont, UAN, and UAN + PK are statistically comparable, while S content measured in UAN + PMgS is significantly higher. Content in AS is significantly higher even than that of UAN + PMgS. The S_t content on the UAN treatment shows the lowest values, significantly lower than Cont, AS, and Fallow. Cont, AS, and UAN + PMgS and Fallow are statistically comparable, but generally the entire Figures 2a,b, 3a, 4 and 5 show that the AS treatment tends to have wider error bars than the other treatments.

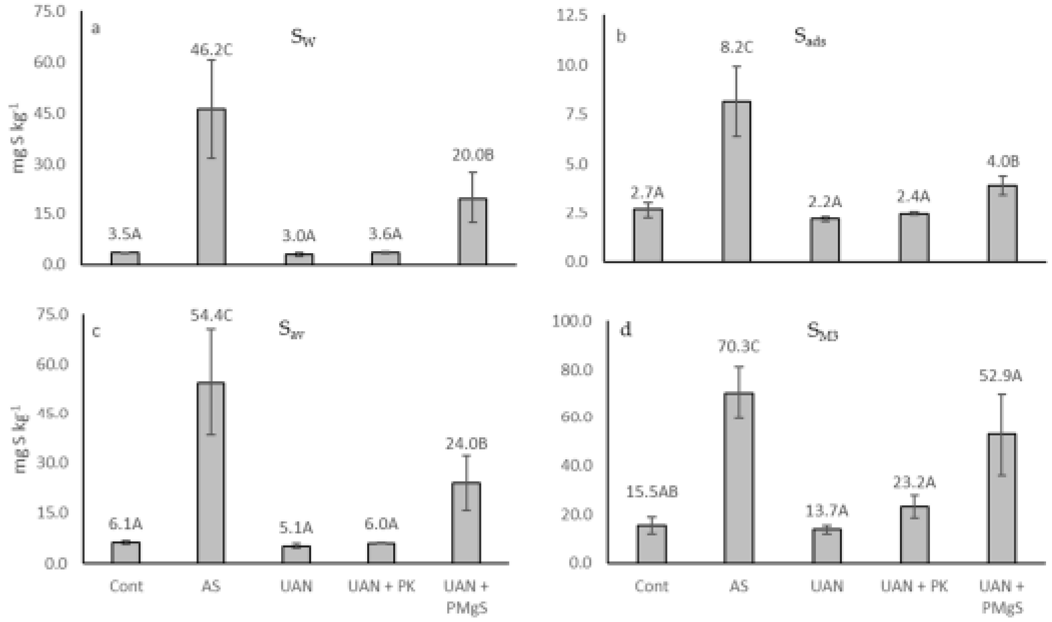


Figure 5. Content of sulfur fractions in subsoil (mg S kg^{-1}) in 2019: S_w (**a**); S_{ads} (**b**); S_{av} (**c**); S_{M3} (**d**). The capital letters describe statistically significant differences between treatments. Tukey HSD test ($p < 0.05$).

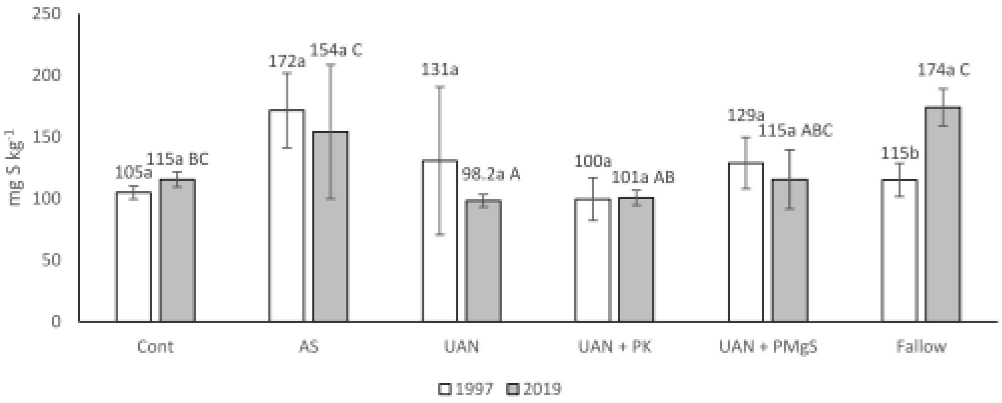


Figure 6. Content of pseudo-total sulfur (S_t) fraction in subsoil (mg S kg^{-1}) in 1997 and 2019; the lowercase letters describe statistically significant differences between years and the capital letters (for 2019) between treatments. Tukey HSD test ($p < 0.05$).

The calculation of different ratios resulted in the following: S_w contributes to S_{av} by 57, 85, 59, 60, and 83% in Cont, AS, UAN, UAN + PK, and UAN + PMgS treatments, respectively. S_{av} makes up 5.3, 35, 5.2, 5.9, and 21% of S_t in Cont, AS, UAN, UAN + PK, and UAN + PMgS treatments, respectively.

AS and UAN + PMgS in S_{M3} treatments show significant increase above other treatments. UAN + PK also seems to be different to UAN. On average, the content of S_{M3} (35.1 mg S kg^{-1}) is higher than that of S_{av} (19.4 mg S kg^{-1}). There is no significant difference according to ANOVA with Tukey HSD post-hoc test at $p < 0.05$; however, the standard deviation for S_{av} is so wide (even after removal of AS from the calculation) that this comparison is not appropriate.

Enough data were present to evaluate S_t content not only among treatments, but also its change in years comparing 1997 with 2019 (Figure 6). Cont, UAN + PK, UAN + PMgS, and AS treatments led to no significant change, while Fallow showed an increase in S content. Error bars present on the UAN treatment do not allow for accurate comparison.

3.4. Comparison of Sulfur Status in Topsoil and Subsoil

It is clear that in the topsoil in 1997, the results of almost all treatments were similar; however, in 2019, the AS shows significantly higher values over Cont, UAN, and UAN + PK treatments (capital letters), while being comparable with UAN + PMgS and Fallow. Cont, UAN, and UAN + PK are not different between each other, but they also have some overlap with UAN + PMgS and Fallow.

In the subsoil in 1997, S content in the AS treatment was already significantly higher than that of other treatments. Cont, UAN + PK, UAN + PMgS, and Fallow are all comparable. The year 2019 in subsoil is the most interesting, as UAN and UAN + PK produced the lowest values of S content, significantly lower than Cont. Cont itself seems to be similar to AS and UAN + PMgS treatments. Fallow produced the highest value of S content in subsoil, statistically comparable to AS.

Differences in S content at the beginning of the trial and its end were also evaluated (Table 8). In subsoil, the only difference in S content between the beginning and the end of the experiment was detected in the Fallow treatment.

Table 8. Balance of S in topsoil and subsoil comparing year 1997 to 2019 (kg S ha^{-1}).

Treatment	**Cont**	**AS**	**UAN**	**UAN + PK**	**UAN + PMgS**	**Fallow**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
			Topsoil			
1997	952 ± 86 A b	900 ± 42 A a	885 ± 52 A b	845 ± 97 A b	943 ± 71 A b	872 ± 75 A a
2019	692 ± 74 A a	911 ± 105 B a	682 ± 74 A a	669 ± 82 A a	716 ± 69 AB a	769 ± 67 AB a
Balance	−260	12	−203	−176	−227	−103
			Subsoil			
1997	471 ± 41 A a	772 ± 109 B a	587 ± 56 AB a	448 ± 32 A a	579 ± 63 A a	519 ± 42 A b
2019	519 ± 26 B a	693 ± 123 BC a	442 ± 208 A a	453 ± 79 A a	520 ± 84 AB a	785 ± 23 C a
Balance	48	−78	−146	5	−60	266

The capital letters describe statistically significant differences between treatments. The lowercase letters describe statistically significant differences between years. Tukey HSD test ($p < 0.05$).

The balance row shows how much sulfur each treatment gained (+) or lost (−) over the course of the trial. In topsoil, the only influx of S was registered on the AS treatment (although only 12 kg of S ha^{-1}), while others lost some of their initial content (ranging between 103 to 260 kg of S ha^{-1}). In subsoil, the Fallow treatment gained 266 kg of S ha^{-1}, while Cont gained 48 kg S ha^{-1} and UAN + PK gained only 5 kg S ha^{-1}. UAN, AS, and UAN + PMgS lost 146, 78, and 60 kg S ha^{-1}, respectively.

3.5. Sulfur Movement in Topsoil and Subsoil

Table 9 provides an insight into the movement of sulfur in soil. The highest losses of sulfur were present in the AS treatment, followed by the UAN + PMgS treatment losing 3193 kg and 2133 kg of S, respectively, in both topsoil and subsoil. Other treatments lost much less, 204 to 356 kg of S ha^{-1}. In subsoil, losses were 199, 308, and 367 kg of S ha^{-1} during the entire experiment for UAN + PK, UAN, and Cont, respectively. Fallow in subsoil lost only 11 kg of S ha^{-1}. The period 1997 to 2019 was selected as there is a more representative dataset allowing us to perform this calculation.

Table 9. S movement in topsoil and subsoil during the experiment.

Treatment	Cont	AS	UAN	UAN + PK	UAN + PMgS	Fallow
				Topsoil (kg S ha^{-1})		
Status in 1997 + inputs 1997–2019 (a)	1127	4340	1060	1020	3050	1047
Status in 2019 + uptake 1997–2019 (b)	771	1147	838	816	917	769
Loss from topsoil (a − b)	356	3193	221	204	2133	277
				Subsoil (kg S ha^{-1})		
Status in 1997 + loss from topsoil (c)	827	3965	808	651	2713	796
Status in 2019 (d)	519	693	442	453	520	785
Loss from subsoil (c − d)	308	3271	367	199	2193	11

4. Discussion

4.1. Biomass Yield and S Uptake

Figure 1a shows relative yields increased in all fertilized treatments. Except for Cont, all fertilized treatments received uniformly 120 kg N ha^{-1} year^{-1} and produced comparable yields, while only two treatments received S fertilizer. Based on the fact, that all N fertilized treatments were statistically the same, we can deduce that S was not a limiting nutrient in our trials.

Relative sulfur uptake (Figure 1b) showed more differences, as treatments amended with S fertilizer have the highest relative S uptake. Cont treatment provided uptake of 3.38 kg S ha^{-1} year^{-1}. Meanwhile application of 142 kg S ha^{-1} year^{-1} in the form of AS resulted in uptake of 9.84 kg S ha^{-1} year^{-1}, while application of UAN + MgS (84 kg S ha^{-1} year^{-1}) increased S uptake to 8.31 kg S ha^{-1} year^{-1} (increase by 291% and 246% over Cont, respectively). A similar increase of S uptake was present in the study of Sakal et al. [38], where a dose of 40 kg S ha^{-1} increased S uptake of maize by up to 9.44 kg S ha^{-1} over unfertilized treatment, which provided uptake of 2.58 kg S ha^{-1} in trials with maize and wheat crop rotation. Overall, the increase in S uptake was also noted on treatments UAN and UAN + PK, providing increase of 192% and 180%, respectively. Weil and Mughogho [30] presented similar results, as they also observed increase in S uptake on treatments fertilized by 80 kg of N ha^{-1}. Generally, the application of N increases yields and, thus, produces higher uptake of other nutrients [39], including S [40].

Table 4 describes these results in more detail. Interestingly, uptake on each treatment shows descending tendencies in periods 2002–2007, 2008–2013, and 2014–2019. During these periods, yields on individual treatments also show a significant decrease (not present in this study); therefore, reduced uptake can be attributed to reduced yields. The reduction of yields over time can be partially attributed to N dose. Our trials were previously investigated by Černý et al. [41], where they focused on N uptake. They found that uptake of N can reach up to 185 kg N ha^{-1}, while yearly inputs are 120 kg N ha^{-1}, so the nitrogen dose might not be high enough to properly supply the crops. Other possible explanations can include a change of the crop hybrid (viz. materials and methods) and precipitation, which can cause mobilization of N in soil and possibly N leaching [42,43], as well as optimal growth of the crop itself and better nutrient availability [44].

Given the results of correlation analysis (Table 5), it seems that the S_{ads} fraction correlates best with total S content in plant biomass ($r = 0.747$; $p < 0.01$), followed closely by S_{av} ($r = 0.725$; $p < 0.01$) and S_W ($r = 0.674$; $p < 0.01$). S_{av} is calculated as the sum of S_W and S_{ads}, thereby explaining the very similar coefficients of these fractions. This is generally in agreement with other works, stating that S_W, S_{ads}, and their sum Sav are very important fractions in plant nutrition [17,35] (as discussed later). S_{M3} correlates only moderately with the content of S in biomass. Since Mehlich 3 includes HNO_3, it is possible that it extracts some part of the S_t content that is usually not available to plants. Relationship between the mentioned fractions and S biomass content may not represent the reality well enough in this study due to the small sample set size. Recently, Sedlář et al. [45] correlated content of S in maize biomass with content of S in S_W and S_{M3} fractions at Pearson´s correlation coefficient values of r = 0.961 and 0.804, respectively, at $p < 0.001$ in a pot experiment. In field trials, correlation coefficients were measured at r = 0.174 (insignificant) and 0.629 ($p < 0.05$) for S_W and S_{M3}, respectively. In their experiments, S biomass content was determined early during vegetation (BBCH 16–18), while in this study, maize was sampled quite a bit later (BBCH 75). Differences in sampling period can explain some of the differences, as content in S is quite variable in soil and plants across the vegetation season [46]. In addition, Sedlář et al. [45] collected maize from farmers scattered across the Czech Republic using both a crop rotation system and monoculture, bringing even more variability into the evaluation. On the other hand, this study focuses solely on maize from long-term monoculture on one station only.

4.2. Topsoil and Subsoil Sulfur Content

Results of two-way ANOVA (Table 6) revealed significant interaction of year and treatment on content of S in all soil S fractions, with the exception of S_t. This was caused by a high content of S on AS treatment in the soil in the year 2019 (Figures 2 and 3), which resulted in significant interaction of (AS treatment × year 2019 pair). Possible causes of such increase in content of S on this treatment are discussed later. These results would suggest that once inputs of S reach such levels, as with the inputs of S on AS treatment in our study (Table 3), the S starts to cumulate in $S_{w,}$ S_{ads}, S_{av}, and S_{M3} fractions over time. UAN + PMgS treatment received S fertilizer as well. Interactions of (UAN + PMgS × any year pairs) were rarely significant and, in almost all cases, not different from pairs with (treatments without S fertilizer and any year). This suggests that the fertilizer rate on UAN + PMgS is not high enough to cause cumulation of S in topsoil.

Soil samples were collected after crop harvest. During crop growth, levels of S (especially S_{av}) would be high enough for sufficient crop growth in spring. Grobler et al. [47] mention that maize is capable of producing sufficient yields with 10 mg S_{av} kg^{-1} of soil. S_{av} in our trials (Figure 3a) never reached lower values than 10 mg S_{av} kg^{-1} even after harvest (on average around 44 kg S_{av} ha^{-1}), when S pools were already drained. Considering the results of S_{M3} extraction (Figure 4 in topsoil and Figure 5d in subsoil), S_{M3} extracted a very similar amount of sulfur to S_{av}. Kulhánek et al. [13] investigated the relationship between S_{av} and S_{M3} and found a very close Pearson correlation (r = 0.882; $p < 0.001$). Using the Pearson correlation analyses, we also found a very close relationship of these two fractions in this study (r = 0.958 in 1997 and 0.990 in 2019, both at $p < 0.001$). This high correlation can be caused by the fact that each treatment received the same amount of fertilizer each year for the entire duration of the trials, as well as all treatments of this study being located on the same soil type, while Kulhánek et al. [13] investigated a wider variety of soil types with much more varied fertilizer inputs. A similar relationship was also reported in other studies [48,49]. As mentioned in the Results, no differences between S_{av} and S_{M3} in topsoil and subsoil (Tukey; $p < 0.05$) were found. This makes the Mehlich 3 method suitable for evaluating available S content in soil.

Kulhánek et al. [50]—in Czech—developed a certified methodology for determination of plant available S using Mehlich 3 extractant. They proposed criteria (5 categories in total) for evaluation of plant available S using S_{M3} content in soil. The results of our study place S_{M3} content in topsoil in the "Satisfactory" category in 1997. Having said this, UAN and UAN + PK treatments have 20.7 and 20.4 mg S kg^{-1}, which technically puts them on the border of the "Low" and "Satisfactory" categories (ranges for "Low" and "Satisfactory" categories are 11–20 and 21–30 mg S kg^{-1}). On the other hand, in the year 2019 the S_{M3} content is ranked in the "Low" category in all treatments, since the content of S_{M3} has decreased in general with the exception of the AS treatment. In subsoil, there is no temporal comparison, since we only have results from 2019 (Figure 5d). Using the categories proposed by Kulhánek et al. [50], Cont and UAN are in the "Low" category, UAN + PK is ranked in the "Satisfactory" category, and UAN + PMgS and AS are ranked in the "High" content category.

Overall, S pools of different S fractions on all treatments in topsoil seem to be decreasing in time with the exception of the AS treatment (Figures 2 and 3), which shows an increase in S_w, S_{ads}, and S_{av} fractions, while maintaining even levels of S_t.

It is obvious that (aside from AS) all treatments suffered a decrease of S pools over time (Table 7). In 2019, the S_w remaining pool in the Cont, UAN, and UAN + PK treatments ranged from 23 to 33% of S_w determined in the samples of the year 1997. S_{ads} content on these treatments ranged 58–73% as compared to 1997 and in 2019. S_{av} content ranged from 31–43% of S_{av} in 1997. Lastly, S_t content for Cont, UAN, and UAN + PK treatments in our trials in 2019 reached 73–79% of those in 1997. This conforms to Balík et al. [9], who investigated sulfur status in soils on 10 different sites in the Czech Republic. They found that S_w, S_{ads}, S_{av}, and S_t content decreased in time to 32, 61, 50, and 92%, respectively (comparing the year 1981 with 2007). Changes in mineral S fractions were evaluated by Kulhánek et al. [19] in a long-term field experiment (comparing the year 1996 with 2014) with a potato, wheat, and barley crop rotation and several fertilizers. They describe a decreasing tendency of S_{av} content in the entire trial; however, S_w content increased following the application of sewage sludge after potatoes. In other crops, S_w always showed a decrease.

Indeed, the application of organic matter into soil can increase its water holding capacity [51] and, therefore, temporarily increase the S_w fraction. In our experiment, no organic fertilizers were applied to the soil during the entire experiment. Changes of C_{SOM} in our monoculture trial were investigated by Balík et al. [52]. Initial C_{SOM} levels decreased from 1.26% in 1993 to 0.98% in Cont and 0.93% in AS in topsoil in 2019. Subsoil C_{SOM} was reported at 0.73% in Cont and 0.65% in AS in 2019. This is caused by cultivation of maize for silage, since very little post-harvest residues are present (just stubble and roots), and C_{SOM} presents net loss. Considering the current content of C_{SOM} in Cont topsoil (0.98%) and subsoil (0.73%) [52], it is possible to calculate their ratio at 1.34 as well as a ratio of current S_t levels in topsoil (157 mg S kg^{-1}–Figure 3b) and subsoil (115 mg S kg^{-1}–Figure 6), where we get the ratio 1.36. It seems that S_t content is bound in the same way in organic matter in topsoil and subsoil. After all, organic bound S can make up to 90% of S_t [12]. Loss of the S_{av} fraction from 1997 to 2019 in topsoil (Figure 3a) amounted roughly to 16.2–24.0 mg S kg^{-1}). Loss of S_t (Figure 3b) ranged from 24 to 59 mg S kg^{-1} in all treatments except AS. This suggests that loss of S from the organic fraction was present and partially contributed to loss in S_t. In arable systems where soil organic matter is not accumulating, there is little opportunity for inorganic $SO_4{}^{2-}$ ions immobilization into organic matter [53]. The S_t loss can probably be attributed to leaching of the $SO_4{}^{2-}$ anion, which is discussed later.

Even the Fallow treatment shows interesting results. Content in all S fractions is highest in 1997, but lower in 2008. This treatment has no crop production, so the decrease in the period 1997–2008 can be attributed to leaching of S from atmospheric depositions. Unlike the other non-AS treatments, the decrease of S was not present from 2008 to 2019. The most likely cause is the decrease of atmospheric S depositions in recent years [10]. These results are similar to those of Gourav et al. [33], where a higher content of S_w and S_t was present on the Fallow treatment than on Cont after 48 years of experiment with maize monoculture. In our study, the Cont treatment of the S_{av} fraction amounted to 16.2, 8.5, and 6.9% of S_t in 1997, 2008, and 2019, respectively. S_{av} for the Fallow treatment made up 16.5, 10.1, and 9.4% in 1997, 2008, and 2019, respectively. UAN, UAN + PK, and UAN + PMgS behaved very similarly to Cont. It is clear, that treatments with maize production and S leaching had a higher influence over the decrease of S_{av} pools than that of Fallow, which is only influenced by leaching.

The AS treatment seems to show opposing results. The increase of S_{ads} in topsoil in this treatment (Figure 2b) can be explained, e.g., by a decrease of pH that causes increased sorption of $SO_4{}^{2-}$ [54]. AS fertilization indeed decreases the pH of soil [55]. This, however, does not explain the increase of S_w and S_t fractions. A possible explanation may be the combination of site and rainfall variability as well as annual application of 142 kg S per hectare (which can be considered as a very high dose), which could cause the accumulation of sulfur in this treatment over time. Influence of time was deemed significant earlier (Table 6) and seems to support this thought.

The S content in all fractions (Figure 5) in subsoil was increased in 2019 on UAN + PMgS and AS treatments over those without S fertilization. Knights et al. [53] and Zayed et al. [56] report that surplus S can be accumulated in subsoil, which could explain that the highest increase overall was present on the AS treatment that received the highest amounts of S inputs, followed by UAN + PMgS, which received the second highest inputs of S. This can also explain the increased content of S_t in 1997 on the AS treatment (Figure 6). Even though we evaluate data from 1997, the experiment and fertilizer applications started in 1993, so AS was applied for several years before the first data are presented in this study. Leaching of S from previous years could have increased S content in the subsoil in 1997 on this treatment in subsoil.

S_w content in subsoil on AS and UAN + PMgS treatments had increased by 42.7 and 16.5 mg S kg^{-1} over Cont (Figure 5a). Since soil samples were taken after harvest, it is possible that soil was sampled during a downward movement of S, which was in surplus in topsoil. This could also explain the increase in S_{ads} (Figure 5b). The S_{av} (Figure 5c) content on Cont, UAN, and UAN + PK is on par; however, S_t content (Figure 6) is somewhat lower for UAN and UAN + PK in comparison with Cont. It is possible that a higher uptake of S by plants in the UAN and UAN + PK treatments could cause mineralization and upward movement of S from the subsoil [51]. Accumulation of total S over time is visible in the subsoil on Fallow in comparison with other non-S-fertilized treatments (Figure 6). Since there is no crop production on Fallow, we assume that maize roots have influenced the other treatments. Indeed, maize roots can grow as deep as the subsoil layer (30–60 cm) [57,58] and could, therefore, cause a decrease in subsoil S content.

4.3. Sulfur Status and Movement in Topsoil and Subsoil

Values of S_t in the topsoil and subsoil (Table 8) are calculated in kg S ha^{-1}. Therefore, temporal changes represent identical tendencies for the soil S content in Figures 3b and 6.

From Table 8, it is clear that all treatments except AS show a decrease in S_t content in the topsoil. Negative S balance can be attributed to crop growth and leaching [24]. Cont is influenced by leaching and maize growth, while Fallow is only influenced by leaching; therefore, it is understandable that Fallow showed the lowest loss of S. A slightly positive balance is noticeable in AS. A possible explanation may be the combination of site and rainfall variability as well as annual application, as was mentioned above in the discussion of topsoil and subsoil sulfur content. In the subsoil Cont, Fallow, and UAN + PK treatments, we measured the increase in S content. It is possible that leached sulfates from the topsoil were absorbed or accumulated in organic matter.

Table 9 describes the movement of S in the topsoil and subsoil. The calculation presented in this table is based on a comparison of the original S status in soil and total S inputs during trials with the status of S at the end of the experiment with total plant uptake during the experiment. Using this method, we calculated that on all treatments in topsoil, an extensive S loss is present. In subsoil, the trend is the same, with the exception of the Fallow treatment that seems to have stable values. We consider the loss from subsoil in Table 9 equal to the total S lost from the topsoil and subsoil profile (0–60 cm depth). The Fallow treatment registered the smallest loss of 11 kg S ha^{-1}, while Cont, UAN, and UAN + PK lost 308, 367, and 199 kg S ha^{-1} during the entire trial, respectively. UAN + MgS and AS provided losses of 2193 and 3271 kg S ha^{-1} during the entire trial or 95.3 and 142 kg S ha^{-1} year^{-1}, respectively. This loss can certainly be attributed to leaching of SO_4^{2-} anions as described in Riley et al. [24], where a three-year pot experiment with AS application and undisturbed soil sample was established with a focus on S leaching. Here, the control treatment received a total input of 22 kg S ha^{-1} in terms of depositions, while the AS treatment received an additional 50 kg S ha^{-1} in the form of AS fertilizer (in total 72 kg S ha^{-1} was added). It was found that 78% of S applied on AS was leached in the first year and it increased to 96% after three years. When loss by leaching and plant uptake were added together (55 and 107 kg S ha^{-1} in Cont and AS, respectively), the total S outputs in AS and even Cont treatments exceeded the total inputs at the end of the trial by 35 and 33 kg S ha^{-1}, respectively. The authors explain this by net S mineralization from organic bound S in soil. Using these data, we calculated a ratio (in%) of outputs (uptake and leaching) over inputs (deposition and fertilizer) and obtained 253% for Cont and 149% for AS. The same calculation was performed for Cont, AS, and UAN + PMgS treatments with the result of 221%, 102%, and 114%, respectively. Furthermore, S uptake (Table 4) did not reach the levels of fertilizer inputs (Table 3) and mobile sulfates were generally susceptible to percolation in AS and UAN + PMgS treatments. Leaching of sulfates was obviously present. Riley et al. [24] measured the presence of organic bound S in leachates. It makes sense in the context of our study, since Balík et al. [52] evaluated changes in organic bound S on the same trials as this study and found a decrease of organic matter content that reduces space for net SO_4^{2-} accumulation. This lack of S retention was also demonstrated in Rothamsted long-term experiments [51], where applications of 52–220 kg S ha^{-1} $year^{-1}$ over 150 years had not increased organic, nor inorganic, pools of S. In addition, we demonstrated (Figure 3b) that S_t content in our trials is decreasing and organic bound S is, of course, part of this fraction [17].

Bergholm and Majdi [25] also report S leaching during a six-year experiment. However, their results differ from those of ours. Out of a total S input of 821 kg S ha^{-1} in the form of depositions and AS fertilizer, up to 339 kg S ha^{-1} was leached (41.2%) in comparison with Cont, where inputs were 133 kg S ha^{-1} in the form of atmospheric S depositions, and leaching amounted to 79 kg S ha^{-1} (59%). Accumulation in AS and Cont reached 317.1 and 8.1 kg S ha^{-1}, respectively. Calculation of outputs (leaching and accumulation) over inputs ratio was performed resulting in 60% and 65% for AS and Cont treatments, respectively. These results differ from those in our study or in Riley et al. [24]. This difference is due to the fact, that the Bergholm and Majdi [25] study was conducted on forest soil. Authors measured accumulation of S in the forest floor (that is very rich in organic matter) and also in mineral soil. On the other hand, Riley et al. [24] as well as our study conducted an experiment on arable soil. They found leaching of organic bound S and a recent study by Balík et al. [52] found a decrease of soil organic matter on our trials. There are other factors that can influence the movement of S in soil, like an increase in precipitation being capable of increasing movement of nutrients in soil [26,27]. Soil texture can have an influence on water retention and, therefore, leaching as well. The soil texture in our trials was Loam with 5.4% clay content. In Riley et al. [24], the soil texture was Sandy loam of 0–40 cm depth with clay content around 7.5% and Loamy sand of 40–60 cm with clay content around 5.5%. In Bergholm and Majdi [25], the soil texture was also Loamy sand with 4% clay. In the end, it is possible to state that the majority of the disparity can be attributed to vastly different conditions between forest and arable soil.

Comparing the results of our maize monoculture long-term field experiment with a pot experiment [24] and forest trial [25] is indeed difficult due to the very different conditions that apply to each of these respective environments. Even though these trials are so different, what they share in common is the fact that a significant part of sulfur fertilizers is leached and unavailable to plants and may even be contaminating ground waters [59].

In general, to reduce the leaching of sulfates, it is recommended to use fertilizers with lower solubility, like elemental S, which is less susceptible to leaching [24,60], or performing the application in several smaller doses [24]. However, Santoso et al. [60] mention that fast growing crops like maize show significantly lower S uptake if fertilized by slowly soluble fertilizers, especially if applied in split doses.

5. Conclusions

In this study, we examined the influence of S fertilization and atmospheric depositions on soil and plants in maize monoculture lasting 27 years. We found that S was not a limiting nutrient and S fertilization did not increase yields, although it created higher S uptake by maize. Generally, we noticed a decrease in topsoil S_w, S_{ads}, S_{av}, and S_t content on all treatments with exception being the AS treatment, which doubled its initial content in mineral fractions and maintained the same levels of S_t, which we attribute to the very high S dose on this treatment. In our conditions as well as similar ones, S_{av} fractions ($S_w + S_{ads}$) determination seems to be most suitable soil tests for plant S uptake prediction.

Using the total S balance, we demonstrate that a significant amount of S is being leached below the soil depth of 60 cm on all treatments over the period of 27 years of the experiment, with the highest values on both S fertilized treatments. We conclude that high atmospheric depositions before the 1990s caused S pools in soil to fill up and contribute to S leaching. Furthermore, the decrease of S depositions in the period of the 1990s to 2019 and leaching of S caused a decrease in S content in the topsoil in almost all treatments (except AS) during the period of 1997–2019. Based on our results, we can propose the use of multiple small S doses or slowly soluble fertilizers as well as organic fertilizers in maize monoculture.

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References

1. Marschner, H. (Ed.) *Marschner's Mineral Nutrition of Higher Plants*, 3rd ed.; Elsevier: London, UK, 2012; p. 672. ISBN 978-0-12-384905-2.
2. Bender, R.R.; Haegele, J.W.; Ruffo, M.L.; Below, F.E. Nutrient uptake, partitioning, and remobilization in modern, transgenic insect-protected maize hybrids. *Agron. J.* **2013**, *105*, 161–170. [CrossRef]
3. Steinke, K.; Rutan, J.; Thurgood, L. Corn Response to Nitrogen at Multiple Sulfur Rates. *Agron. J.* **2015**, *107*, 1347–1354. [CrossRef]
4. Lehmann, J.; Solomon, D.; Zhao, F.-J.; McGrath, S.P. Atmospheric SO_2 Emissions Since the Late 1800s Change Organic Sulfur Forms in Humic Substance Extracts of Soils. *Environ. Sci. Technol.* **2008**, *42*, 3550–3555. [CrossRef] [PubMed]
5. Yang, Z.; Singh, B.R.; Hansen, S.; Hu, Z.; Riley, H. Aggregate Associated Sulfur Fractions in Long-Term (>80 Years) Fertilized Soils. *SSSA J.* **2007**, *71*, 163–170. [CrossRef]
6. Chien, S.H.; Gearhart, M.M.; Villagarcía, S. Comparison of Ammonium Sulfate with Other Nitrogen and Sulfur Fertilizers in Increasing Crop Production and Minimizing Environmental Impact: A review. *Soil Sci.* **2011**, *176*, 327–335. [CrossRef]
7. Ciampitti, I.A.; Vyn, T.J. Physiological perspectives of changes over time in maize yield dependency on nitrogen uptake and associated nitrogen efficiencies: A review. *Field Crop. Res.* **2012**, *133*, 48–67. [CrossRef]
8. Gao, Y.; Ma, M.; Yang, T.; Chen, W.; Yang, T. Global atmospheric sulfur deposition and associated impaction on nitrogen cycling in ecosystems. *J. Clean. Prod.* **2018**, *195*, 1–9. [CrossRef]
9. Balík, J.; Kulhanek, M.; Cerny, J.; Száková, J.; Pavlíková, D.; Cermak, P. Differences in soil sulfur fractions due to limitation of atmospheric deposition. *Plant Soil Environ.* **2009**, *55*, 344–352. [CrossRef]
10. Czech Hydrometeorological Institute. (In Czech). Available online: https://www.chmi.cz/files/portal/docs/uoco/isko/grafroc/19groc/gr19cz/09_depozice_v1.pdf (accessed on 10 August 2020).
11. Krupová, D.; Pavlendová, H.; Pavlenda, P.; Šrámek, V. Atmospheric deposition of sulphur and nitrogen in forests of the Czech and Slovak Republic. *J. For.* **2018**, *64*, 249–258. [CrossRef]
12. Scherer, W.H. Sulfur in soils. *J. Plant Nutr. Soil Sci.* **2009**, *172*, 326–335. [CrossRef]
13. Kulhánek, M.; Černý, J.; Balík, J.; Sedlář, O.; Suran, P. Potential of Mehlich 3 method for extracting plant available sulfur on the Czech agricultural soils. *Plant Soil Environ.* **2018**, *64*, 455–462. [CrossRef]
14. McLaren, R.G.; Cameron, K.C. Soil, plant and fertilizer sulphur. In *Soil Science*; McLaren, R.G., Cameron, K.C., Eds.; Oxford University Press: Auckland, New Zealand, 1996; pp. 221–228. ISBN 9780195583458.

15. Tisdale, S.L.; Nelson, W.L.; Beaton, J.D.; Havlin, J.L. *Soil Fertility and Fertilizers*, 5th ed.; Macmillan Publishing Company: New York, NY, USA, 1993; p. 634. ISBN 978-0024208354.
16. Blum, S.C.; Lehmann, J.; Solomon, D.; Caires, E.F.; Alleoni, L.R.F. Sulfur forms in organic substrates affecting S mineralization in soil. *Geoderma* **2013**, *200–201*, 156–164. [CrossRef]
17. Boye, K.; Eriksen, J.; Nilsson, I.; Mattson, L. Sulfur flow in a soil-plant system—effects of long-term treatment history and soil properties. *Plant Soil* **2010**, *334*, 323–334. [CrossRef]
18. Vermien, C.; Smolder, R.; McLaughlin, M.J.; Degryse, F. Model-based rationalization of sulphur mineralization in soils using 35S isotope dilution. *Soil Biol. Biochem.* **2018**, *120*, 1–11. [CrossRef]
19. Kulhánek, M.; Balík, J.; Černý, J.; Sedlář, O.; Vašák, F. Evaluating of soil sulfur forms changes under different fertilizing systems during long-term field experiments. *Plant Soil Environ.* **2016**, *62*, 408–415. [CrossRef]
20. Eriksen, J.; Olesen, J.E.; Askegaard, M. Sulphate leaching and sulphur balances of an organic cereal crop rotation on three Danish soils. *Eur. J. Agron.* **2002**, *17*, 1–9. [CrossRef]
21. Cooper, M.D.; Jenkins, A. Response of acid lakes in the UK to reductions in atmospheric deposition of sulfur. *Sci. Total. Environ.* **2003**, *313*, 91–100. [CrossRef]
22. Mörth, C.-M.; Torssander, P.; Kjønaas, O.J.; Stuanes, A.O.; Moldan, F.; Giesler, R. Mineralization of Organic Sulfur Delays Recovery from Anthropogenic Acidification. *Environ. Sci. Technol.* **2005**, *39*, 5234–5240. [CrossRef]
23. Dijksterhuis, H.G.; Oenema, O. Studies on the effectiveness of various sulfur fertilizers under controlled conditions. *Fertil. Res.* **1990**, *22*, 147–159. [CrossRef]
24. Riley, N.G.; McGrath, S.P. Leaching losses of sulphur from different forms of sulphur fertilizers: A field lysimeter study. *Soil Use Manag.* **2002**, *18*, 120–126. [CrossRef]
25. Bergholm, J.; Majdi, H. Accumulation of nutrients in above and below ground biomass in response to ammonium sulfate addition in a Norway spruce stand in southwest Sweden. *Water Air Soil Pollut.* **2001**, *130*, 1049–1054. [CrossRef]
26. Ponette, Q.; Dufey, J.E.; Weissen, F. Downward movement of dolomite, kieserite or a mixture of $CaCO_3$ and kieserite through the upper layers of an acid forest soil. *Water Air Soil Pollut.* **1997**, *95*, 353–379. [CrossRef]
27. Frank, J.; Stuanes, A.O. Short-term effects of liming and vitality fertilization on forest soil and nutrient leaching in a Scots pine ecosystem in Norway. *For. Ecol. Manag.* **2003**, *176*, 370–386. [CrossRef]
28. Kim, K.; Kaiser, D.E.; Lamb, J. Corn response to starter fertilizer and broadcast sulfur evaluated using strip trials. *Agron. J.* **2013**, *105*, 401–411. [CrossRef]
29. Khan, M.J.; Khan, M.H.; Khattak, R.A.; Jan, M.T. Response of maize to different levels of sulfur. *Comm. Soil Sci. Plant Anal.* **2006**, *37*, 41–51. [CrossRef]
30. Weil, R.R.; Mughogho, S.K. Sulfur nutrition of maize in four regions of Malawi. *Agron. J.* **2000**, *92*, 649–656. [CrossRef]
31. Carciochi, W.D.; Divito, G.A.; Fernández, L.A.; Echeverría, H.E. Sulfur affects root growth and improves nitrogen recovery and internal efficiency in wheat. *J. Plant Nutr.* **2017**, *40*, 1231–1242. [CrossRef]
32. Timsina, J.; Singh, U.; Badaruddin, M.; Mesner, C.; Amin, M.R. Cultivar, nitrogen, and water effects on productivity, and nitrogen-use efficiency and balance for rice-wheat sequnces of Bangladesh. *Field Crop. Res.* **2001**, *72*, 143–161. [CrossRef]
33. Gourav, N.K.S.; Shrama, R.P.; Sharma, G.D. Vertical distribution of sulfur fractions in a continuously fertilized acid alfisol under maize-wheat cropping system. *Commun. Soil Sci. Plant Anal.* **2018**, *49*, 923–933. [CrossRef]
34. Tlustoš, P.; Hejcman, M.; Kunzová, E.; Hlisinkovský, L.; Zámečníková, H.; Száková, J. Nutrient status of soil and winter wheat (*Triticum aestivum* L.) in response to long-term farmyard manure application under different climatic and soil physicochemical conditions in the Czech Republic. *Arch. Agron. Soil Sci.* **2018**, *64*, 70–83. [CrossRef]
35. Morche, L. S-Fluxes and Spatial Alterations of Inorganic and Organic Sulfur Fractions in Soil as Well as Their Accumulation and Depletion in the Rhizosphere of Agricultural Crops by Partial Use of the Radioisotope 35S. Ph.D. Thesis, Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany, 2008; p. 321. (In German)
36. International Organization for Standardization. Soil Quality–Extraction of Trace Elements Soluble in Aqua Regia (ISO standard no. 11466:1995). 1995. Available online: https://www.iso.org/standard/19418.html (accessed on 9 October 2021).
37. Mehlich, A. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Commun. Soil Sci. Plant Anal.* **1984**, *15*, 1409–1416. [CrossRef]
38. Sakal, R.; Sinha, R.B.; Singh, A.O.; Bhogal, N.S.; Ismail, M.D. Influence of sulphur on yield and mineral nutrition of crops in maize-wheat semence. *J. Indian Soc. Soil Sci.* **2000**, *48*, 325–329.
39. Pasley, H.R.; Cairns, J.E.; Camberate, J.J.; Vyn, T.J. Nitrogen fertilizer rate increases rate increases plant uptake and soil availability of essential nutrients in continuous maize production in Kenya and Zimbabwe. *Nutr. Cycl. Agroecosyst.* **2012**, *115*, 373–389. [CrossRef]
40. Carciochi, W.D.; Salvagiotti, F.; Pagani, A.; Calvo, N.I.R.; Eyherabide, M.; Rozas, H.R.S.; Ciampitti, I.A. Nitrogen and sulfur interaction on nutrient use efficiencies and diagnostic tools in maize. *Eur. J. Agron.* **2020**, *116*, 126045. [CrossRef]
41. Černý, J.; Balík, J.; Kulhánek, M.; Vašák, F.; Peklová, L.; Sedlář, O. The effect of mineral N fertilizer and sewage sludge on yield and nitrogen efficiency of silage maize. *Plant Soil Environ.* **2012**, *58*, 76–83. [CrossRef]
42. Nie, S.; Eneji, A.E.; Chen, Y.; Sui, P.; Huang, J.; Huang, S. Nitrate leaching from maize intercropping systems with N fertilizer overdose. *J. Integr. Agric.* **2012**, *11*, 1555–1565. [CrossRef]

43. Simeonova, T.; Stoicheva, D.; Koleva, V.; Sokolowska, Z.; Hajnos, M. Effect of long-term fertilizer application in maize crop growing on chemical element leaching in Fluvisol. *Int. Agrophys.* **2017**, *31*, 243–249. [CrossRef]
44. Li, C.; Zheng, Z.; Peng, Y.; Nie, X.; Yang, L.; Xiao, Y.; Zhou, G. Precipitation and nitrogen addition enhance biomass allocation to aboveground in an alpine steppe. *Ecol. Evol.* **2019**, *9*, 12193–12201. [CrossRef] [PubMed]
45. Sedlář, O.; Balík, J.; Kulhánek, M.; Černý, J.; Matěchová, M.; Suran, P. Crop sulfur status in relation to soil sulfur determined using anion exchange membranes and Mehlich 3. *J. Plant Nutr.* **2021**, *44*, 1563–1570. [CrossRef]
46. Blake-Kalff, M.M.A.; Hawkesford, M.J.; Zhao, F.J.; McGrath, S.P. Diagnosing sulfur deficiency in field-grown oilseed rape (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). *Plant Soil.* **2000**, *225*, 95–107. [CrossRef]
47. Grobler, L.; Bloem, A.A.; Claassens, A.S. A critical soil sulphur level for maize (*Zea mays* L.) grown in a glasshouse. *S. Afr. J. Plant Soil.* **1999**, *16*, 204–206. [CrossRef]
48. Rogers, C.W.; Dari, B.; Schroeder, K.L. Comparison of soil-test extractants for potassium, calcium, magnesium, sulfur, and micronutrients in Idaho soils. *Agrosyst. Geosci. Environ.* **2019**, *2*, 190067. [CrossRef]
49. Kowalenko, C.G.; Bittman, S.; Neilsen, G.H.; Kenney, E.; Hunt, D.E.; Neilsen, D. Potential for improving sulfur tests on agricultural soils in contrasting ecoregions of British Columbia, Canada. *Geoderma Reg.* **2014**, *1*, 10–20. [CrossRef]
50. Kulhánek, M.; Balík, J.; Sedlář, O.; Zbíral, J.; Smatanová, M.; Suran, P. *Determining of Bioavailable Sulfur in Soil Using Mehlich 3 Method*; Czech University of Life Sciences: Prague, Czech Republic, 2018; p. 32. ISBN 978-80-213-2893-8. (In Czech)
51. Lal, R. Soil organic matter and water retention. *Agron. J.* **2020**, *112*, 3265–3277. [CrossRef]
52. Balík, J.; Kulhánek, M.; Černý, J.; Sedlář, O.; Suran, P. Soil organic matter degradation in long-term maize cultivation and insufficient organic fertilization. *Plants* **2020**, *9*, 1217. [CrossRef]
53. Knights, J.S.; Zhao, F.J.; Spiro, B.; McGrath, S.P. Long-term effects of land use and fertilizer treatments on sulfur cycling. *J. Environ. Qual.* **2000**, *29*, 1867–1874. [CrossRef]
54. Prietzel, J.; Weick, C.; Korintenberg, J.; Seybold, G.; Thumerer, T.; Treml, B. Effects of repeated $(NH_4)_2SO_4$ application on sulfur pool in soil, soil microbial biomass, and ground vegetation of two watersheds in the Black Forest/Germany. *Plant Soil.* **2001**, *230*, 287–305. [CrossRef]
55. Fageria, N.K.; dos Santos, A.B.; Moraes, M.F. Influence of urea and ammonium sulfate on soil acidity indices in lowland rice production. *Commun. Soil Sci. Plant Anal.* **2010**, *41*, 1565–1575. [CrossRef]
56. Zayed, B.A.; Naeem, E.S.; Sharkawi, H.M.E.; Gewaily, E.E. Efficiency of sulfur fertilizer on yield and soil properties of salt stressed paddy fields. *Egypt. J. Agric. Res.* **2010**, *88*, 1–11.
57. Gao, Y.; Duan, A.; Qiu, X.; Liu, Z.; Sun, J.; Zhang, J.; Wang, H. Distribution of roots and root length density in a maize/soybean strip intercropping system. *Agric. Water Manag.* **2010**, *98*, 199–212. [CrossRef]
58. Han, Y.; Zhang, X.; Ma, X. Fine root length of maize decreases in response to elevated CO_2 levels in soil. *Appl. Sci.* **2020**, *10*, 968. [CrossRef]
59. Sharma, M.K.; Kumar, M. Sulphate contamination in groundwater and its remediation: An overview. *Environ. Monit. Assess.* **2020**, *192*, 74. [CrossRef] [PubMed]
60. Santoso, D.; Lefroy, R.D.B.; Blair, G.J. Sulfur and phosphorus dynamics in an Acid soil/crop system. *Aust. J. Soil Res.* **1995**, *33*, 113–124. [CrossRef]

4.2 Influence of Long-Term Organic Fertilization on Changes in the Content of Various Forms of Sulfur in the Soil under Maize Monoculture

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Influence of Long-Term Organic Fertilization on Changes in the Content of Various Forms of Sulfur in the Soil under Maize Monoculture

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Abstract: Sulfur nutrition is a crucial part of proper crop growth. In this study, we investigated the influence of organic fertilizers in a long-term field experiment (23 years) with continuous maize monoculture. We focused on: (a) changes in the soil sulfur fraction pools, (b) the balance of total sulfur inputs and outputs, and (c) sulfur uptake by maize. The following treatments were selected: unfertilized control (Control), urea and ammonium nitrate (UAN), UAN and wheat straw (UAN + St), sewage sludge (SS), farmyard manure (FYM), and slurry (Slurry). Using sequential extraction, we determined water-soluble (S_W—in water), adsorbed (S_{ads}—in 0.032 mol L^{-1} NaH_2PO_4), and available ($S_{av} = S_W + S_{ads}$) sulfur content. Microwave-assisted digestion in an Aqua regia solution was used to measure the pseudo-total sulfur content (S_{pt}). Organic-bound sulfur (S_{org}) was calculated as a difference between S_{pt} and S_{av}. We found that average biomass yields responded to a uniform 120 kg N ha^{-1} year^{-1} dose, rather than the dose of S in fertilizers, with an increase over the Control by 34–49%. The effect of an additional 33.5 kg N ha^{-1} year^{-1} on UAN + St treatment was not significant. Average sulfur uptake responded to increased yields (69-121% higher than Control), rather than the sulfur application, with the exception of SS, where the dose of sulfur was high enough to cause an additional uptake. In the topsoil, we discovered a significant decrease over time (from 1997 to 2019) in water-soluble (S_W), adsorbed (S_{ads}), available (S_{av}), and pseudo-total (S_{pt}) fractions on all treatments to 29, 59, 37, and 82% of their initial values, respectively. For all treatments, the proportion of S_{org} in S_{pt} increased over time, which was caused by the decrease in mineral fractions (S_W, S_{ads}, S_{av}). The absolute content of S_{org} decreased over time for all treatments except SS and FYM to about 85% of the initial value. Using the simple balancing method, we calculated that UAN + St, SS, FYM, and Slurry treatments annually lost 8.04, 66.1, 21.4, and 26.8 kg of S ha^{-1}, respectively. This loss was attributed to the decrease in atmospheric depositions, as well as the release of sulfur from soil organic matter (for UAN + St and Slurry treatments) and a high proportion of easily mineralizable and inorganic sulfur from the SS treatment. Generally, the FYM fertilizer provided the highest potential for maintaining soil S_{pt} status.

Keywords: maize monoculture; sewage sludge; slurry; farmyard manure; straw; organic fertilizer; soil sulfur

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1. Introduction

Sulfur is a key nutrient in agriculture. It influences the metabolism of the plant itself [1], as well as crop yields and quality [2]. Some soil sulfur inputs are required for proper crop production.

In the most recent history, one of the biggest avenues of sulfur inputs was atmospheric depositions, but, due to desulfurization technologies in the industry, this path is no longer sufficient [3,4]. In the Czech Republic, the amount of sulfur supplied through wet and dry atmospheric depositions decreased from the hundreds to tens of kilograms of sulfur per hectare in the late 1980s to the 1990s, respectively [5,6]. Currently, in the Czech Republic, the sulfur inputs through the atmosphere are in the range of 4–5 kg per hectare annually [7]. This trend is reflected throughout all of Europe, where a similar decrease was reported by Gao et al., [8]. This decrease is a consequence of the reduction of sulfur-containing emissions from the industry. The European environmental agency reported a decrease in emissions in Europe by 74% between 1990 and 2011 [9]. Similarly, a 90% decrease in sulfur-containing emissions has also been present in the Czech Republic [7,10]. The development in the sulfur depositions, as well as the use of high-analysis fertilizers with a low content of sulfur, has caused the crops to show symptoms of sulfur deficiency [3]. On top of that, another contributing factor is also high pure nitrogen fertilization, which leads to an increase in yields and can lead to an increase in the uptake of other nutrients [11–13]. The combination of these factors can lead to deficiencies in plant sulfur nutrition [4] for crops such as maize (*Zea mays* L.), which seem to respond well to sulfur fertilizers at doses in the range of 25 to 60 kg of sulfur [14–16].

Sulfur in the soil is present either in the organic or mineral (inorganic) form [1]. The mineral fraction represents around 5–10% of the total sulfur content, and the content of mineral sulfate ($SO_4{}^{-2}$) anions is important for plant nutrition [17,18]. Mineral sulfates can be present in three forms: (i) water-soluble and most easily available to plants, which usually represents around 1% of the total S content, (ii) adsorbed sulfur on the soil particles, which can be released easily into the water-soluble fraction and supply the plants with sulfur (together, water-soluble and adsorbed sulfate fractions are regarded as plant available), and (iii) as a co-precipitated sulfur fraction occluded in the precipitates of magnesium and calcium carbonates [17,19], which are generally unavailable to plants, yet can be very slowly released [20].

Mineral sulfates are very mobile and susceptible to leaching into the subsoil [21–24], or even the ground waters [25]. Due to high mineral S inputs from atmospheric depositions, this release was measured in the European soils [21,22], as well as the Czech Republic [23,26]. On the other hand, organic-bounded sulfur is generally unavailable to plants, as it is less soluble; nevertheless, throughout the vegetation, a certain mass can be mineralized and released [20,21,27] to help with maintaining a steady supply of sulfur. This process can lead to a significant increase in sulfates in water streams [3].

A decrease in total sulfur content in the soil was reported in the Czech Republic between the years 1981 and 2007, from 221 mg S kg^{-1} to 204 mg S kg^{-1} [28]. This decrease does not seem dramatic at first, but in the study, the authors also measured an average decrease in water-soluble, adsorbed, and available sulfur content by 66.5, 41.5, and 50%, respectively. A similar investigation between the years 1996 and 2014 was carried out in the study by [29], and the same trend was found. Mehlich 3 sulfur extraction [30] has recently been introduced as a possible determination of plant-available sulfur in soil in the Czech Republic [18,31], and there is also a significant decrease in Mehlich 3 extractable sulfur in the Czech soils [31], which further confirms the trends.

The application of sulfur-containing fertilizers is helpful in sustaining good crop nutritional status. Generally, mineral fertilizers contain sulfur in the sulfate anion (for example, ammonium sulfate or magnesium sulfate) [11], but these are quite mobile [21,22,24] or different forms of elemental sulfur (S^0), such as micronized or mixed with bentonite clay [21], that are less mobile and less susceptible to leaching, yet might be problematic in sustaining proper sulfur supply for fast-growing crops such as maize [32].

On the other hand, the application of organic wastes in agriculture can be of great importance to maintain soil fertility [33]. It was found that, in order to maintain or even increase total sulfur content (and organic-bound sulfur as well) to a steady supply of sulfur in the soil, there must be a steady input of carbon in the organic matter [34], and this has been demonstrated in farmyard manure and sewage sludge application. This is something that Knights et al. [35] did not find in their experiment, where the annual application of mineral sulfur fertilizers over the course of 150 years did not, in fact, increase total sulfur content. The ability of organic fertilizers to maintain steady levels of sulfur in the soil can be different and is based on the kind of fertilizer and its origin. Therefore, more stable materials might prove more effective than materials with more unstable compositions (such as slurries, [36,37]). For example, the sewage sludge produced by water treatment facilities is an environmental problem and can partially be resolved by using the sludge as a fertilizer [38]. Out of total sewage sludge production, about 37% is used in agriculture in Europe [39]. Out of all the countries in the European Union, more than half recycle over 50% of their sewage sludge production into their agriculture [40]. This material has a large pool of potentially mineralizable sulfur [34] and is water soluble [41,42].

This study aimed to investigate the soil sulfur status under the conditions of 23 years of continuous maize monoculture fertilized by several organic fertilizers. Specifically, the assessment included: (a) changes in the soil sulfur fraction pools, (b) the balance of total sulfur inputs and outputs, and (c) sulfur uptake by maize.

2. Materials and Methods

The experiment was conducted at the long-term stationary experiment site of the Czech University of Life Sciences in Prague located at Červený Újezd. The field trials were initiated in the year 1993. The experimental site characteristics at the beginning of the trials are presented in Table 1. Information about current nutrient levels, as well as pH, is presented in Table 2. The trials were conducted in a complete block design. There were four blocks; each block had all fertilizer treatments arranged into individual plots. This means each treatment was replicated 4 times. Area of plot was 170 m^2. The silage maize hybrids (*Zea mays* L.) were planted at a density of 80,000 plants per ha. Average monthly air temperature and precipitation are displayed in Figure 1.

Table 1. Experimental site characteristics.

GPS Coordinates	**50°4′22″ N** **14°10′19″ E**
Altitude (meters above sea level)	410
Mean annual precipitation (mm)	493
Mean annual temperature (°C)	7.7
Soil type	Haplic luvisol
Soil texture	Silty Loam
pH ($CaCl_2$)	6.5
Clay (%) (<0.002 mm)	5.4
Silt (%) (0.002–0.05 mm)	68.1
Sand (%) (0.05–2 mm)	26.5
Bulk density topsoil (g cm^{-3})	1.47
Bulk density subsoil (g cm^{-3})	1.50
C_{SOM} (%)	1.26
Cation exchange capacity ($mmol_{(+)}$/kg)	118

Table 2. Content of Mehlich 3 extractable nutrients (P, K, Ca, and Mg) and other soil properties in 2019, topsoil.

Properties	**Treatments**					
	Control	**UAN**	**UAN + St**	**SS**	**FYM**	**Slurry**
P (mg P kg^{-1})	141	102	125	366	317	303
K (mg K kg^{-1})	141	115	138	129	247	228
Ca (mg Ca kg^{-1})	2512	2000	2041	2435	3032	2797
Mg (mg P kg^{-1})	111	78.1	152	166	178	153
C_{SOM} (%)	0.98	0.95	1.16	1.20	1.49	1.12
$C_{HA/FA}$	0.56	0.63	0.82	0.78	1.04	0.84
pH ($CaCl_2$)	6.36	5.91	5.88	6.25	6.81	6.70

P—phosphorus, K—potassium, Ca—calcium, Mg—magnesium, C_{SOM}—carbon soil organic matter, $C_{HA/FA}$—humic and fulvic acid ratio, UAN—urea and ammonium nitrate, UAN + St—UAN and wheat straw, SS—sewage sludge, FYM—farmyard manure.

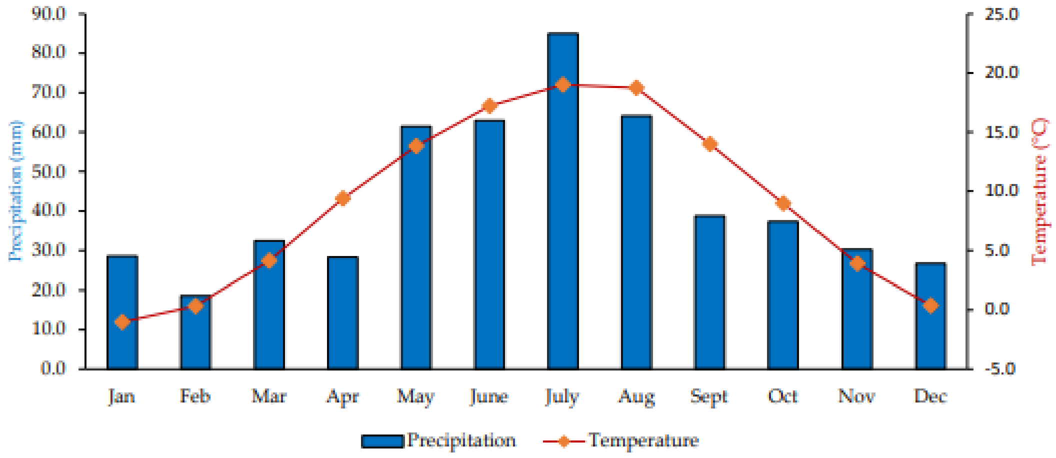

Figure 1. A climograph of average air temperatures and monthly precipitation calculated as average of 1997 to 2019 values.

2.1. Treatments

Six fertilizer treatments were selected, namely: (1) unfertilized control (Control); (2) urea and ammonium nitrate (UAN); (3) UAN and wheat straw (UAN + St); (4) sewage sludge from municipal water treatment facility (SS); (5) cattle farmyard manure (FYM); and (6) cow slurry (Slurry). Annual nutrient inputs from fertilizers are described in Table 3. UAN fertilizer was applied in spring before sowing. Other fertilizers were applied in the autumn before tillage. All organic fertilizers were immediately incorporated into the soil by ploughing. Every fertilizer was applied in a single dose. No additional amendments were added to individual treatments; only stubble from the previous year was incorporated into the soil. Sulfur content analysis in stubble and roots was not performed.

Table 3. Experimental design and nutrient inputs with fertilizers.

Treatment	kg Nutrient ha^{-1} Year^{-1}				C:N:S *	C:S
	N	S	P	K		
Control	0	0	0	0	-	-
UAN	120	0	0	0	-	-
UAN + St	120 + 33.5	2.61	4.34	45.0	14.6:1:0.02	728
SS	120	52.4	82.1	16.0	7.32:1:0.43	17.0
FYM	120	16.4	32.6	129	13.4:1:0.14	95.7
Slurry	120	16.3	23.2	105	5.08:1:0.14	36.3

UAN—urea ammonium nitrate solution, St—wheat straw in average dose of 5000 kg DM (dry matter) ha^{-1} year^{-1}, SS—sewage sludge in average dose of 3101 kg DM ha^{-1} year^{-1}, 30.3% DM. FYM—farmyard manure in average dose of 5027 kg DM ha^{-1} year^{-1}, 23.7% DM. Slurry—slurry in the average dose of 2280 kg DM ha^{-1} year^{-1}, 5.7% DM. * Nutrient ratios in fertilizers calculated based on C:N ratio results in Balík et al. [43] and internal results.

Total sulfur inputs including annual sulfur deposition (dry and wet) are described in Table 4. Precipitation (as the only source of soil water) was measured directly at the experimental site; however, the site was not equipped to detect S depositions. The sulfur deposition data were provided by the meteorological station at Prague in Ruzyně that belonged to the Czech Hydrometeorological Institute. This was the nearest professional station measuring S depositions (the distance is about 10 km by air; GPS: 50°6′0.6″ N, 14°15′19.8″ E).

Table 4. Total sulfur inputs (depositions and fertilizer) during the experiment.

Input Period	Control	UAN	UAN + St	SS	FYM	Slurry
	kg S ha^{-1} per Period					
1993–1996	80	80	90	290	146	145
1997–2001	61	61	74	323	143	143
2002–2007	48	48	64	362	146	146
2008–2013	37	37	53	351	135	135
2014–2019	29	29	45	343	127	127
1993–2019	254	254	325	1670	697	696
1997–2019	175	175	235	1380	552	551

Numbers in the Control and UAN treatments represent the atmospheric depositions that every treatment received. UAN + St, SS, FYM, and Slurry treatments also include inputs from the application of their respective fertilizer.

2.2. *Plant Analyses*

Every year, once the silage maize reached maturity (BBCH 75 or R4 vegetative stage, roughly 65% biomass moisture), the aboveground maize biomass from every plot was harvested and weighed to obtain the biomass yield (BY)—more precisely, two middle rows were harvested (area of 20 m^2). Dry matter content was determined at 105 °C.

To determine the plant sulfur content, the dried samples (for 72 h at 40 °C) were fine milled (<1 mm) (Retsch SM100, Haan, Germany) in laboratory. To determine the plant sulfur content, a wet digestion analysis was performed. Briefly, an aliquot 0.25 g of milled sample was weighed and immersed in nitric acid (7 mL of 65% HNO_3) and hydrogen peroxide (2 mL of 30% H_2O_2). Samples were then digested in a microwave-assisted high-pressure environment. The whole procedure is further described in Tlustoš et al. [44]. This analysis was performed twice for every plot, that is, 8 times per treatment.

2.3. *Soil Analyses*

Topsoil subsamples (0–30 cm depth) were collected in 1997, 2008, and 2019 after the biomass harvest from every plot and pooled together to get one soil sample per plot. Subsoil subsamples (30–60 cm depth) were collected in 1997 and 2019 after harvest from every plot and pooled together to get one subsoil sample. Every sample was later air dried in a forced-air oven until reaching constant weight at 40 °C; then, samples were ground and sieved for particles <2 mm. These samples were archived until further analysis.

To study the changes of different sulfur fractions in topsoil and subsoil, the following methods were selected: A sequential extraction method by Morche [20] and modified by Kulhánek et al. [18] for available sulfur fractions determination. Briefly, samples were extracted with demineralized water (1/10 *w*/*v*) to extract the readily available S (S_W) fraction and subsequently with 0.032 mol L^{-1} NaH_2PO_4 to extract the adsorbed sulfur (S_{ads}) fraction After the distilled water was added, the sample was shaken for 30 min, then centrifuged for 5 min at 8000 *g*. The supernatant was removed and stored in the freezer at 5 °C until further analysis. The same soil sample was then extracted with sodium dihydrogen phosphate, and the process was repeated. The sum of sulfur in these fractions was then the bioavailable sulfur (S_{av}). Usually, extraction by 1 mol L^{-1} HCl follows the extraction of S_{ads} to determine carbonate-occluded S, but this determination was omitted due to the low carbonate content in the investigated soil.

The pseudo-total sulfur (S_{pt}) concentration in the soil was determined by the modified ISO 11466 1995 [45] method using Aqua regia extraction. The modification was microwave-assisted high-pressure digestion and evaporation of samples using a heating plate (150 °C) and subsequent quantitative transfer with distilled water to a final volume of 25 mL glass tube, which were topped up by deionized water and kept at laboratory temperature until measurements were taken.

The organic sulfur content (S_{org}) was calculated as the difference between the pseudo-total content (S_{pt}) and available (S_{av}) content. Mehlich 3 extraction was also performed following Mehlich [30] in order to also evaluate the S_{M3} fraction. Briefly, an aliquot of the soil sample was extracted by Mehlich 3 solution (1/10 *w*/*v*). The sample was then shaken for 5 min and filtered. The filtered extract was stored in a freezer at 5 °C until further analysis. Sequential extraction, Mehlich 3 extraction, and Aqua regia digestion were performed once on every plot, that is, four times per treatment. In 1997 and 2008, the sequential and Mehlich 3 extractions were not performed for subsoil. Aqua regia digestion was not performed in 2008.

Sulfur concentrations in all 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 (Mulgrave, Australia). The operating measurement wavelength for ICP-OES was 180.7 nm for S.

All statistical evaluations were performed using STATISTICA software, version 13 (TIBCO, Palo Alto, CA, USA). Even though trials started in 1993, we evaluated the period of 1997–2019, since a more representative dataset was available. Two-way ANOVA was performed to investigate the interaction of year x treatment and its influence on topsoil S fractions. Since we did not have historical data for the subsoil S content (except for S_{pt}), a two-way ANOVA was not performed. The one-way ANOVA analysis was performed to individually test the influence of year on the topsoil and subsoil sulfur fractions, as well as biomass sulfur content. The same was performed to test the influence of the fertilizer treatment over topsoil and subsoil sulfur fraction content, plant biomass sulfur, uptake, and yield. The differences between means were determined using Tukey´s HSD post-hoc test at $p < 0.05$. The Spearman rank correlation was performed to determine which topsoil sulfur fraction was most related to the content of sulfur in biomass. Only results for $p < 0.05$ are presented, as lower *p*-values (0.01) produced no significant results.

3. Results

3.1. Biomass Yield and S Uptake

Statistical analysis of the average maize yield during the period 1993–2019 (Figure 2a) revealed significant differences between the unfertilized Control treatment and all other fertilized treatments. Meanwhile, differences between the fertilized treatments were all within variance and were not significant.

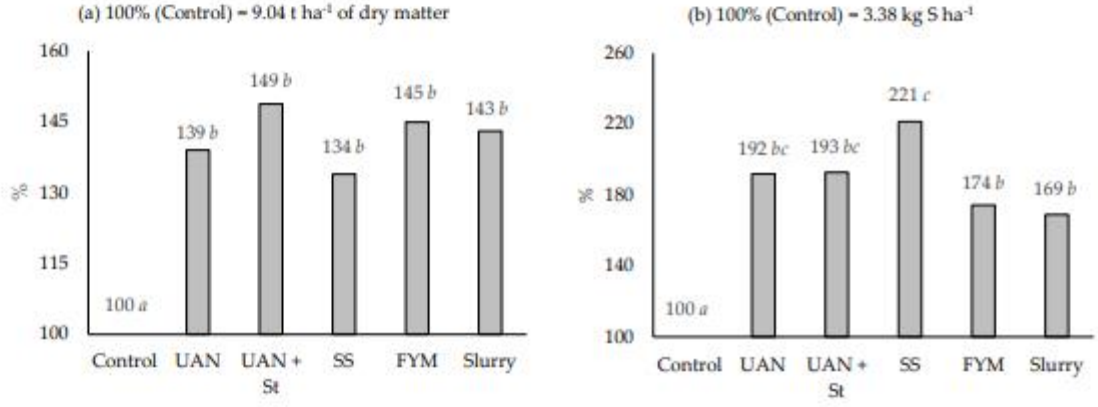

Figure 2. Relative average yields (**a**) and relative average sulfur uptake (**b**) at harvest during the period 1993-2019; the different *italic* letters describe statistically significant differences between treatments. Tukey HSD test; $p < 0.05$. $n = 27$.

Further data are shown in Figure 2b, where the average sulfur uptake in the period of 1993–2019 is reported. The Control treatment showed the significantly lowest values of sulfur uptake by maize in comparison with the fertilized treatments. During this time, there were significant differences, even between the fertilized treatments. Maize with FYM and Slurry treatments had significantly lower S uptakes than plants on SS treatment (which reached the highest uptake of S). UAN and UAN + St treatments were comparable to Slurry and FYM, as well as SS treatments.

Table 5 shows plant sulfur content in the years 1997, 2008, and 2019. Judging by the *italic* letters (comparison of treatments) the Control treatment always reached the significantly lowest S content, while the SS treatment reached the highest content in 2008 and 2019. Judging by the standard letters (comparing the difference between the year in one treatment), it is evident that the S content for all treatments decreased as time moved on.

Table 5. Plant sulfur content (mg S kg^{-1}) after harvest in dry biomass ($n = 8$).

Year	Control	UAN	UAN + St	SS	FYM	Slurry
	Mean	Mean	Mean	Mean	Mean	Mean
1997	568 *a* c	714 *b* c	712 *b* c	681 *b* b	717 *b* c	710 *b* c
2008	472 *a* b	637 *b* b	590 *b* b	754 *c* b	545 *b* b	538 *ab* b
2019	381 *a* a	522 *b* a	484 *b* a	618 *c* a	447 *ab* a	441 *ab* a

The different *italic* letters describe statistically significant differences between treatments (in rows). The different standard letters describe statistically significant differences between years (in columns). Tukey HSD test $p < 0.05$.

The maize sulfur uptake is further examined in Table 6. Judging by the *italic* letters, there were significant differences between treatments, even at the beginning of the experiment. UAN and SS treatments already differed enough from the Control treatment and kept this trend for the duration of the entire experiment. UAN + St was comparable with the Control in the first period; however, in the second and all the following periods, it became significantly different from the Control. Generally, during all of the following periods, all treatments produced significantly higher values than the Control. Overall, the highest uptake was always present in the SS treatment.

Table 6. Detailed sulfur plant uptake in the period during the experiment (kg S ha^{-1} per period).

Uptake Period	Control	UAN	UAN + St	SS	FYM	Slurry
	Mean	Mean	Mean	Mean	Mean	Mean
1993–1996	15.7 *a*	25.3 *b*	24.2 *ab*	28.8 *b*	23.0 *ab*	21.7 *ab*
1997–2001	24.8 *a*	42.7 *bc*	35.4 *bc*	43.6 *b*	34.7 *b*	33.5 *b*
2002–2007	22.2 *a* b	43.2 *b* b	42.9 *bc* b	48.2 *b* b	39.5 *b* b	40.6 *b* b
2008–2013	17.6 *a* ab	39.8 *bc* b	40.3 *bc* b	42.9 *c* ab	32.5 *bc* a	28.3 *ab* a
2014–2019	16.1 *a* a	32.3 *b* a	33.1 *bc* a	38.6 *b* a	29.4 *ab* a	30.1 *ab* a
1993–2019	96.3	183	176	202	159	154
1997–2019	80.6	158	152	173	136	133

The different *italic* letters describe statistically significant differences among treatments (in rows). The different standard letters describe statistically significant differences among years (in columns). Tukey HSD test ($p < 0.05$); $n = 4$ for years 1993–1996 and 1997 and 2001; $n = 6$ for following periods.

Evaluating the changes in treatments in years (judging by standard letters) shows that all treatments produced lower uptakes in later periods than the earlier ones, even in the fertilized treatments. Only results from the last three periods were compared, as these periods consisted of an equal number of years.

Spearman's correlation coefficient was calculated in order to evaluate the importance of each soil sulfur fraction in maize nutrition (Table 7, results of 1997, 2008, and 2019 were pooled together for this analysis). Given the influence of the fertilizer treatment over the values, the relationship between the values was monotonic. Therefore, Spearman's correlation was chosen. The test revealed that S_W, S_{ads}, S_{av}, S_{M3}, and S_{org} fractions were significant in this relationship. The relationship between plant S content and soil S_{pt} fractions was not significant.

Table 7. Spearman's correlation coefficient values comparing the relationship of plant sulfur content with soil sulfur content.

	S_W	S_{ads}	S_{av}	S_{M3}	S_{pt}	S_{org}
Plant S content	0.741 *	0.669	0.743 *	0.694 *	0.245	0.564 *

Values marked with * were significant at $p < 0.05$; $n = 72$.

3.2. *Topsoil Sulfur Content*

A two-way ANOVA was performed for topsoil in order to establish the influence of the year and treatment and their interaction with the content of S fractions in topsoil. The results in Table 8. show that there was no significant interaction for year and treatment. On the other hand, individually, these effects had significant influences on the content of S fractions in topsoil.

Table 8. Results for two-way ANOVA comparing the effects of treatment, year, and their interaction on the S content in individual S fractions in topsoil.

Topsoil S Fraction	**Treatment**		**Year**		**Treatment × Year**	
	F	**df**	**F**	**df**	**F**	**df**
S_W	6.62 *	5	206.96 *	2	0.44	10
S_{ads}	7.80 *	5	91.99 *	2	0.84	10
S_{av}	7.51 *	5	205.69 *	2	0.49	10
S_{M3}	15.19 *	5	108.07 *	2	0.46	10
S_{org}	19.76 *	5	5.80 *	2	0.98	10
S_{pt}	22.43 *	5	23.13 *	2	0.95	10

The values marked with asterisk (*) are significant effect at $p < 0.05$.

To further investigate the effect of the year on the content of S fractions in topsoil, a one-way ANOVA was performed. The results of the soil S content analysis revealed the same pattern across all soil sulfur fractions. The mineral fraction, namely S_W, S_{ads}, S_{av}, and S_{M3} in Figures 3–6, respectively showed a significant decrease in sulfur pools in all treatments, whether fertilized or not. Generally, the biggest significant decrease happened between the years 1997 and 2008 for S_W and $S_{av,}$ while the decrease from 2008 and 2019 was smaller and, in many cases, was not significant. The rate of decrease in S pools for S_{ads} and S_{M3} fractions from 1997 to 2008 was not as big as the rate for S_W and S_{av}. The S_{av} content resembled the changes in the S_W and S_{ads} fractions, because the S_{av} fraction content was calculated as a sum of water-soluble and adsorbed content. The proportion of S_W in S_{av} changed over the years. In 1997, the proportion of S_W in S_{av} from all treatments around was, on average, 76%. In 2019, this proportion was, on average, around 60%. On the other hand, the proportion of S_{ads} in S_{av} increased accordingly.

The significantly decreasing soil sulfur pools were found even in the S_{pt} (Figure 7) fraction with few exceptions. Firstly, although the SS treatment showed a decreasing trend, due to variability in results, the decrease was insignificant. Given several more years, the situation would be likely to change. The second exception was the FYM treatment, which produced insignificant changes. The FYM and SS treatments had, in general, the highest contents of S_{pt} fractions. The percentages present in each column of Figure 7 show the proportion of organic sulfur out of the pseudo-total. It is evident that, in all treatments, the proportion of organic sulfur increased over time. This means that mineral S pools accordingly decreased over the course of the experiment. On average, the proportion of S_{av} in S_{pt} in 1997 was 15.4%, while the same proportion in 2019 was only 7.7%.

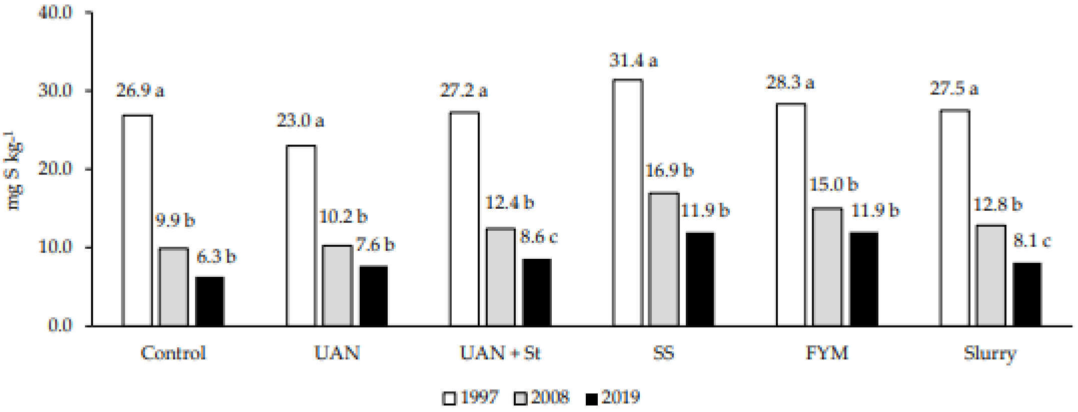

Figure 3. Content of water-soluble sulfur fraction (S_W) in topsoil. Different letters describe significant differences between years within the treatment. Tukey HSD test ($p < 0.05$), ($n = 4$).

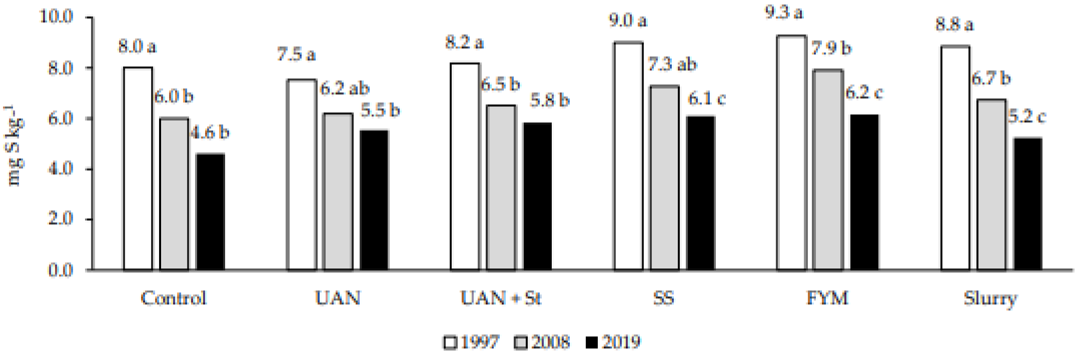

Figure 4. Content of adsorbed sulfur fraction (S_{ads}) in topsoil. Different letters describe significant differences between years within the treatment. Tukey HSD test ($p < 0.05$), ($n = 4$).

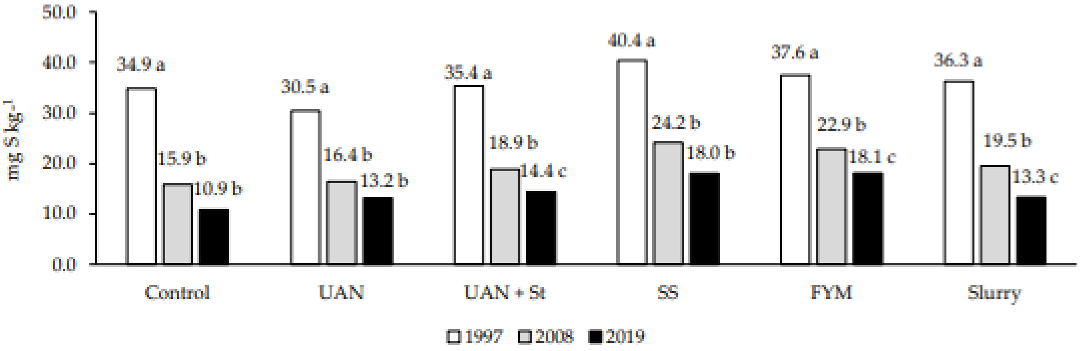

Figure 5. Content of available sulfur fraction (S_{av}) in topsoil. Different letters describe significant differences between years within the treatment. Tukey HSD test ($p < 0.05$), ($n = 4$).

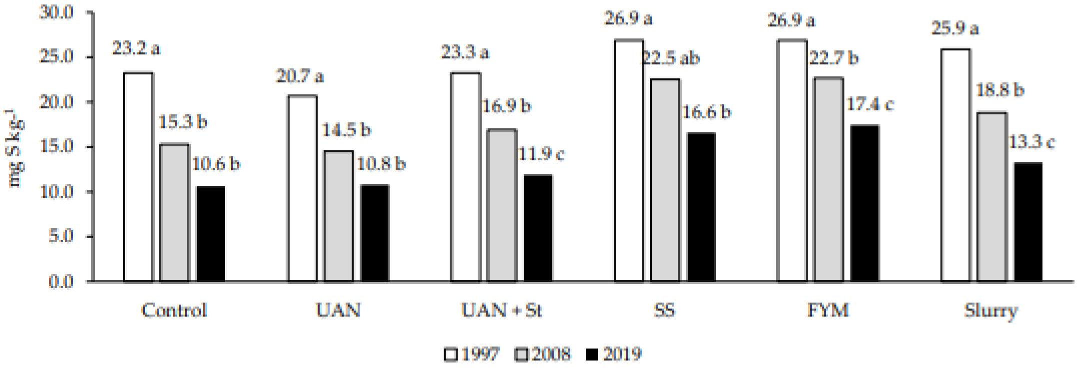

Figure 6. Content of Mehlich 3 extractable sulfur fraction (S_{M3}) in topsoil. Different letters describe significant differences between years within the treatment. Tukey HSD test ($p < 0.05$), ($n = 4$).

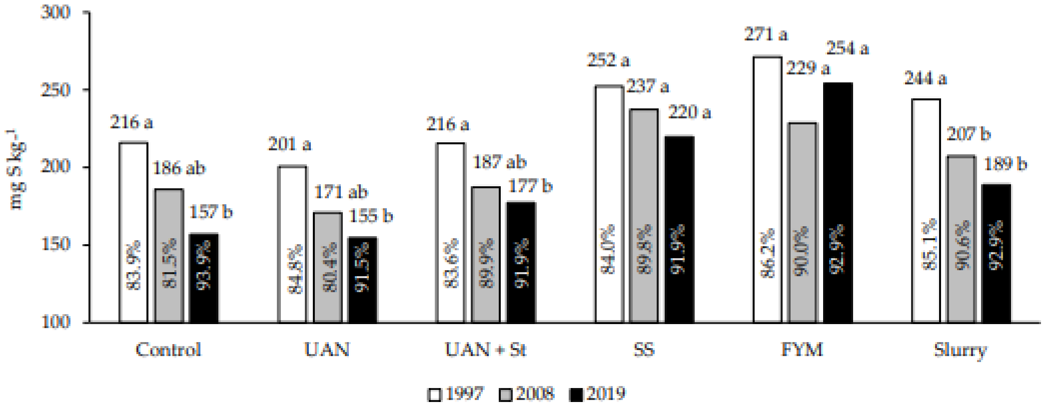

Figure 7. Content of pseudo-total sulfur fraction (S_{pt}) in topsoil. Different letters describe statistically significant differences between years within the treatment. Tukey HSD test ($p < 0.05$), ($n = 4$). Percentages shown inside the columns are a proportion of organic sulfur fraction out of pseudo-total sulfur content.

The results present in Figure 8 describe changes in the S_{org} fraction over time for each treatment (calculated as the difference between the pseudo-total content and available content). The lowest content of sulfur in this fraction, as well as the most significant decreases, were present in the Control and UAN. On the other hand, the organic sulfur content did not produce any significant change in organic fertilized treatments, namely, the SS, FYM, and Slurry. It is important to note that the Slurry treatment showed a decreasing trend. This trend might become significant over more time.

The data presented in Table 9 allow further investigation of the soil S changes in individual fractions. The presented values are a ratio comparing the 1997 S content to the 2019 S content. Generally, we can see that the biggest decrease in soil S happened for the S_W fraction, down to 23–42%, of the 1997 content. The FYM S_W content for this treatment was significantly higher than that of the control. The content of S in the S_{av} fraction decreased to 31–48%; however, there were no differences between treatments, which is likely due to the fact that the S_{av} fraction has to reflect changes in both the S_W (where some changes were significant) and S_{ads} fractions (where all the values were not statistically different from each other). Sulfur in the S_{org} fraction showed a change to 81–101% of the 1997 values. Organic fertilizer treatments in this fraction produced a smaller decrease than the Control and UAN treatments. On the other hand, statistical differences were only present between the control and FYM treatments. This also corresponded to a decrease in the S_{pt} fraction, where the SS, FYM and UAN + St fractions showed a smaller decrease than the other treatments.

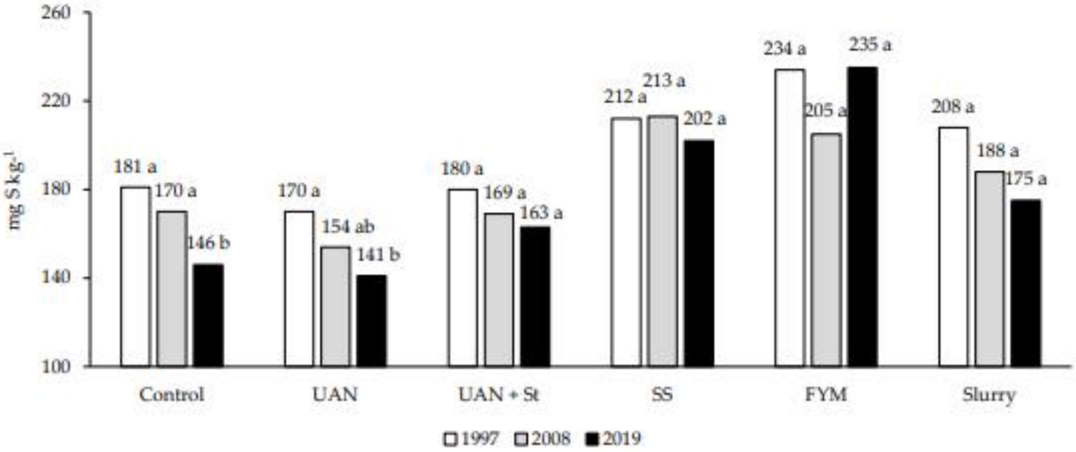

Figure 8. Content of organic sulfur fraction (S_{org}) in topsoil. Different letters describe statistically significant differences between years within the treatment. Tukey HSD test ($p < 0.05$), ($n = 4$).

Table 9. Relative changes (%) in the content of respective sulfur fractions in topsoil. Values represent relative content in the year 2019 in comparison with the year 1997 (100% = content in 1997). Mean plus standard deviation (SD).

Treatment	S_W	S_{ads}	S_{av}	S_{org}	S_{pt}
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Control	23 ± 8.00 *a*	58 ± 9.40 *a*	31 ± 9.44 *a*	81 ± 7.93 *a*	73 ± 5.05 *a*
UAN	33 ± 10.1 *ab*	73 ± 11.4 *a*	43 ± 10.7 *a*	83 ± 9.85 *ab*	77 ± 10.5 *a*
UAN + St	32 ± 4.52 *ab*	71 ± 11.0 *a*	41 ± 5.18 *a*	90 ± 6.18 *ab*	82 ± 4.17 *ab*
SS	38 ± 3.44 *ab*	68 ± 4.43 *a*	45 ± 2.49 *a*	95 ± 6.43 *ab*	87 ± 5.41 *ab*
FYM	42 ± 7.93 *b*	67 ± 5.08 *a*	48 ± 7.13 *a*	101 ± 3.29 *b*	94 ± 2.95 *b*
Slurry	29 ± 2.42 *ab*	59 ± 3.17 *a*	37 ± 1.90 *a*	85 ± 6.85 *ab*	77 ± 5.25 *a*

The different *italic* letters describe statistically significant differences between treatments per soil sulfur fraction. Tukey HSD test $p < 0.05$ ($n = 4$).

3.3. *Subsoil*

The results of the subsoil S content analysis are presented in Table 10. Since the results in previous years are incomplete, we decided to only present the findings of the year 2019. Table 10 shows differences between the treatments in each of the investigated S fractions. For the S_W fraction, the Control was comparable to the UAN and UAN + St, while it was significantly lower than the FYM and Slurry. It is also important to note that the SS treatment was significantly higher than all of the previous treatments (with a roughly six times greater value than the Control). For the S_{av} fraction, the S content in the Control reached higher values than the UAN and UAN + St and lower values than the FYM and Slurry. Nevertheless, the Control was statistically comparable to those treatments. The UAN and UAN + St had significantly lower S contents than the FYM and Slurry. All treatments showed also significantly lower values than the content of S in the SS treatment. Interestingly, the sulfur content of the SS treatment was comparable to the FYM, Slurry, and UAN + St treatments in the S_{ads} fraction and, furthermore, the SS treatment in the S_{org} fraction was comparable to the content of the Control, UAN + St, and UAN. The highest

S_{org} content reached by the FYM treatment was significantly different from all fractions, except for the Slurry. Out of the S_{av} fraction, the S_W fraction for the Control, UAN, and UAN + St treatments comprised 56.7–59.3%. The FYM and Slurry comprised 68.2–68.7%, and the SS reached up to 81.3%.

Table 10. The content of sulfur fractions in subsoil (mg S kg^{-1}) in the year 2019.

	S_W	S_{ads}	S_{av}	S_{org}
Control	3.5 *a*	2.7 *a*	6.1 *ab*	109 *ab*
UAN	3.0 *a*	2.2 *a*	5.1 *a*	93 *a*
UAN + St	4.6 *a*	3.1 *ab*	7.7 *a*	112 *ab*
SS	21.8 *c*	5.0 *b*	26.8 *c*	112 *ab*
FYM	8.9 *b*	4.0 *b*	12.9 *b*	138 *c*
Slurry	7.5 *b*	3.5 *ab*	11.1 *b*	119 *bc*

The different *italic* letters describe statistically significant differences between treatments in a fraction at $p < 0.05$ Tukey HSD test, $n = 4$.

Changes in the S_{pt} fraction in the subsoil are described in Figure 9. Both the SS and UAN treatments presented a decrease in sulfur from the year 1997 to 2019 in this fraction. Due to variability, this decrease is not significant. The Control, UAN + St, and Slurry treatments showed an insignificant increase, while the FYM content stayed the same in both years. Judging by the *italic* letters, the content of S in this fraction in the year 2019 was lowest for the UAN treatment, which was statistically comparable to the Control, UAN + St, and Slurry treatments. Significantly high S contents were produced by the SS and FYM treatments.

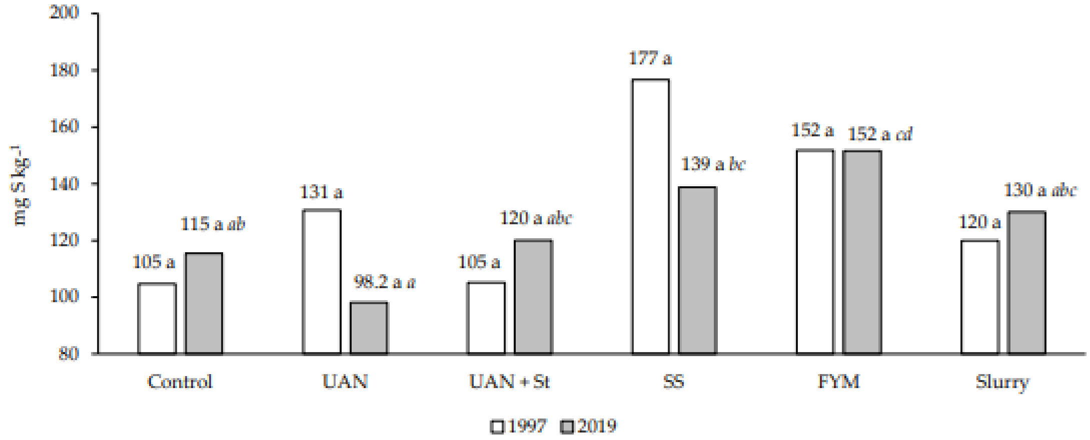

Figure 9. Content of pseudo-total sulfur (S_{pt}) fraction in the subsoil in 1997 and 2019; the different standard letters describe statistically significant differences between years. The different *italic* letters (for 2019) describe significant differences between treatments. Tukey HSD test ($p < 0.05$), ($n = 4$).

The S_W and S_{av} content proportion of the S_{pt} in 2019 subsoil was, on average, 6.2% and 8.9%, respectively. It is important to note that, for the SS treatment, this proportion was 15.7% and 19.3% for the S_W and S_{av}, respectively. Other values fell close to the average.

3.4. Comparing the Pseudo-Total Sulfur Pools in Topsoil and Subsoil

Pseudo-total sulfur content in the topsoil (0–30 cm depth) and subsoil (30–60 cm depth) is present in Table 11 in kilograms of sulfur per hectare. These values were calculated from the S_{pt} content, which is also presented in Figure 7 (for topsoil) and Figure 9 (for subsoil).

Table 11. Balance of pseudo-total sulfur in topsoil and subsoil comparing the year 1997 to 2019.

Treatment	Control	UAN	UAN + St	SS	FYM	Slurry
	kg S ha^{-1}					
Topsoil						
1997	952 *ab* b	885 *a* b	951 *ab* a	1113 *bc* a	1197 *c* a	1076 *abc* b
2019	692 *ab* a	682 *a* a	782 *ab* a	969 *bc* a	1121 *c* a	832 *abc* a
Balance	−260	−203	−169	−144	−76	−243
Subsoil						
1997	471 *a* a	587 *a* a	473 *a* a	795 *a* a	683 *a* a	540 *a* a
2019	519 *ab* a	442 *a* a	540 *abc* a	625 *bc* a	682 *c* a	585 *abc* a
Balance	+48	−146	+67	−171	−1	+45

The different *italic* letters describe statistically significant differences between treatments in a year (in rows). The different standard letters describe statistically significant differences between years of treatment (in columns). Tukey HSD test, $p < 0.05$, $n = 4$.

Judging by the *italic* letters for the topsoil, the differences between treatments were the same for both years 1997 and 2019. Generally, the Control, UAN, and UAN + St treatments had statistically comparable contents of S. The SS and FYM significantly had the highest contents of S and were comparable to each other. The Slurry treatment was comparable to both groups. When focusing on the development of the S content over the years (judging by the standard letters) it is visible that the Control, UAN, and Slurry treatments showed a significant decrease in soil S.

Regarding the subsoil, there were no statistically significant differences between treatments in the year 1997. In the year 2019, the lowest values were produced by the UAN treatment, which was comparable to the Control. The SS and FYM reached the highest significant contents of S. The Slurry and UAN + St were comparable to all groups, due to their high variability.

The balance row describes how much sulfur each of the treatments gained (+) or lost (−) during the entire experiment. Overall, in the topsoil, only the loss of pseudo-total sulfur content was registered (ranging from −76 to −260 kg S ha^{-1}). For the subsoil, the only treatments that registered the influx were the UAN + St and Slurry (+67 and +45 kg S ha^{-1}, respectively), while all other treatments lost S content (ranging from −1 to −171 kg S ha^{-1}).

3.5. Sulfur Movement in Topsoil and Subsoil

Table 12 describes the vertical movement of the pseudo-total sulfur in topsoil and subsoil during the trials. In the topsoil, the smallest loss was present for the UAN treatment, followed by the UAN + St and Control treatments at 221, 252, and 356 kg S ha^{-1}, respectively. The FYM and Slurry reached the loss of 492 and 661 kg S ha^{-1}, respectively. The greatest S loss was calculated for the SS treatment with 1350 kg S ha^{-1}. The trend was similar for the subsoil, where the smallest loss was present for the UAN + St, Control, and UAN treatments at 186, 309, and 366 kg S ha^{-1}, respectively. The FYM and Slurry lost 493 and 616 kg S ha^{-1}, respectively. The SS treatment also lost the most S in the subsoil at 1521 kg S ha^{-1}.

Table 12. Movement of sulfur in topsoil and subsoil during the trials.

Treatment	Control	UAN	UAN + St	SS	FYM	Slurry
Topsoil (kg S ha^{-1})						
Status 1997 and inputs in 1997 to 2019 (a)	1127	1060	1186	2493	1749	1626
Status in 2019 and uptake 1997 to 2019 (b)	771	838	934	1143	1257	965
Loss from topsoil (a,b)	356	221	252	1350	492	661
Subsoil (kg S ha^{-1})						
Status in 1997 and loss from topsoil (c)	828	808	726	2146	1175	1201
Status in 2019 (d)	519	442	540	625	682	585
Loss from subsoil (c,d)	309	366	186	1521	493	616

4. Discussion

4.1. Biomass Yields and Maize Sulfur Uptake

The average relative biomass yields from the entire experiment are displayed in Figure 2a. The Control treatment significantly produced the lowest yields of biomass from the fertilizer treatments. The differences in yields between all fertilizer treatments were insignificant. All treatments, with the exception of the Control, received 120 kg of N ha^{-1} year^{-1} as fertilizer. On top of that, the UAN + St treatment received an additional dose of 33.5 kg of N ha^{-1} year^{-1} from the straw. Even though the yields for this treatment were, on average, the highest, there was no significant difference from other fertilizer treatments. Different sulfur inputs played no role in the production of yields, as maize seemed to respond to nitrogen fertilizer. The soil P, K, Mg, and Ca supply (Mehlich 3 extractables) was sufficient for maize growth for all treatments (Table 2).

The relative sulfur uptake (Figure 2b) from the entire experiment showed more varying results than yields. Maize from the Control treatment without any fertilizer inputs significantly produced the lowest uptake. On the other hand, the highest uptake was produced by maize from the SS treatment, which received 52.4 kg of S ha^{-1} year^{-1} as fertilizer (the highest dose in this experiment).

Godlewska [46] reported an increase of 140% and 130% in S uptake over their control by plants in composted sewage sludge and fresh sewage sludge treatments, respectively. An increase in the uptake of sulfur by maize was also reported by Sakal et al. [47]. An increased uptake of S was also presented by Knights et al. [35], where a long-term application of FYM in a maize monoculture caused an increase in uptake to 11 kg of S ha^{-1} year^{-1} over their control (2.0 kg of S ha^{-1} year^{-1}).

Statistically comparable to the uptake of S for the SS treatment were the UAN and UAN + St treatments, although the UAN + St received 2.61 kg of S ha^{-1} year^{-1}. Interestingly, the increases in the uptake of S for the UAN and UAN + St treatments were most likely produced by the dose of 120 kg of N ha^{-1} year^{-1} on these treatments. This phenomenon was also measured by Weil and Mughogho [12], where a significant increase in S uptake was produced for a treatment that received 80 kg of N ha^{-1} year^{-1} and no sulfur as fertilizer. Similarly, Knights et al. [35] also determined that pure mineral N fertilization (96 kg N ha^{-1} year^{-1}) with zero sulfur caused a 2.6 fold increase in S uptake.

Table 7 shows a correlation analysis of the plant sulfur content with the soil sulfur content in individual fractions. S_W, S_{ads}, S_{av}, and S_{org} content correlated with plant $p < 0.05$. The results of correlation analysis agree with Boye et al. [27] and Morche [20], who mentioned that these fractions are very critical for proper plant nutrition. These authors also mentioned that S_{org} content can be mineralized throughout the period of crop growth and resupply the mineral fractions, thus explaining the correlation of plant S content with soil S_{org} content. There was a significant correlation between S_{M3} content and plant sulfur content. Sedlář et al. [48] also describe this relationship between plant sulfur content and S_W and S_{M3} soil content on a wide variety of soil types and crop rotation systems that included maize across the entire Czech Republic.

Generally, the content of plant S was greater for fertilizer treatments in comparison with the Control (Table 5), although, in some cases, in years 2008 and more so in 2019, the difference was insignificant. This is an important factor to note, since S is part of the primary and secondary metabolism in crops [1] and generally can influence yield quality [2]. Since we analyzed the entire aboveground biomass and not just the grain, we cannot comment on the influence of fertilization on the S content in the grains. In summary, the increase in yields can be attributed to the dose of nitrogen, and the sulfur dose had no significant effect on yields. The overall increase in S uptake for all N fertilizer treatments in our experiment can be mostly attributed to the application of nitrogen. The application of nitrogen fertilizer in sufficient doses increases crop yields [12,13,47] and, thus, also increases the uptake of other nutrients [13], including sulfur [49]. On the other hand, the application of sulfur in the SS treatment at the dose of 52.4 kg of S ha^{-1} year^{-1} was sufficient enough to produce a significant increase in biomass sulfur concentration and, in turn, increase the sulfur uptake over the FYM and Slurry treatments, which both received approximately only 16 kg of S ha^{-1} $year^{-1}$ and the same dose of N as the SS treatment.

4.2. Topsoil and Subsoil Sulfur Content

For maize to have a sufficient supply of sulfur, there needs to be at least 10 mg of S kg^{-1} of soil in the plant available fraction (S_{av}) [50]. In our experiment, the S_{av} content was not lower than that amount (Figure 5), even though the soil samples were collected after the maize biomass harvest when the S pools were drained. This means that, during vegetation, the content of S_{av} should be high enough to supply the crop with a sufficient amount of sulfur.

The results displayed in Figures 3–6 show that there was a significant decrease in sulfur content in the soil from 1997 to 2019 in terms of S_W, S_{ads}, S_{av}, and S_{M3} fractions.

Balík et al. [28] investigated soil sulfur status changes from 1981 to 2007 from a wide variety of sites and crop rotations under FYM, FYM + NPK, and unfertilized Control treatments. The authors found a decrease in the S_W, S_{ads}, S_{av}, and S_{pt} fraction contents to 32, 61, 50, and 92% of their initial values in 1981, respectively. Furthermore, they also found an increase in the proportion of S_{org} in the S_{pt} content from 79.3% in 1981 to 88% in 2007. The results in our study (Table 9, Figure 7) show a very similar trend to the results of Balík et al. [28]. Table 9 also shows a slight decrease in S_{org} content for all treatments except the FYM, which seemed to stay near the original value. The differences between treatments in this sulfur fraction were insignificant with the exception of the Control, which reached the significantly lowest value. This is in accord with Forster et al. [34], who showed an increase in S_{org} for treatments amended with FYM. Figure 8 shows the decrease in S_{org} in absolute numbers for the Control, UAN, and UAN + St in 2019 in comparison to 1997. The SS, FYM, and Slurry treatments had rather high initial values of S_{org} content in comparison with the Control, UAN, and UAN + St treatments. This was caused by the fact that the site was already fertilized since 1993, and inputs of organic carbon and sulfur influenced the S_{org} content. The same can be said for the S_{pt} content (Figure 7). Interestingly, the slurry treatment also showed a decrease in S_{org} (unlike the SS and FYM treatments), but it was insignificant. If given enough time, this trend might become significant in the future.

Foster et al. [34] evaluated the status of soil S pools in the long-term experiment initiated in 1962 under crop rotation using different fertilizer treatments. They found that sewage sludge and farmyard manure fertilization increased total sulfur content significantly, which is generally in agreement with the results of S_{pt} in our study. Furthermore, the authors determined the S_{av} content in the interval between 10.0 and 17.8 mg of S kg^{-1}, which also confirms our results (Figure 5) with the interval of 10.9 to 18.1 mg S kg^{-1}. The S_W content in the soil is the most labile and susceptible to changes [20] and is most influenced by plant uptake [34,35], available soil sulfur supply [28,29], sulfur added in terms of fertilizers [51], and, most notably, the decrease in inorganic sulfur inputs from the atmosphere [3,4,31]. Another major factor that comes into play here is organic fertilization; this effect is later discussed in Section 4.3.

In the Czech Republic, the Mehlich 3 extraction method has been used to determine plant-available nutrients (e.g., P, Mg, Ca, K), and, recently, this method has been adopted in order to evaluate the plant-available S in soil. The methodology according to [52] puts arable soil into five categories according to the S_{M3} status. In 1997, all of the treatments in our study would have fit into the "satisfactory" category (ranging from 21–30 mg S_{M3} kg^{-1}). In 2008, all treatments except the SS and FYM shifted into the "low" category (ranging from 11–20 mg S_{M3} kg^{-1}), and, by 2019, even the SS and FYM would place in the same category as the others. For the subsoil, there is no comparison. Zbíral et al. [31] evaluated sulfur soil pools for a wide range of Czech soils using the Mehlich 3 method. The authors found that, on average, there was a decrease in S_{M3} content in the soil from 33 mg of S kg^{-1} in 1981 to 8 mg of S kg^{-1} in 2017 for control treatments with no fertilizers. They also reported a decrease from 26 mg of S kg^{-1} in 1995 to 17 mg of S kg^{-1} in 2013. This corresponds well to the results in our study, where Zbíral et al. [31] attributed this decrease to the reduction in SO_2 emissions and, in turn, the reduction in total sulfur depositions that occurred in the 1990s in the Czech Republic.

In consideration of the S_{M3} fraction (Figure 6), the extracted S content was almost identical to the S_{av} fraction (Figure 5). The correlation coefficient between plant S content and soil S_{M3} content (Table 7) was 0.694 (significant at $p < 0.05$), which suggests that a Mehlich 3 extractant could release plant-available sulfur content. Kulhánek et al. [18] determined the correlation coefficient at 0.882 (significant at $p < 0.001$) for the S_{M3} and S_{av} fractions. Unlike our results, the results in Kulhánek et al. [18] were collected for a wider range of soil types and from farms, which included crop rotation, as well as monocultures. A significant positive correlation between the S_{av} and S_{M3} content has also been reported outside of the Czech Republic [53,54], thereby making this method suitable for determining the plant-available sulfur.

The general decrease in S in mineral sulfur fractions (including S_{M3}) in topsoil from all organic, mineral, or even control treatments can only be attributed to the decrease in SO_2 emissions, as suggested by Zbíral et al. [31] or Balík et al. [28], from hundreds of kg of S ha^{-1} $year^{-1}$ in the late 1980s or early 1990s to just units of kg of S ha^{-1} $year^{-1}$ [6], which was even reflected by authors in Europe [34,35].

The results of the subsoil S_{pt} content are presented in Figure 9; however, the variability of the results does not help the interpretation. When comparing the S_{pt} content in 1997 and 2019, there seems to be no statistical differences when judging by standard letters, yet the content for the SS treatment decreased by 21.5%. It seems to resemble the results of the topsoil (Figure 7). The initial content of S_{pt} for this treatment was, however, 1.68 fold that of the Control. Given the fact that sewage sludge contains a lot of potentially mineralizable S [34], it is possible that the content of sulfur was mineralized over time and was susceptible to upward movement caused by plant uptake (maize roots can reach a depth greater than 60 cm) [55] or downward movement in terms of the leaching of sulfate anions [21,22]. Indeed, the mineral S_W content in this treatment sustained a 6.23-fold increase (Table 10) over the Control in 2019, while the S_{org} content was almost identical to the Control. The UAN treatment produced a decrease in S_{pt} content (Figure 9). This can be explained by the fact that the initial S_{pt} content of this treatment was higher than the Control and also by the fact that the C_{SOM} content in this treatment decreased in the subsoil [56]. This is further supported by Table 10, where the lowest content of S_{org} was present in the UAN treatment. The Control, UAN + St, and Slurry treatments produced a slight increase in S_{pt} content from 1997 to 2019 (Figure 9). These treatments had comparable contents of S_{org} (Table 10). This would suggest that the doses of S and C in the UAN + St and Slurry were not enough to have a significant effect on the topsoil and subsoil S_{pt} and S_{org} pools, and the S supplied by the Slurry was easily mineralized [36,37]. This is further confirmed in Table 10, where we can see a 2.14-fold increase in S_W for the Slurry over the Control. The levels of S_{pt} in the FYM in the subsoil did not change over time. The S_W and S_{org} contents for this treatment in 2019 was also significantly higher than those in the Control, which suggest that FYM plays an important role in maintaining sufficient S levels in the subsoil. The trend for the FYM in the subsoil was identical to that in the topsoil.

4.3. *Influence of Organic Carbon*

One of the main effects of organic matter application is the sustaining and/or improvement of the soil organic matter (C_{SOM}) content. Balík et al. [56] reported changes in the C_{SOM} content for their current experimental site in a prior paper. At the beginning of the experiment in 1993, the C_{SOM} content was 1.26%. In 2018, the C_{SOM} content changed for the Control, UAN, UAN + St, and FYM treatments to 0.98, 0.95, 1.16, and 1.49%, respectively. This was due to the fact that maize biomass was harvested, and only stubble and roots were again incorporated into the soil. The dose of straw for the UAN + St treatment was also not sufficient to maintain the levels of C_{SOM} and produced an insignificant decrease in the C_{SOM}. On the other hand, the FYM treatment was supplied with enough carbon to produce a significant increase.

The content and quality of organic matter supplied with fertilizers has influence over the content of S_{org} and S_{pt} fractions in the topsoil. Although the dose of S was greater in the SS (52.4 kg of S ha^{-1} year^{-1}) than in the FYM (16.4 kg of S ha^{-1} year^{-1}), the S_{pt} and S_{org} content was greater in the FYM than in the SS. Meanwhile, the Slurry treatment received almost identical doses as the FYM and reached a lower content of S for the S_{pt} and S_{org} fractions in 2019, respectively. Generally, organic materials with higher C:S ratios are prone to immobilization [34], which includes manures. Sewage sludge has a large pool of potentially mineralizable S [34], where a significant portion of inorganic sulfur is present in sulfate and metal sulfides [41]. The SS also had the highest overall S content. Slurries have generally much lower C:S ratios [36] and are more prone to mineralization than other organic fertilizers [37]. In Table 3, we can see the C:S ratios for organic fertilizers. The highest ratio (over 700) was present in the UAN + St treatment, which would suggest immobilization according to Zhang et al. [57]. Based on Figure 8, it is obvious that the S_{org} content did not, in fact, significantly decrease. However, given the trend, it is very likely that the change will be significant over time. After all, the dose of sulfur was only 2.61 kg of S ha^{-1} year$^{\cdot}$. The trend of S_{org} content for the Slurry treatment was similar to that of the UAN + St, where the dose of applied sulfur was higher (16.3 kg of S ha^{-1} year^{-1}); however, the C:S ratio was 36.3. The combination of a relatively higher fulvic acid content, which is less stable (discussed later, Section 4.4.), can explain the trend in S_{org} content. The SS treatment had the lowest C:S ratio (17.0), which means that the mineralization of sulfur should occur [57]. According to Dewill et al. [41] and Sommers et al. [42], a significant percentage of sulfur in SS fertilizer is inorganic and could possibly be released [21,22]. The S_{org} content (Figure 8) did not change over the course of the experiment, which suggests that the dose of sulfur in the SS was right to maintain the content of organic-bound sulfur. The FYM treatment produced no change in the S_{org} content (Figure 8) at a much lower sulfur dose than the SS. This is due to the fact that FYM materials have a higher C:S ratio and are more stable than many other organic materials [34]. Generally, the increase in total soil sulfur is mainly driven by the increase in organic carbon [34], which is in accordance with our results. In a different experiment, with over 150 years of constant pure mineral S application, there was no significant increase in soil sulfur content [35].

The application of organic matter into the soil can generally increase the water-holding capacity [58] and, in turn, increase the content of the water-soluble S_W fraction. The results in Figure 3 show that the S_W content was indeed higher for the organic fertilized treatments; most notably, the SS and FYM treatments reached 1.89-fold increases over the Control treatment. Kulhánek et al. [29] investigated changes in the S_W and S_{av} content from 1996 to 2014 with a crop rotation through long-term field experiments. They found a decreasing tendency in the S_{av} fraction, but for the sludge fertilized treatment of potatoes, there was an increase in S_W content, while a decreasing tendency was observed for the following crops, thereby suggesting that the effectiveness of SS deteriorates over time.

4.4. Topsoil and Subsoil Sulfur Movement

Table 11 shows the change in topsoil and subsoil pseudo-total sulfur content from 1997 to 2019 in kg of S ha^{-1}. These values were calculated from Figures 7 and 9 for the topsoil and subsoil, respectively, using the bulk density of soil.

The balance of sulfur in the soil is, of course, influenced by maize sulfur uptake and leaching. [21,22]. From among the organic fertilizer treatments, the FYM produced a lower decrease in soil sulfur pools, followed by the SS and UAN + St treatments. This was due to inputs of organic carbon in fertilizers that cause an increase in organic carbon in soil [35,38,58] and, in turn, increase organic-bound sulfur in the soil [34,35]. The Control and mineral-only UAN produced a similar, high decrease in balance, as these treatments received no organic carbon. The Slurry treatment showed interesting results. It also received organic carbon but balanced more similarly to the Control and UAN treatments. A possible criterion for the evaluation of the stability of soil organic carbon can be the humic and fulvic acid ratio (C_{HA}/C_{FA}) [59,60], where a higher content of C_{FA} represents a lower stability of the soil organic carbon [61]. The C_{SOM} content and C_{HA}/C_{FA} in the Slurry treatment were 1.12% and 0.84, respectively, in 2019 (Table 2) in comparison with the FYM treatment, where the same parameters were measured at 1.49% and 1.04, respectively, in 2019. The Slurry treatment, overall, had a lower content of organic carbon as well as a higher proportion of a less stable carbon fraction. Our original expectations for balance regarding the UAN + St and SS treatments were also surpassed—in a negative way. We did not expect the balance of these treatments to be this low. Looking at the C_{SOM} content and C_{HA}/C_{FA} ratio might shed some light on the results (Table 2). The C_{SOM} content and C_{HA}/C_{FA} ratios for the UAN + St were 1.16% and 0.82, respectively. For the SS treatment, the C_{SOM} content and C_{HA}/C_{FA} ratio were 1.20% and 0.78, respectively. This could explain their similar behavior. On top of that, in Table 3, we can see that the Slurry and SS had similar C:N ratios (5.05 and 7.32, respectively), which means that the carbon content in these fertilizers was similar, yet the C:S ratio for the SS was double in comparison to the Slurry. If we consider that the sulfur in the Slurry is organic in nature at the dose of 16.3 kg of S ha^{-1} $year^{-1}$, and the closeness of the C:N ratio with SS treatment, we can also consider that 16.3 kg of S out of the annual dose of SS (52.4 kg of S ha^{-1} $year^{-1}$) was organic bound, which would mean that 68% of the SS dose was in fact inorganic, which is not in opposition with the literature. For example, Dewill et al. [41] mention that sludges can have inorganic sulfates as their dominant sulfur species.

In the subsoil, the situation is more complicated. None of the treatments (Table 11, subsoil section) showed significant differences in time due to variability in results, which was influenced by the variability in subsoil on site, as well as the fact that the experiment was fertilized for 4 years before the trial evaluation started. The FYM was in balance in 2019, while the UAN + St and Slurry were in positive balance. A possible explanation might be the mobilization of fulvic acids from the topsoil and their release, thus inducing the mobilization of sulfur into the subsoil, since they are less stable than organic carbon in the FYM [34]. The negative balance for the UAN treatment could be caused by the plant uptake, even in the subsoil (30–60 cm depth). Maize roots can even grow into the subsoil [55]. This would also explain why the balance for the UAN was lower than for the Control, as the UAN treatment produced a higher yield (Figure 2a), which caused an increased uptake of sulfur (Figure 2b) [13,16] and, thus, caused a decrease in the subsoil sulfur content. The problematic treatment was the SS. We can see high initial values (Table 11) for this treatment. This treatment received 52.4 kg of S ha^{-1} $year^{-1}$ in fertilizer 4 years prior to the beginning of the experiment, as well as atmospheric inputs from the deposition. Judging by the 1997 value, it is clear that both factors led to an increase in the 1997 value. The negative balance of this treatment can, therefore, be attributed to maize uptake, as well as to the decrease in atmospheric sulfur depositions and leaching [21], since the SS contained a significant portion of easily mineralizable sulfur [34] and possibly a major portion of inorganic sulfur [41] that could be made up from water-soluble sulfates [42]. The significant decrease in depositions led to a significant response in soil and crops. However, this response takes time [62], and it is possible that, even though the biggest decrease happened in the late 80s and early 90s [5–7,31], the response in subsoil could lag behind. On top of these, the sludge materials have higher amounts of easily mineralizable sulfur [34], which could cause a decrease through the leaching of sulfate anions. Before venturing forth, this would be an appropriate moment to mention inputs for the SS treatment. Our experiment received the annual supply of sewage sludge from the same water treatment facility every year. The inputs of sulfur for the SS treatment from the sewage sludge were 52.4 kg of S ha^{-1} $year^{-1}$, which is substantially more than other organic fertilizers had. Sulfates are a significant primary ion present in municipal and industrial waters [63] that can reach the water treatment facility, where inorganic sulfates can be added as water softeners or for phosphate precipitation [64]. As a result, sewage sludge can have a high content of sulfur [41]. This explains the rather high content of sulfur in this organic fertilizer.

In Table 12, we can see the vertical movement of the total sulfur balance in the topsoil and subsoil calculated based on inputs (Table 4), uptakes (Table 6), and status of the topsoil and subsoil (Table 11). The content of sulfur that could not be accounted for by inputs, uptakes, or pseudo-total content was considered to be lost by leaching. It can be argued that a possible loss of sulfur can happen from the emissions of H_2S and SO_2 gases, as was reported by Kinsela et al. [65]. The problem is that it is hard to estimate the exact values. Taking the work of Kinsela et al. [65] into consideration, the authors conducted an experiment where the emission of SO_2 and H_2S gases was measured in Australia with sulfur-rich soils and 1445 L m^{-2} of precipitation, and they measured a release of 3.66 kg of S ha^{-1} $year^{-1}$. By taking this value and applying it to the SS treatment in our experiment (as it had the highest S inputs), we can calculate that, over the course of 23 years, 84.2 kg of S ha^{-1} after 23 years would be released in gases. Given the fact that the precipitation in our experiment was 493 L m^{-2}. (Table 1) and considering the precipitation trend (Figure 1) at the time of fertilizer application, we assumed that the potential for sulfur volatilization was negligible, so a decision to omit it was made—therefore, leaching would be the most dominant cause of sulfur loss. Additional water input was calculated in terms of organic fertilizers. The highest moisture content was measured for the Slurry treatment (5.7% of dry matter). Given the moisture content and applied doses of dry matter (Table 3), we can calculate that this treatment received 3.77 L m^{-2} $year^{-1}$ with fertilizers. This would not be enough to influence the soil moisture content. The same applied to the other treatments as well.

The results in Table 12 show that a loss was present in all treatments. The total loss from the entire investigated profile (0–60 cm depth) is online (d). At this point, it was not surprising that even the organic fertilized treatments produced a loss of sulfur. The decrease in mineral fractions could be attributed to the decrease in atmospheric depositions that supplied mineral sulfur into the soil [31]. Interestingly, even the organic fertilized treatments sustained a loss of sulfur. The decrease in organic-bound sulfur can be explained by findings in Riley et al. [21]. In our experiment, the outputs were also greater than the inputs.

When comparing just the fertilizer inputs and annual loss, the SS annually received 52.4 kg of S ha^{-1} $year^{-1}$ in fertilizer, while the annual loss was calculated (loss from subsoil divided by 23) at 66.1 kg of S ha^{-1} $year^{-1}$. The same could be calculated for the FYM, Slurry, and UAN + St treatments, which received 16.4, 16.3, and 2.61 kg of S ha^{-1} $year^{-1}$ as a fertilizer, respectively. After the calculation, we obtained 21.4, 26.8, and 8.04 kg of S ha^{-1} $year^{-1}$ of lost sulfur for the FYM, Slurry, and UAN + St, respectively. All fertilized treatments produced higher annual losses than the annual fertilizer inputs.

5. Conclusions

This study examined the influence of organic sulfur fertilization and atmospheric depositions on soil and plants in a 23-year-long maize monoculture for Control, UAN, UAN + St, SS, FYM, and Slurry treatments. In terms of maize, the sulfur supply was not the limiting factor, and yields were influenced by the nitrogen dose on all treatments. Sulfur fertilization, however, caused a significant increase in sulfur uptake for the SS treatment, as it received the highest sulfur dose, while the increased uptake of the other treatments was mostly caused by the increase in yields.

In the soil, we found a decrease in S_{pt} for all treatments except the FYM. For all treatments, the proportion of the S_{org} in S_{pt} increased over time, which was caused by the decrease in mineral fractions (S_W, S_{ads}, S_{av}) for all treatments. The absolute content of the S_{org} was decreasing over time for all treatments except the SS and FYM, as the dose of sulfur for the SS was highest, and the FYM was rich in stable organic compounds. The Slurry and UAN + St in our experiment were relatively richer in labile forms of carbon, which resulted in the decrease in the S_{org}.

By using the total sulfur balance, we demonstrated significant leaching for all treatments (even the unfertilized Control) of sulfur below the 60 cm depth over the period of the entire experiment. We conclude that high inputs of sulfur from atmospheric depositions in the 1990s were the major cause for this decrease, as well as the possible release of sulfur from the organic fertilizer treatments. The highest leaching was measured for the SS treatment, where the highest inputs in terms of S were present. We contribute the leaching to the dose and the fact that sewage sludges are generally rich in inorganic and easily releasable sulfur. If considered for fertilization, we would recommend a much lower dose of sewage sludge than the amounts administered in our work to reduce sulfur leaching and inputs of potentially toxic elements. Based on these results, it is possible to say that the periodical application of farmyard manure is the best choice for maintaining a proper sulfur nutrient status.

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Abbreviations

Control	Control treatment
UAN	Urea and ammonium nitrate
UAN + Wheat	Urea and ammonium nitrate + wheat straw
SS	Sewage sludge
Slurry	Cow slurry
FYM	Farmyard manure
S_W	Water-soluble sulfur
S_{ads}	Adsorbed sulfur
S_{av}	Plant-available sulfur
S_{pt}	Pseudo-total sulfur
S_{org}	Organic-bound sulfur
S_{M3}	Mehlich 3 extractable sulfur
C_{SOM}	Soil organic matter carbon
$C_{HA/FA}$	Humic and fulvic acid ratio
DM	Dry matter
C:N:S	Carbon:nitrogen:sulfur ratio in fertilizer
BY	Biomass yield
SD	Standard deviation

References

1. Marschner, H. (Ed.) *Marschner's Mineral Nutrition of Higher Plants*, 3rd ed.; Elsevier: London, UK, 2012; p. 672, ISBN 978-0-12-384905-2.
2. Steinke, K.; Rutan, J.; Thurgood, L. Corn Response to Nitrogen at Multiple Sulfur Rates. *Agron. J.* **2015**, *107*, 1347–1354. [CrossRef]
3. Lehmann, J.; Solomon, D.; Zhao, F.J.; McGrath, S.P. Atmospheric SO_2 Emissions Since the Late 1800s Change Organic Sulfur Forms in Humic Substance Extracts of Soils. *Environ. Sci. Technol.* **2008**, *42*, 3550–3555. [CrossRef]
4. Yang, Z.; Singh, B.R.; Hansen, S.; Hu, Z.; Riley, H. Aggregate Associated Sulfur Fractions in Long-Term (>80 Years) Fertilized Soils. *SSSA J.* **2007**, *71*, 163–170. [CrossRef]

5. Tlustoš, P.; Pavlíková, D.; Balík, J.; Száková, J. Sulphur cycle in the soil and in the environment. In Proceedings of the 7th International Conference Reasonable Use of Fertilizers Focused on Sulphur in Plant Production, Prague, Czech Republic, 29 November 2001.
6. Krupová, D.; Pavlendová, H.; Pavlenda, P.; Šrámek, V. Atmospheric deposition of sulphur and nitrogen in forests of the Czech and Slovak Republic. *J. For.* **2018**, *64*, 249–258. [CrossRef]
7. Czech Hydrometeorological Institute. Available online: https://www.chmi.cz/files/portal/docs/uoco/isko/grafroc/19groc/gr19cz/09_depozice_v1.pdf (accessed on 10 August 2020). (In Czech).
8. Gao, Y.; Ma, M.; Yang, T.; Chen, W.; Yang, T. Global atmospheric sulfur deposition and associated impaction on nitrogen cycling in ecosystems. *J. Clean. Prod.* **2018**, *195*, 1–9. [CrossRef]
9. European Environmental Agency. Sulphur Dioxide Emissions. European Environment Agency. 2015. Available online: https://www.eea.europa.eu/data-and-maps/indicators/eea-32-sulphur-dioxide-so2-emissions-1/assessment-3#tab-related-briefings (accessed on 1 March 2022).
10. Ministry of the Environemtn of the Czech Republic. Národní Program Snižování Emisí České Republiky. 2007. Available online: https://www.mzp.cz/C1257458002F0DC7/cz/strategicke_dokumenty/$FILE/OOO-NPSE_2007-20200217.pdf (accessed on 28 February 2022). (In Czech).
11. Chien, S.H.; Gearhart, M.M.; Villagarcía, S. Comparison of Ammonium Sulfate with Other Nitrogen and Sulfur Fertilizers in Increasing Crop Production and Minimizing Environmental Impact: A review. *Soil Sci.* **2011**, *176*, 327–335. [CrossRef]
12. Weil, R.R.; Mughogho, S.K. Sulfur nutrition of maize in four regions of Malawi. *Agron. J.* **2000**, *92*, 649–656. [CrossRef]
13. Pasley, H.R.; Cairns, J.E.; Camberate, J.J.; Vyn, T.J. Nitrogen fertilizer rate increases rate increases plant uptake and soil availability of essential nutrients in continuous maize production in Kenya and Zimbabwe. *Nutr. Cycl. Agroecosyst.* **2012**, *115*, 373–389. [CrossRef]
14. Kim, K.; Kaiser, D.E.; Lamb, J. Corn response to starter fertilizer and broadcast sulfur evaluated using strip trials. *Agron. J.* **2013**, *105*, 401–411. [CrossRef]
15. Khan, M.J.; Khan, M.H.; Khattak, R.A.; Jan, M.T. Response of maize to different levels of sulfur. *Commun. Soil Sci. Plant Anal.* **2006**, *37*, 41–51. [CrossRef]
16. Carciochi, W.D.; Divito, G.A.; Fernández, L.A.; Echeverría, H.E. Sulfur affects root growth and improves nitrogen recovery and internal efficiency in wheat. *J. Plant Nutr.* **2017**, *40*, 1231–1242. [CrossRef]
17. McLaren, R.G.; Cameron, K.C. Soil, plant and fertilizer sulphur. In *Soil Science*; McLaren, R.G., Cameron, K.C., Eds.; Oxford University Press: Auckland, New Zealand, 1996; pp. 221–228, ISBN 13 9780195583458.
18. Kulhánek, M.; Černý, J.; Balík, J.; Sedlář, O.; Suran, P. Potential of Mehlich 3 method for extracting plant available sulfur on the Czech agricultural soils. *Plant Soil Environ.* **2018**, *64*, 455–462. [CrossRef]
19. Tisdale, S.L.; Nelson, W.L.; Beaton, J.D.; Havlin, J.L. *Soil Fertility and Fertilizers*, 5th ed.; Macmillan Publishing Company: New York, NY, USA, 1993; p. 634, ISBN 13 978-0024208354.
20. Morche, L. S-Fluxes and Spatial Alterations of Inorganic and Organic Sulfur Fractions in Soil as Well as Their Accumulation and Depletion in the Rhizosphere of Agricultural Crops by Partial Use of the Radioisotope ^{35}S. Ph.D. Thesis, Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany, 2008; p. 321. (In German)
21. Riley, N.G.; McGrath, S.P. Leaching losses of sulphur from different forms of sulphur fertilizers: A field lysimeter study. *Soil Use Manag.* **2002**, *18*, 120–126. [CrossRef]
22. Bergholm, J.; Majdi, H. Accumulation of nutrients in above and below ground biomass in response to ammonium sulfate addition in a Norway spruce stand in southwest Sweden. *Water Air Soil Pollut.* **2001**, *130*, 1049–1054. [CrossRef]
23. Suran, P.; Kulhánek, M.; Balík, J.; Černý, J.; Sedlář, O. Evaluation of Soil S pools under 23 Years of Maize Monoculture. *Agronomy* **2021**, *11*, 2376. [CrossRef]
24. Dijksterhuis, H.G.; Oenema, O. Studies on the effectiveness of various sulfur fertilizers under controlled conditions. *Fertil. Res.* **1990**, *22*, 147–159. [CrossRef]
25. Sharma, M.K.; Kumar, M. Sulphate contamination in groundwater and its remediation: An overview. *Environ. Monit. Assess.* **2020**, *192*, 74. [CrossRef]
26. Kopáček, J.; Hejzlar, J.; Porcal, P.; Posch, M. Sulphate leaching from diffuse agricultural and forest sources in a large central European catchment during 1900–2010. *Sci. Total Environ.* **2014**, *470–471*, 543–550. [CrossRef]
27. Boye, K.; Eriksen, J.; Nilsson, I.; Mattson, L. Sulfur flow in a soil-plant system—Effects of long-term treatment history and soil properties. *Plant Soil* **2010**, *334*, 323–334. [CrossRef]
28. Balik, J.; Kulhánek, M.; Černý, J.; Száková, J.; Pavlíková, D.; Čermák, P. Differences in soil sulfur fractions due to limitation of atmospheric deposition. *Plant Soil Environ.* **2009**, *55*, 344–352. [CrossRef]
29. Kulhánek, M.; Balík, J.; Černý, J.; Sedlář, O.; Vašák, F. Evaluating of soil sulfur forms changes under different fertilizing systems during long-term field experiments. *Plant Soil Environ.* **2016**, *62*, 408–415. [CrossRef]
30. Mehlich, A. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Commun. Soil Sci. Plant Anal.* **1984**, *15*, 1409–1416. [CrossRef]
31. Zbíral, J.; Smatanová, M.; Němec, P. Sulphur status in agricultural soils determined using the Mehlich 3 method. *Plant Soil Environ.* **2018**, *64*, 255–259. [CrossRef]

32. -Santoso, D.; Lefroy, R.D.B.; Blair, G.J. Sulfur and phosphorus dynamics in an Acid soil/crop system. *Aust. J. Soil Res.* **1995**, *33*, 113–124. [CrossRef]
33. Kayikcioglu, H.H.; Yener, H.; Ongun, A.R.; Okur, B. Evaluation of soil and plant health associated with successive three-year sewage sludge field application under semi-arid biodegradation condition. *Arch. Agron. Soil Sci.* **2019**, *12*, 1659–1676. [CrossRef]
34. Förster, S.; Welp, G.; Scherer, H.W. Sulfur speciation in bulk soil as influenced by long-term application of mineral and organic fertilizers. *Plant Soil Environ.* **2012**, *58*, 316–321. [CrossRef]
35. Knights, J.S.; Zhao, F.J.; Spiro, B.; McGrath, S.P. Long-term effects of land use and fertilizer treatments on sulfur cycling. *J. Environ. Qual.* **2000**, *29*, 1867–1874. [CrossRef]
36. Islam, M.R.; Bilkis, S.; Hoque, T.S.; Uddin, S.; Jahiruddin, M.; Rahman, M.M.; Siddique, A.B.; Hossain, M.A.; Marfo, T.D.; Danish, S.; et al. Mineralization of Farm Manures and Slurries under Aerobic and Anaerobic Conditions for Subsequent release of Phosphorus and Sulphur in Soil. *Sustainability* **2021**, *13*, 8605. [CrossRef]
37. Barlóg, P.; Hlisnikovský, L.; Kunzová, E. Effect of Digestate on Soil Organic Carbon and Plant-Available Nutrient Content Compared to Cattle Slurry and Mineral Fertilization. *Agronomy* **2020**, *10*, 379. [CrossRef]
38. Van Vuuren, D.P.; Bouwman, A.F.; Beusen, A.H.W. Phosphorus demand for the 1970–2100 period: A scenario analysis of resource depletion. *Glob. Environ. Chang.* **2010**, *20*, 428–439. [CrossRef]
39. European Commission. *Environmental, Economic and Social Impacts of the Use of Sewage Sludge on Land*; Final Report; Part II: Report on Options and Impacts (10/02/2010, DG ENV.G.4/ETU/2008/0076r); Milieu Ltd.: Brussels, Belgium; WRc and Risk & Policy Analysts Ltd. (RPA): London, UK, 2010; p. 158.
40. European Commission. *Environmental, Economic and Social Impacts of the Use of Sewage Sludge on Land*; Final Report; Part III: Project interim reports (10/02/2010, DG ENV.G.4/ETU/2008/0076r); Milieu Ltd.: Brussels, Belgium; WRc and Risk & Policy Analysts Ltd. (RPA): London, UK, 2010; p. 116.
41. Dewil, R.; Baeyens, J.; Roles, J.; Van De Steene, B. Distribution of Sulphur Compounds in Sewage Sludge Treatment. *Environ. Eng. Sci.* **2008**, *25*, 879–886. [CrossRef]
42. Sommers, L.E.; Tabatabai, M.A.; Nelson, D.W. Forms of sulphur in sewage sludge. *J. Environ. Qual.* **1977**, *6*, 42. [CrossRef]
43. Balík, J.; Kulhánek, M.; Černý, J.; Sedlář, O.; Suran, P.; Asrade, D.A. The Influence of Organic and Mineral Fertilizers on the Quality of Soil Organic Matter and Glomalin Content. *Agronomy* **2022**, *12*, 1375. [CrossRef]
44. Tlustoš, P.; Hejcman, M.; Kunzová, E.; Hlisinkovský, L.; Zámečníková, H.; Száková, J. Nutrient status of soil and winter wheat (*Triticum aestivum* L.) in response to long-term farmyard manure application under different climatic and soil physicochemical conditions in the Czech Republic. *Arch. Agron. Soil Sci.* **2018**, *64*, 70–83. [CrossRef]
45. International Organization for Standardization. Soil Quality—Extraction of Trace Elements Soluble in Aqua Regia (ISO Standard No. 11466:1995). 1995. Available online: https://www.iso.org/standard/19418.html (accessed on 20 December 2022).
46. Godlewska, A. Sulphur content in test plants and arylsulfatase activity in soil after application of waste materials. *Appl. Ecol. Environ. Res.* **2018**, *16*, 145–152. [CrossRef]
47. Sakal, R.; Sinha, R.B.; Singh, A.O.; Bhogal, N.S.; Ismail, M.D. Influence of sulphur on yield and mineral nutrition of crops in maize-wheat semence. *J. Indian Soc. Soil Sci.* **2000**, *48*, 325–329. [CrossRef]
48. Sedlář, O.; Balík, J.; Kulhánek, M.; Černý, J.; Matěchová, M.; Suran, P. Crop sulfur status in relation to soil sulfur determined using anion exchange membranes and Mehlich 3. *J. Plant Nutr.* **2021**, *44*, 1563–1570. [CrossRef]
49. Prietzel, J.; Weick, C.; Korintenberg, J.; Seybold, G.; Thumerer, T.; Treml, B. Effects of repeated $(NH_4)_2SO_4$ application on sulfur pool in soil, soil microbial biomass, and ground vegetation of two watersheds in the Black Forest/Germany. *Plant Soil* **2001**, *230*, 287–305. [CrossRef]
50. Grobler, L.; Bloem, A.A.; Claassens, A.S. A critical soil sulphur level for maize (*Zea mays* L.) grown in a glasshouse. *S. Afr. J. Plant Soil* **1999**, *16*, 204–206. [CrossRef]
51. Scherer, W.H. Sulfur in soils. *J. Plant Nutr. Soil Sci.* **2009**, *172*, 326–335. [CrossRef]
52. Kulhánek, M.; Balík, J.; Sedlář, O.; Zbíral, J.; Smatanová, M.; Suran, P. *Determining of Bioavailable Sulfur in Soil Using Mehlich 3 Method*; Czech University of Life Sciences: Prague, Czech Republic, 2018; p. 32, ISBN 978-80-213-2893-8. (In Czech)
53. Rogers, C.W.; Dari, B.; Schroeder, K.L. Comparison of soil-test extractants for potassium, calcium, magnesium, sulfur, and micronutrients in Idaho soils. *Agrosyst. Geosci. Environ.* **2019**, *2*, 190067. [CrossRef]
54. Kowalenko, C.G.; Bittman, S.; Neilsen, G.H.; Kenney, E.; Hunt, D.E.; Neilsen, D. Potential for improving sulfur tests on agricultural soils in contrasting ecoregions of British Columbia, Canada. *Geoderma Reg.* **2014**, *1*, 10–20. [CrossRef]
55. Gao, Y.; Duan, A.; Qiu, X.; Liu, Z.; Sun, J.; Zhang, J.; Wang, H. Distribution of roots and root length density in a maize/soybean strip intercropping system. *Agric. Water Manag.* **2010**, *98*, 199–212. [CrossRef]
56. Balík, J.; Kulhánek, M.; Černý, J.; Sedlář, O.; Suran, P. Soil organic matter degradation in long-term maize cultivation and insufficient organic fertilization. *Plants* **2020**, *9*, 1217. [CrossRef] [PubMed]
57. Zhang, H.; Kariman, K.; Zhu, L.; Liu, L.; Chen, J.J.; Zhu, L.; Li, D. Spatial distribution of carbon, nitrogen and sulfur in surface soil across the Pearl River Delta area, South China. *Geoderma Reg.* **2021**, *25*, e00390. [CrossRef]
58. Lal, R. Soil organic matter and water retention. *Agron. J.* **2020**, *112*, 3265–3277. [CrossRef]
59. Schnitzer, M. Organic matter, Principles and Processes. In *Encyclopedia of Soils in the Environment*, 3rd ed.; Hillel, D., Ed.; Elsevier Ltd.: Amsterdam, The Netherlands, 2005; pp. 85–93.

60. Menšík, L.; Hlisnikovský, L.; Pospíšilová, L.; Kunzová, E. The e_ect of application of organic manures and mineral fertilizers on the state of soil organic matter and nutrients in the long-term field experiment. *J. Soil Sediment* **2018**, *18*, 2813–2822. [CrossRef]
61. Klučáková, M. Size and charge evaluation of standard humic and fulvic acids as crucial factors to determine their environmental behavior and impact. *Front. Chem.* **2018**, *6*, 265. [CrossRef]
62. Mitchell, R.J.; Hewison, R.L.; Fielding, D.A.; Fisher, J.M.; Gilbert, D.J.; Hurskainen, S.; Pakeman, R.J.; Potts, J.M.; Riach, D. Decline in atmospheric sulphur deposition and changes in climate are the major drivers of long-term change in grassland plant communities in Scotland. *Environ. Pollut.* **2018**, *235*, 956–964. [CrossRef]
63. Tang, J.; He, J.; Liu, T.; Xin, X.; Hu, H. Removal of heavy metal from sludge by the combined application of a biodegradable biosurfactant and complexing agent in enhanced electrokinetic treatment. *Chemosphere* **2017**, *187*, 599–608. [CrossRef]
64. Dai, Y.; Li, Y.; Ke, Y.; Li, B. Efficiency and mechanism of advanced treatment for phosphate wastewater by high efficiency and low consumption coagulation and phosphorus removal system. *IOP Conf. Sef. Earth Environ. Sci.* **2021**, *631*, 012002. [CrossRef]
65. Kinsela, A.S.; Denmead, T.O.; Macdonald, B.C.T.; Melville, M.D.; Reynolds, J.K.; White, I. Field-based measurements of sulfur gas emissions from an agricultural coastal acid sulfate soil, eastern Australia. *Soil Res.* **2011**, *49*, 471–480. [CrossRef]

4.3 Soil Organic Matter Degradation in Long-Term Maize Cultivation and Insufficient Organic Fertilization

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Soil Organic Matter Degradation in Long-Term Maize Cultivation and Insufficient Organic Fertilization

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Abstract: Soil organic matter carbon (C_{SOM}) compounds degradation was observed in long-term field experiments with silage maize monoculture. Over a period of 26 years, the content of carbon in topsoil decreased by 22% in control unfertilized plots compared to 25% and 26% in treatments fertilized annually with mineral nitrogen. With annual wheat straw application (together with mineral N), the content of C_{SOM} decreased by 8%. Contrary to that, the annual application of farmyard manure resulted in a C_{SOM} increase of 16%. The ratio of carbon produced by maize related to total topsoil C_{SOM} content ranged between 8.1–11.8%. In plots with mineral N fertilization, this ratio was always higher than in the unfertilized control plots. With the weaker soil extraction agent ($CaCl_2$), the ratio of carbon produced by maize was determined to be 17.9–20.7%. With stronger extraction agent (pyrophosphate) it was only 10.2–14.6%. This shows that maize produced mostly unstable carbon compounds. Mineral N application resulted in stronger mineralization of original and stable organic matter compared to the unfertilized control. However, the increase of maize-produced carbon content in fertilized plots did not compensate for the decrease of "old" organic matter. As a result, a tendency to decrease total C_{SOM} content in plots with mineral N applied was observed.

Keywords: C balance; humic acids; fulvic acids; mineral N; farmyard manure; straw

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1. Introduction

The amount and quality of soil organic matter (C_{SOM}) are very significant parameters for assessing soil fertility. C_{SOM} is influenced by a range of soil-climatic conditions, systems of soil management, cultivated crops, fertilization, agrotechnical issues, etc. Agricultural crops differ significantly in the amount and quality of post-harvest residues and root biomass. The crops with high potential to decrease the content of organic matter in soil comprise maize, especially if grown for silage [1]. Loges et al. [2] compared the average amount of post-harvest residues and roots among maize monoculture and rotated crops (clover grass–maize–wheat). The amount of carbon supplied into the soil (post-harvest residues + roots) was 1.3 t ha^{-1} year^{-1} in the case of monoculture, compared to 1.6 t ha^{-1} year^{-1} in crop rotation. As a result of mineralization, the annual carbon decrease was 530 kg C ha^{-1} year^{-1} in monoculture, whereas in crop rotation it was only 120 kg C ha^{-1} year^{-1}.

C_{SOM} can be divided into stable and labile fractions. Stable carbon forms are represented by humic acids (C_{HA}), fulvic acids (C_{FA}), and humins (C_{HU}). Carbon sequestration in terms of C_{HA}, C_{FA}, and C_{HU} content and quality is very important to understand soil quality degradation [3,4].

Gregorich et al. [5] found that after seven years of maize cultivation as a monoculture, the ratio of carbon originating from maize (C_4) related to C_{SOM} (C_4/C_{SOM}) was only 15% of total C_{SOM}. The previous crop was permanent grass stand. Collins et al. [6] determined the C_4 ratio in C_{SOM} as 23–60% in fertilized plots and 9–32% in unfertilized control plots. These results were obtained in the main

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maize-growing regions in the USA in fields with maize cultivated for 8–35 years. Similarly, [7,8] reported higher C_4 ratios in fertilized plots compared to unfertilized ones; this was affected mainly by higher biomass yields (including root biomass) in the fertilized crop. Bettina et al. [9] evaluated experiments lasting 40 years with maize monoculture, which followed after rye monoculture. The C_4 ratio in C_{SOM} was 9.5% and 14.1% in unfertilized crops and fertilized crops in topsoil, respectively; the values for subsoil were 5.7% (no fertilization) and 7.2% (fertilization). Bettina et al. [9] presumed that the main reason for the lower C_4 ratio compared to other studies was the growing of maize for silage in their experiments. Almost all the biomass was thus harvested and removed from the fields and only low stubble remained. Similarly, low C_4 ratios were also reported by [10,11].

Maize biomass carbon is easily mineralized in the soil; hence, the increased C_4 ratio is determined mostly in easily hydrolysable fractions (C_{DOC}). Depending on the purpose of cultivation (silage/grain), length of monoculture cultivation, a system of fertilization, soil-climatic conditions, the C_4 ratio in C_{DOC} was almost 1/3 [5,9,12]. Fertilized plots also had higher total content of C_{DOC} [13]. Even higher values than in C_{DOC} were observed for C_4 in microbial biomass carbon [5,9]), again with the values of fertilized plots being higher. The results also show that "younger" organic matter (plant residues) is mineralized more quickly than "older" organic matter in the soil. C_4 thus accounts for the main share of carbon loss in the form of CO_2; Bettina et al. [9] reported almost 80.0%.

The trials of Loges et al. [2] suggest the ratio of root biomass and exudates as 18% compared to the aboveground biomass. Out of the C amount in roots and post-harvest residues, the main part remains in the topsoil and a lesser amount in subsoil [9,14–16]. Loges et al. [2] presumed that it was sufficient to examine the 0–30 cm soil layer to determine the impacts of different cultivation systems on post-harvest residues' C input into soil. Rasse et al. [10] found almost all C_4 in the depth 0–90 cm, 63% of it being in the 0–30 cm layer. In the subsoil, the content of C_{SOM} is significantly lower and more stable [17]. The reason is a tighter relationship of organic matter to clay particles [18] and also a lower air content, i.e., lower mineralization intensity.

The aim of this paper was to assess the changes in C_{SOM} amount and quality in long-term silage maize monoculture, as well as compare the effects of different mineral and organic fertilizers. This research is also important from the viewpoint of long-term sustainable soil management in the Czech Republic (CR), with respect to increasing usage of maize in biogas plants [19], low intensity of organic fertilization of maize and other crops, and unbalanced C in agricultural systems. These issues are very important, as shown by the high ratio of maize in crop rotations. Moreover, maize is often grown for several consecutive years without rotation. In the CR, its acreage is 10.5% of agricultural soils—about 232,400 ha being silage maize + 74,800 ha for grain [20]. Furthermore, luvisol (the soil type chosen for our experiment) is representing 4.29% of the area of agriculturally used soils in the CR, which means 179,167 ha [21].

2. Results

The explanation of abbreviations is mentioned above in the list of abbreviations and further in the chapter materials and methods.

2.1. *Yield Parameters*

Differences in yields of harvested biomass over a period of 26 years are shown in Figure 1. Although the differences among fertilized treatments are relatively high (sometime even 18%), statistically significant differences were not observed. This is mainly due to the variability of climatic conditions during experimental seasons, where the effect of individual fertilization varied according to related season.

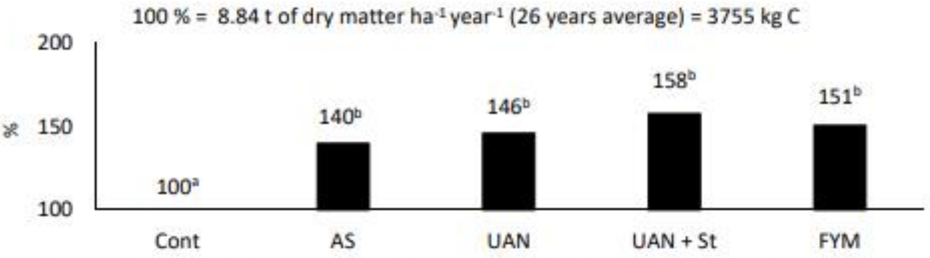

Figure 1. Average maize biomass yield; different letters behind the values are meaning significant differences among investigated treatments (Tukey test; $p < 0.05$); number of replications per treatment $n = 4$.

The values in fertilized plots reached 12.2–14.0 t DM ha^{-1} and correspond to site conditions. The highest yields were obtained in the urea ammonium nitrate (UAN) + straw (St) treatment (Figure 1) with the highest N dose applied, i.e., 153.5 kg N ha^{-1} year^{-1} (120 + 33.5) (see the chapter methodology).

2.2. Carbon Produced by Maize

Table 1 summarizes the maize carbon (C_4) results. The obtained average yields of dry matter and its C content were used to calculate the amount of carbon transported every year from the field. The yield of 8.84 t DM ha^{-1} also represents 3755 kg C ha^{-1}. The amount of carbon in post-harvest residues (stubble) was determined experimentally in 2018. The total carbon accumulated in the aboveground biomass was calculated as the sum of "harvest" + "stubble" values. Similarly, the carbon amount in topsoil roots was obtained by analyses in 2018 (at the harvest period). To compare our experimentally determined results, the values calculated based on various algorithms reported in the literature are presented in Table 1.

Table 1. Carbon balance of maize (kg C ha year^{-1}); AS–ammonium sulfate, UAN–urea ammonium nitrate, UAN + st–urea ammonium nitrate + straw, FYM–farmyard manure.

Treatment	Above Ground Biomass				Roots + Exudates [3]
	Transport by Harvest [1]	Stubble [2]	Σ	Roots [2]	Loges et al. [2] Rasse et al. [10]
Control	3755	208	3963	394	548
AS	5210	255	5466	736	756
UAN	5421	322	5743	696	794
UAN + St	5854	293	6147	809	850
FYM	5615	360	5975	768	826

[1] Average value of 26 year period; [2] Determined in the year 2018; [3] Calculated according to Rasse et al. [10] and Loges et al. [2].

2.3. The Changes of Soil Organic Matter Carbon (C_{SOM}) Contents

In 1993, the amount of C_{SOM} in the topsoil was 1.26%. After harvest in 2018, the following values were determined: control 0.98%, ammonium sulfate (AS) 0.93%, UAN 0.95%, UAN + St 1.16%, manure 1.49% (Figure 2). There was a significant decrease of C_{SOM} in the topsoil of all treatments, except for farmyard manure. In relative values, the C_{SOM} content decreased by 22% in the control and mostly in AS by 26%. C_{SOM} content in subsoil was not determined at the beginning of the experiment, but Figure 2 clearly shows a lower C_{SOM} content in the subsoil in the AS treatment compared to other treatments. On the contrary, treatment with farmyard manure resulted in significantly higher C_{SOM} content also in the subsoil, which is probably related to rather deep tillage (28 cm).

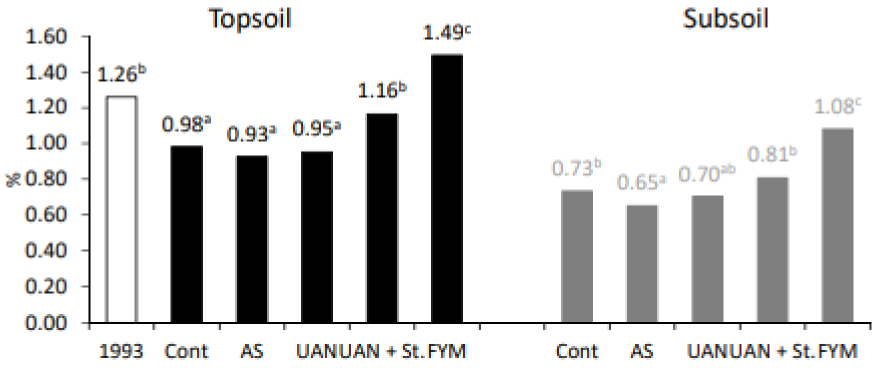

Figure 2. Soil organic matter carbon (C_{SOM}) content in topsoil and subsoil; different letters behind the values are meaning significant differences among investigated treatments (Tukey test; $p < 0.05$); number of replications per treatment $n = 4$.

2.4. Carbon Balance

Table 2 presents the score of carbon losses as a result of mineralization as well as respiration of CO_2. Column 2 shows the calculated difference of C_{SOM} content at the beginning of the experiment (1993) and after harvest in 2018. For example, the loss in the AS treatment was—571 kg C ha^{-1} year^{-1}. Further, the C amount supplied with organic fertilization was calculated, e.g., for UAN + St treatment it was 2140 kg C ha^{-1} year^{-1}.

Table 2. C_{SOM} loses due to the mineralization (soil organic matter + fertilizer + crop residues) (in kg C ha^{-1}year^{-1}); AS—ammonium sulfate, UAN–urea ammonium nitrate, UAN + st–urea ammonium nitrate + straw, FYM–farmyard manure, C–Carbon.

Treatment	C in Topsoil [1]	C from Org. Fertilizer	C in Topsoil + C from Org. Fert.	C in Stubble [2]	C in Roots [2]	Total C	Loses/Harvest (%) [3]
Control	−485	0	485	208	394	1087	28.9
AS	−571	0	571	255	736	1562	29.7
UAN	−537	0	537	322	696	1555	28.4
UAN + St	−173	2140	2313	293	809	3415	57.6
FYM	398	1603	1205	360	768	2333	41.1

[1] Calculated as difference among C_{SOM} at the beginning of experiment (1993) and year 2018; [2] determined in 2018; [3] Ratio of C loses compared with carbon content in biomass of harvested maize.

The data concerning the carbon amount ploughed into the soil in the form of post-harvest residues (stubble) and roots (topsoil only) originate from our analyses in 2018. It was evident that the highest carbon losses are in the treatment UAN + St, i.e., 3415 kg C ha^{-1} year^{-1}. These results confirm the dynamics of straw mineralization in soil. The losses in the treatment with farmyard manure were 2333 kg C ha^{-1} year^{-1}. Manure contains organic matter, which is significantly more stable compared to straw and affects C_{SOM} stability considerably. Presuming that stubble residues do not significantly affect C content in soil and are mineralized quickly (about three times faster than manure) and further that root biomass needs a similar time to decompose as manure, about 16% transformation of carbon from manure to stable soil compounds may be deduced. The residual amount then mineralizes or may counterbalance the losses by mineralization of the original organic matter.

Methods using stable carbon isotopes ($^{13}C/^{12}C$) allowed us to determine the ratio of maize carbon (C_4) in the total topsoil and subsoil C_{SOM}. The reported analyses were assessed only for the unfertilized control plots and for mineral N treatments. In the fertilized plots, the content of maize carbon in total C_{SOM} in topsoil and subsoil increased. In the topsoil of the control plots, the C_4 ratio in C_{SOM} was 8.1%, in the case of AS it was 10.3%, and in UAN 11.8%, respectively (calculated as $C_4/(C_3 + C_4)$ from Table 3.

* 100%). The C_4 ratio in C_{SOM} in subsoil was about 25–50% lower compared to topsoil (control 5.5%, AS 7.9%, UAN 6.0%).

Table 3. Carbon content in topsoil and subsoil; AS—ammonium sulfate, UAN—urea ammonium nitrate, C—carbon, C_{SOM}—soil organic matter carbon, C_{CaCl2}—carbon determined with 0.01 mol L^{-1} $CaCl_2$, C_{PF}—carbon determined with 0.1. mol L^{-1} $Na_4P_2O_7$, C_3—"old" soil organic matter, C_4—carbon from maize.

Treatment	Soil Depth (cm)	Origin of C	C_{SOM} (g m^{-2})	C_{CaCl2} (g m^{-2})	C_{PF} (g m^{-2})
Control	0–30	$C_3 + C_4$	4322 ^{a}	1.2 ^{a}	970 ^{a}
		C_3	3973 ^{e}	0.99 ^{d}	872 ^{d}
		C_4	349 ^{g}	0.7 ^{g}	98 ^{g}
	30–60	$C_3 + C_4$	3408 ^{o}		
		C_3	3221 ^{r}		
		C_4	187 ^{s}		
AS	0–30	$C_3 + C_4$	4101 ^{a}	4.65 ^{c}	1147 ^{b}
		C_3	3679 ^{d}	3.75 ^{f}	1005 ^{f}
		C_4	422 ^{h}	0.9 ^{h}	142 ^{h}
	30–60	$C_3 + C_4$	3023 ^{p}		
		C_3	2785 ^{q}		
		C_4	238 ^{s}		
UAN	0–30	$C_3 + C_4$	4190 ^{a}	2.99 ^{b}	1058 ^{a,b}
		C_3	3697 ^{d}	2.46 ^{e}	904 ^{e,f}
		C_4	493 ^{h}	0.53 ^{h}	154 ^{h}
	30–60	$C_3 + C_4$	3269 ^{o,p}		
		C_3	3073 ^{q}		
		C_4	196 ^{s}		

Within columns, values followed by the same letter (for topsoil: a–c for total amounts; d–f for C_3 derived carbon; g–h for C_4 derived carbon and for subsoil o–p total amount; q–r for C_3, s–t for C_4), are not significantly different (Tukey test, $p < 0.05$) between experiment plots. Number of replications $n = 4$.

Table 3 shows statistically conclusive differences in the content of "old" organic matter (C_3) among control and N fertilization treatments. In the case of AS and UAN treatments, stronger mineralization of original and stable C_{SOM} occurred compared to the control. Contrariwise, N mineral fertilization resulted in a significant increase in C_4 content. This process also occurs in the subsoil, yet it is less dynamic. The values reported in Table 3 further demonstrate that mineral N fertilization alone increases hydrolysis of C_{SOM}, which is manifested by a significant increase of extractable organic compounds. C_{CaCl2} represents the carbon determined with the weak extracting agent and C_{PF} with strong one, respectively. It was evident that C_4 comprises mostly unstable and easily hydrolysable forms. The ratio of C_4 in C_{CaCl2} was 17.9–20.7%; in C_{PF} it was 10.2–14.6%.

2.5. The Soil Organic Matter Quality Parameters

The relationship between the amount of extractable carbon and total C_{SOM} is shown in Figure 3. The highest values were obtained in the AS treatment. The lowest ratios of C_{CaCl2} and C_{PF} to C_{SOM} were always obtained in the control and farmyard manure treatments, which suggests higher stability of organic matter in soil compared to other treatments. High values in the UAN + St treatment confirm previous results of rather fast mineralization of straw in soil (Table 2). Figure 4 shows the total amount of extractable carbon using $CaCl_2$ or $Na_4P_2O_7$. Considering the high total C_{SOM} content in the farmyard manure treatment, high carbon values were also obtained at the extraction with a strong solution of $Na_4P_2O_7$.

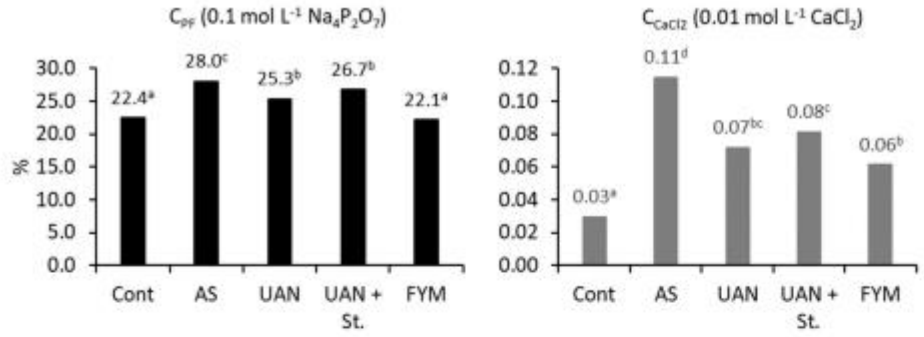

Figure 3. Ratio of extractable carbon of total soil organic matter carbon (C_{SOM}); topsoil; different letters behind the values mean significant differences among investigated treatments (Tukey test; $p < 0.05$); number of replications per treatment $n = 4$.

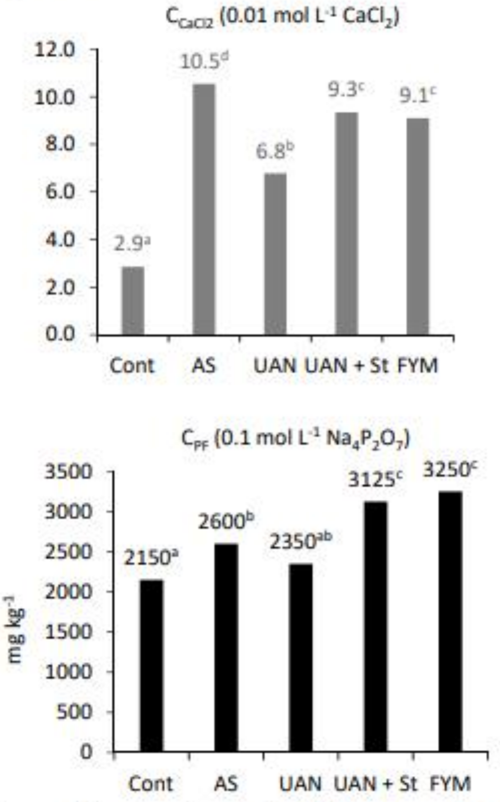

Figure 4. Content of extractable carbon determined with different extraction procedures; topsoil; different letters behind the values mean significant differences among investigated treatments (Tukey test; $p < 0.05$); number of replications per treatment $n = 4$.

Over the period of 26 years, a significant decrease of the C_{HA} content in the control and AS plots (by 50%) was observed. By contrast, the farmyard manure treatment showed significantly higher C_{HA} compared to other treatments. A similar tendency may be observed in case of the C_{HA}/C_{FA} ratio (Figure 5).

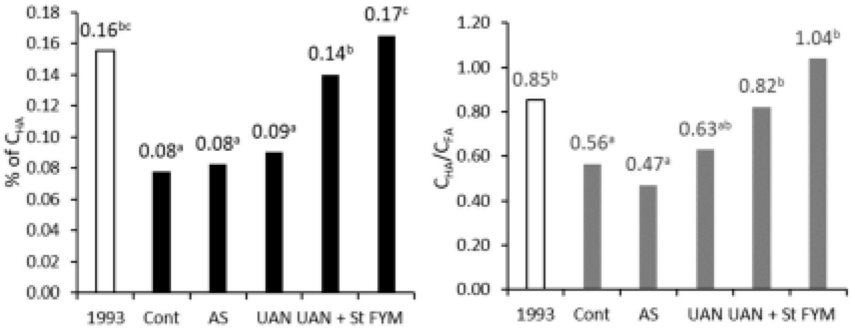

Figure 5. Content of humic acids (C_{HA}) and their ratio to fulvic acids (C_{FA}); topsoil; different letters behind the values mean significant differences among investigated treatments (Tukey test; $p < 0.05$); number of replications per treatment $n = 4$.

2.6. *The Quality of Post-Harvest Residues*

Mineral N fertilization destabilized organic matter in the soil. This was demonstrated by values of the C_{SOM}/N_t ratio in topsoil and subsoil (Figure 6). In topsoil, a significant decrease in the C_{SOM}/N_t ratio was observed in AS and UAN treatments compared to the control. The reason for the change in this ratio is mainly a decrease of C_{SOM} content in mineral N fertilization treatments, because N_t content was the same in all three compared treatments (0.10%). In the subsoil, this difference was not significant, but there was a real tendency to its decrease. It may be presumed that during subsequent years the differences will also become more significant. The highest C_{SOM}/N_t ratio was always observed in the farmyard manure treatment.

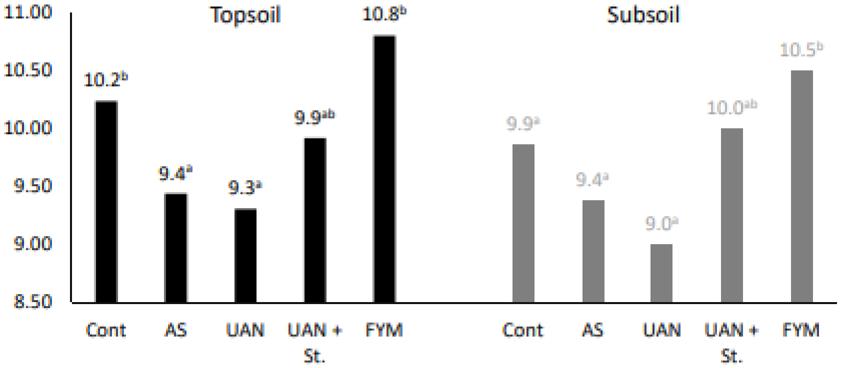

Figure 6. Soil organic matter carbon (C_{SOM})/soil total nitrogen (N_t) ratio in topsoil and subsoil; different letters behind the values mean significant differences among investigated treatments (Tukey test; $p < 0.05$); number of replications per treatment $n = 4$.

The reason for the C_{SOM}/N_t ratio change may be inferred from the increased mineral N content in soil and, furthermore, from the quality of the tilled post-harvest residues (stubble) and root biomass (Figure 7). In stubble, the carbon/nitrogen (C/N) ratio in control plots was 315/1, in AS it was 107/1, and in UAN 88/1, respectively. The lowest (70/1) value was determined in the UAN + St treatment, which corresponds to the highest N fertilization intensity within this treatment. Similarly high differences among the treatments were observed in the analysis of roots in topsoil; the highest ratio was also observed in the control treatment (C/N = 123/1), whereas it was very low in the mineral N treatments (C/N = 49/1). These differences reflect the fact that both root biomass and plant residues were more stable in control plots compared to N fertilization.

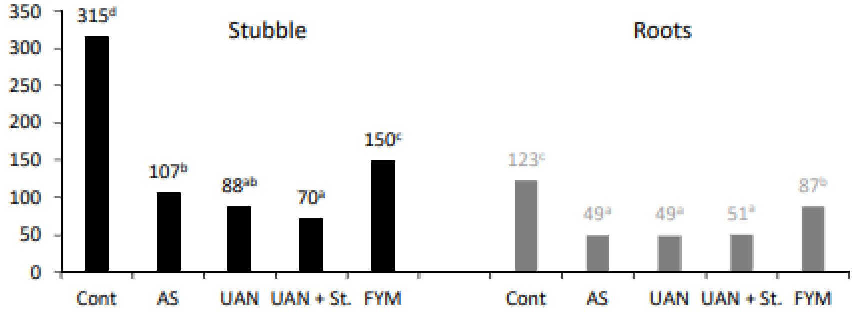

Figure 7. Carbon/nitrogen (C/N) ratio in crop residues of maize; different letters behind the values mean significant differences among investigated treatments (Tukey test; $p < 0.05$); number of replications per treatment $n = 4$.

Figure 8 demonstrates that this was due to post-harvest residues and roots that supplied 90–100% more carbon in mineral N treatments compared to the control. The highest amount of post-harvest residues was in the farmyard manure treatment (stubble: 360 kg C ha^{-1} + roots: 768 kg C ha^{-1} = 1128 kg C ha^{-1}).

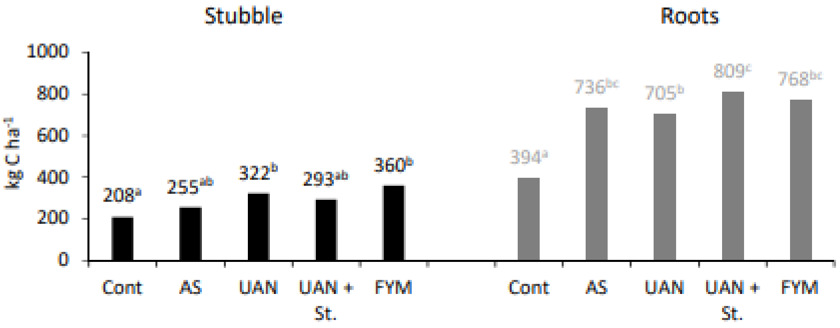

Figure 8. Carbon contents in crop residues of maize; different letters behind the values mean significant differences among investigated treatments (Tukey test; $p < 0.05$); number of replications per treatment $n = 4$.

3. Discussion

The luvisols comprise over 500–600 million ha worldwide and are situated mainly in temperate regions such as in the East European Plains and parts of West Siberian Plain, the North East of the United States of America and Central Europe, but also in the Mediterranean region and southern Australia. Most luvisols are fertile soils and suitable for a wide range of agricultural uses [22]. The worldwide area used for maize forage is about 16.8 million ha [23]. Our results, which are presenting the soil carbon transformations in the maize monoculture cropped on luvisol also provide information that could be useful not only for regional purposes. Similar results can be expected in similar soil conditions by cropping the silage maize in long-term monoculture.

The average yield of the harvested biomass in the control plots over a period of 26 years was 8.84 t DM ha^{-1} year^{-1}, which represents 3755 kg C ha^{-1} year^{-1}. In fertilized plots, the biomass yield was 40–58% greater compared to the control. Although the intensity of mineral nitrogen fertilization (120 kg N ha^{-1} year^{-1}) corresponded to the yields obtained and from the long-term perspective, it was in compliance with N uptake by plants, mineral N resulted in a C_{SOM} decrease in soil. During the experimental period (26 years), the most significant decrease was that of C_{SOM} in the AS treatment

(26%) and the UAN treatment (25%). It is almost alarming that in an annual application of wheat straw (5 t DM ha^{-1} = 2140 kg C ha^{-1}) C_{SOM} also decreased (by 8%). It is obvious that straw is relatively quickly mineralized after its incorporation into the soil [24–27]. Due to this fact it is clear that cereal straw application itself cannot improve the soil organic matter content and quality. This was confirmed in our experiments by the aforementioned decrease of C_{SOM} at this treatment.

The annual average dose of manure was 18.7 t ha^{-1}, which was above average, but not extremely high. Cattle manure with a C/N ratio of 13.4/1 was used. The relatively low C/N ratio in manure fully corresponds with stable technologies currently used in CR. This means a decrease in the C/N ratio compared to the past when the standard values were 15–18/1. However, manure application contributed to a significant increase in the C_{SOM} values (from 1.26% to 1.46%). A significantly positive effect of manure may also be observed in the study of Menšík et al. [4]. Schmidt et al. [28] reported an increase of C_{SOM} values by 32% (from 1.24% to 1.64%) at a dose of 12 t ha^{-1} year^{-1} and a period of 50 years for rye cultivated in a monoculture. The importance of the C/N ratio is evident, but the quality of the incorporated organic matter is important as well. For example, in the UAN + St treatment, the resulting ratio in the fertilizers applied, C/N = 14.6/1, was higher compared to manure, but it did not result in a C_{SOM} increase. Manure contains significantly more stable organic matter than straw and thus helps to control C_{SOM} stability.

Furthermore, in the control plots, a decrease of C_{SOM} content was observed, namely by 22%, which corresponds to losses of 485 kg C ha^{-1} year^{-1}. In the case of the AS treatment, this represents 571 kg C ha^{-1} year^{-1} compared to UAN with 537 kg N ha^{-1} year^{-1}. These values do not include C losses due to mineralization of post-harvest residues. The values are high but fully correspond with the results of [2] concerning long-term silage maize cultivation. These authors reported average losses of 530 kg C ha^{-1} year^{-1} in an unfertilized plot and 550 kg C ha^{-1} year^{-1} in a fertilized one. A decrease of C_{SOM} content in maize monoculture was also documented by [7,9]. A C_{SOM} content increase was reported mostly in maize cultivated for grain [6,10]. For example, Liang and MacKenzie [29] recorded increased C_{SOM} content in topsoil by 18% after six-year maize cultivation for grain. Basically, silage maize cultivation results in a small amount of aboveground post-harvest residues (stubble). In the present study, the height of stubble was about 10 cm. The carbon content in stubble ranged between 208 kg C ha^{-1} (control) up to 360 kg C ha^{-1} (manure). This is in good agreement with the assessment of [15] who reported a value of 290 kg C ha^{-1}.

To evaluate C balance, it is necessary to determine the number of roots. In our experiments, the amount of carbon in roots was determined after harvest. Determined values were in a range of 394 kg C ha^{-1} year^{-1} (control) up to 809 kg C ha^{-1} year^{-1} (UAN + St). Balesdent and Balabane [15] determined the number of roots at the level of 12% of the aboveground biomass. Using this model for calculations, determined values were in the range of 476 kg C ha^{-1} year^{-1} (control) up to 738 kg C ha^{-1} year^{-1} (UAN + St). The aforementioned calculations considered the whole root biomass, including subsoil. The experiments of [2] demonstrated a ratio of root biomass and root exudates of 18% compared to aboveground biomass. Using this model of calculation, the determined values were in the interval from 870 kg C ha^{-1} year^{-1} (control) up to 1349 kg C ha^{-1} year^{-1} (UAN + St). The values for the topsoil and subsoil calculated according to Rasse et al. [10] were as follows: 63% topsoil, 37% subsoil. The comparison of calculated values and those obtained in our measurements show good agreement (Table 1). Both methods show the lowest values in the control and the highest in the UAN + St treatment. To conclude, the values determined by measurements were about 10% lower than the calculated ones. There are several possible reasons: (i) the amount of roots was determined after 26 years of monoculture. As reported by [28], in the case of long-term monoculture, the amount of roots and rhizodeposition decrease by as much as 30%; (ii) the calculations by [2] also comprise exudate carbon that was not determined in our experiments; and (iii) the highest difference among the calculated and measured values was observed mostly in the control; in this variant, a significant tendency to lower yields of aboveground biomass and, thus, roots were observed over the years. For calculation models, the average biomass yield value over the period of 26 years was used.

Studying the root biomass in field conditions always involves error, but it is possible to assess 30 the C ratio using the values determined directly in our study (Table 2). It is evident that the amount of post-harvest residues in silage maize is very small (stubble: 208–360 kg C ha^{-1}; roots 394–809 kg C ha^{-1}) and cannot replace the losses due to C_{SOM} mineralization [2]. In post-harvest residues, the C/N ratio was observed (Figure 7). The results obtained prove that root biomass as well as the stubble residues were more stable (higher C/N ratio) in control plots than in mineral N-fertilized ones. Additionally, the manure treatment showed a statistically significant increase in the C/N ratio compared to the mineral N fertilization. Similar to our experiments, [2] also identified a significantly higher C/N ratio in stubble (197/1) compared to roots (28/1).

N applied in mineral fertilizers showed a significant decrease in the C_{SOM}/N_t ratio in topsoil, which confirms the hypothesis that mineral N fertilization leads to soil organic matter (SOM) destabilization. Contrariwise, manure treatment showed an increase in this ratio.

The overall C ratio (Table 2) clearly shows that the lowest carbon losses due to mineralization were recorded in the control (1087 kg ha^{-1} $year^{-1}$). Contrariwise, the highest losses were determined in the UAN + St treatment—3415 kg C ha^{-1} $year^{-1}$. The evaluation of carbon losses due to the mineralization process related to the total carbon amount in the harvested biomass is very interesting. The results clearly show that the control (28.9%), AS (29.7%), and UAN (28.4%) treatments do not differ significantly. The application of organic fertilizers turns this ratio significantly more negative: UAN + St (57.6%) and manure (41.1%) (Table 2). High losses of C in soil highlight the importance of this research and at the same time the necessity of a complex approach to its solution.

The relationship between the amount of extractable carbon (C_{CaCl2}, C_{PF}) and the total amount of C_{SOM} documents the fact that the control plots and farmyard manure treatment had more stable organic matter and consequently, a lower intensity of mineralization [29]. The highest values were recorded in the AS treatment—the ratio of C_{CaCl2}/C_{SOM} was 0.11% and that of C_{PF}/C_{SOM} 28%. Similarly, a very low extraction strength (0.01 mol L^{-1} $CaCl_2$) was also determined in our previous experiments at different sites [30]. In contrast, a lower ratio of C_{PF}/C_{SOM} was recorded in our trials compared to the results of Ellerbrock and Kaiser [31]. In their trials, about 40% of C_{SOM} was extracted while in our trials it was only 22–28%. Furthermore, [4] used the C_{PF}/C_{SOM} ratio to assess the stability of SOM, suggesting that the higher this ratio is, the less stable the C_{SOM}. Similar to our experiments, [4] also determined the lowest value in the manure treatment (27.6%) and the highest in the mineral nitrogen, phosphorus and potassium (NPK) treatment (34.7%). Over the period of 26 years, C_{SOM} degradation occurred in almost all treatments, except farmyard manure. Both the content of C_{HA} and the C_{HA}/C_{FA} decreased. This process was the fastest in the AS treatment. It is evident that manure helps to increase the content of C_{HA} as well as its ratio in C_{HS} [4]. In the UAN + St treatment, the content of C_{HA} did not increase; the C_{HA}/C_{FA} did not improve, even with the application of high doses of wheat straw.

Using the analyses of $^{13}C/^{12}C$ allowed us to determine the ratio of C_4 in the C_{SOM} and C_4 ratio in different extractable fractions. This was realized only for the mineral fertilizing treatments for two reasons: (i) the $^{13}C/^{12}C$ was not evaluated at organic inputs (FYM, straw), because we have not the data for $^{13}C/^{12}C$ in input materials; (ii) the results originate from the long-term experiments with maize monoculture, which allows us to make a reliable evaluation. It was evident that the C_4 ratio was higher in the fertilized plots compared to the control (control 8.10%, AS 10.3%, UAN 11.8%). The higher ratio in fertilized plots is reported by many authors [6–9]. However, the C_4 ratios determined in these studies differ significantly. There is a significant influence of the monoculture duration—from six years [11], up to 40 years [9]. The purpose of cultivation (grain/silage) is also very important. Our values are in good compliance with [9], who reported a C_4 ratio in C_{SOM} of 9.5% in the unfertilized control and 14.1% in the fertilized treatments. Our experiments and the work by [9] focused on silage maize, where almost all aboveground biomass is taken off the field. A similarly low C_4/C_{SOM} ratio (13%) was also determined by [10]. Ellerbrock and Kaiser [31] also determined 14.2%. By contrast, [6] published results where C_4 represented 60% of C_{SOM} in topsoil. However, it was in long-term grain-maize cultivation. Our results show that the C_4 ratio in the C_{SOM} of the subsoil is significantly lower. In the control, it was 5.5%, in AS 7.9%, and in UAN 6.0%, respectively. Similarly low C_4 ratios in C_{SOM} (5–10%) were recorded for subsoil in the studies of [8,9].

A significantly higher C_4 ratio compared to C_{SOM} was recorded in the hydrolysable fractions of organic compounds (C_{DOC}). The 0.01 mol L^{-1} $CaCl_2$ extraction solution had a C_4 ratio in C_{CaCl2} in the interval of 17.9–20.7% and the 0.1 mol L^{-1} $Na_4P_2O_4$ extractable fraction was in the interval of 10.20–14.60% (C_4 ratio in C_{PF}). It was evident that the weaker the extraction agent, the higher the ratio of maize carbon. Similarly, Ellerbrock and Kaiser [31] determined a ratio of 22% C_4 for water extractable carbon and 16% C_4 for C_{PF}. Higher ratios than those determined in our experiment have been reported, e.g., by [5] ratio 34% C_4 in C_{DOC}; [12] ratio 30%; [9] ratio 5–30%. A very high ratio of C_4 in C_{DOC} (29–54%) was reported by [5]; however, it was in long-term grain-maize cultivation. In our experiments, the ratio of C_4 in C_{DOC} was always higher in fertilized plots compared to the control with no fertilization, which complies with previous results. The results further show that fertilization increases the amount of easily extractable and thus less stable C fraction [13].

Statistically conclusive differences in the content of C_3 between control and N-fertilized plots document the fact that AS or UAN applications result in stronger mineralization of original and stable organic matter compared to a non-fertilized control (Table 3). The increase of C_4 content in these treatments does not compensate for the decrease of C_3 content; the result is a tendency to decrease total C_{SOM} content [2,7]. This process also takes place in the subsoil, but less dynamically. Fertilized plots in our experiments had about 9% lower C_3 content in topsoil.

4. Materials and Methods

4.1. Field Experiments

Long-term stationary field trials with maize monoculture were set up at the Czech University of Life Sciences experimental site Červený Újezd in 1993. Maize was grown for silage. The characteristics of the site are shown in Table 4.

Table 4. Basic description of investigated location; C_{SOM}—soil organic matter carbon.

GPS Coordinates	**50°4′22″ N; 14°10′19″ E**
Altitude (m above sea level)	410
Mean annual temperature (°C)	7.7
Mean annual precipitation (mm)	493
Soil type [32]	Haplic Luvisol
Soil texture [32]	Loam
Clay (%) (<0.002 mm)	5.4
Silt (%) (0.002–0.05 mm)	68.1
Sand (%) (0.05–2 mm)	26.5
Bulk density (g cm^{-3}) topsoil [33]	1.47
Bulk density (g cm^{-3}) subsoil [33]	1.55
C_{SOM} (%)	1.26
pH ($CaCl_2$)	6.5
Cation exchange capacity ($mmol_{(+)}$ kg^{-1})	118

The size of individual experimental plots was 170 m^2 (20 m length × 8.5 m width) and the size of harvested plots 20 m^2 (two middle rows of the experimental plot). Each treatment (experimental plot) was conducted in four replications in a randomized design. Nitrogen was applied in the same dose (120 kg N ha^{-1} $year^{-1}$) using ammonium sulfate (AS), urea ammonium nitrate (UAN) and farmyard manure (FYM), except for the unfertilized control plots (Cont) and treatment UAN with straw (UAN + St) (Table 5). Nitrogen fertilizers were applied in spring, before maize sowing. In the UAN + St treatment, wheat straw (5 t dry matter (DM) ha^{-1}) was applied just before the autumn tillage. Similarly, FYM was applied in the autumn and immediately incorporated with ploughing to minimize nitrogen losses. The amount of FYM corresponded to 120 kg N ha^{-1} (always according to FYM nitrogen content analysis).

Table 5. Fertilizing design of the experiment; AS—ammonium sulfate, UAN—urea ammonium nitrate, UAN + st–urea ammonium nitrate + straw, FYM–farmyard manure, DM—dry matter, C—carbon, N—nitrogen.

Treatment	kg N ha^{-1} year^{-1}	Organic Fertilizer (kg DM ha year^{-1})	C Content in DM (%)	C Supplied (kg ha^{-1}year^{-1})	C Supplied during 26 Years (kg ha^{-1})	C/N in Org. Fert.
Control	—	—	—	—	—	—
AS	120	—	—	—	—	—
UAN	120	—	—	—	—	—
UAN + St	120 + 33.5 [1]	5000	42.8	2140	55,640	79.3/1
FYM	120	5752	27.9	1603	41,678	13.4/1

[1] N content in wheat straw; after application of UAN to UAN + St treatment the final C/N ratio changed to 14.6:1.

All aboveground biomass was harvested and removed from the harvested plot, except for about 10 cm stubble. The roots were extracted by the sampling of topsoil blocks (area 40 × 40 cm; 30 cm depth; 4 subsamples per plot) and were subsequently washed and separated.

Soil samples (topsoil from 0–30 cm and subsoil 30–60 cm depth) were taken up after maize harvest from twelve sampling points per plot, subsequently, mixed, sieved through 5 mm mesh and frozen. To the further described soil analysis, samples from the years 1993 and 2018 were thawed, air-dried, sieved through 2 mm mesh and analyzed (except for $CaCl_2$ extraction, where the fresh soil samples were analyzed). The C_{SOM} content was determined in the samples taken up in both years (1993 and 2018). The remaining soil analysis proceeded only in the samples taken up in 2018.

4.2. *Analysis*

A solution of 0.01 mol L^{-1} $CaCl_2$ (C_{CaCl2}) was used (1:10, w/v) to determine one of the less stable soil C_{CaCl2} fractions. The content of C_{CaCl2} was determined in fresh soil samples by segmental flow-analysis using the infrared detection on a Skalar^{plus}System (Skalar, Breda, The Netherlands).

Fractionation of humic substances (C_{HS}) was undertaken according to the [34] method to obtain the pyrophosphate extractable fraction (C_{PF}), which represents the sum of the carbon in humic acids (C_{HA}) and fulvic acids (C_{FA}). In brief, C_{HA} and C_{FA} were extracted from a 5 g soil sample with a mixture of 0.1 mol L^{-1} NaOH and 0.1 mol L^{-1} $Na_4P_2O_7$ (1:20, *v/v*) solution. Carbon of humic substances C_{HS} and C_{HA} was determined by an oxidimetric titration method. The content of C_{FA} was calculated as the difference between C_{HS} and C_{HA}. The degree of humification was calculated as the ratio of C_{HS} content and C_{SOM} content multiplied by 100 [4].

Stable isotope $\delta^{13}C$ analyses were performed by flash combustion in a Fisons 1108 elemental analyzer coupled with an isotope ratio mass spectrometer Delta V Advantage (ThermoFisher, Bremen, Germany) in a continuous flow regime. The sample size was adjusted to contain a sufficient amount of carbon. Results are reported as $\delta^{13}C$ values (in per mil ‰) relative to Vienna Pee-Dee Belemite (V-PDB). International standards NBS 22 (−30.031 ‰), IAEA-CH-7 (−32.151 ‰), and in-house standard soil (−27.80 ‰) were used as reference material. The long-term reproducibility was better than 0.15‰. $^{13}C/^{12}C$ isotope ratios were expressed as $\delta\ ^{13}C$ values.

$$\delta^{13}C\ (‰) = [(\delta_{sam}/\delta_{std}) - 1] \times 10^3, \tag{1}$$

where δ_{sam} = $^{13}C/^{12}C$ ratio of sample, and δ_{std} = $^{13}C/^{12}C$ ratio of the reference standard (PDB). The $\delta^{13}C$ values of dissolved organic carbon (DOC) were determined by the method of Buzek et al. [35]. In brief, the solution was acidified with diluted phosphoric acid to remove bicarbonates, and further concentrated by evaporation (at 50 °C) for DOC $\delta^{13}C$ measurements (Fisons 1108 and Delta V with NBS 22 as internal reference).

The content of total organic carbon and nitrogen in air-dried soils, in farmyard manure, and in plants was determined using oxidation on a CNS analyzer (Elementar Vario Macro, Elementar Analysensysteme, Hanau-Frankfurt am Main, Germany).

Data were processed using basic tests for normal distribution and subsequently one-way analysis of variance (ANOVA, Tukey test; $p < 0.05$) using the STATISTICA 12 (Dell–StatSoft Inc., Austin, TX, USA) program.

5. Conclusions

Soil organic matter carbon (C_{SOM}) compounds' degradation was observed in long-term field experiments with silage maize monoculture. Over a relatively short period of 26 years, the content of carbon in topsoil decreased by 22% in control plots compared to 26% in treatments fertilized with mineral N. It is almost alarming that in an annual application of wheat straw (5 t DM.ha^{-1}) C_{SOM} also decreased (by 8%). It is obvious that straw was relatively quickly mineralized after its incorporation into the soil. By contrast, the application of farmyard manure resulted in a C_{SOM} increase of 16%.

The ratio of carbon produced by maize from total topsoil C_{SOM} content ranged between 8.1–11.8%. In plots with mineral N fertilization, this ratio was always higher than in the unfertilized control plots. The weaker the soil extraction agent, the higher ratio of maize carbon, which shows that maize produced mostly unstable carbon compounds. Furthermore, mineral N application resulted in stronger mineralization of original and stable organic matter compared to the unfertilized control. However, the increase of maize carbon content in fertilized plots did not compensate the decrease of "old" organic matter. As a result, a tendency of decreasing total C_{SOM} content in plots with mineral N applied was observed.

Generally, it is obvious that silage maize monoculture cropping in a relatively short time period is significantly decreasing the C_{SOM} content as well as its quality expressed by decreasing: (i) content of humic acids and (ii) ratio of humic/fulvic acids.

Author Contributions: Author of the main idea and the draft of the manuscript J.B. Conceptualization, analysis M.K.; methodology, basic data evaluation and analysis J.Č.; Important draft corrections and new ideas O.S. Further statistical evaluation, preparing figures and tables P.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

C_{SOM}	Soil organic matter carbon compounds
C_3	"Old" soil organic matter
C_4	Carbon from maize
CHA	Carbon in humic acids
C_{FA}	Carbon in fulvic acids
C_{HU}	Carbon in humines
C_{HS}	Carbon of humic substances
C_{CaCl2}	Carbon determined with 0.01 mol L^{-1} $CaCl_2$
C_{PF}	Carbon determined with 0.1. mol L^{-1} $Na_4P_2O_7$
C_{DOC}	Easily hydrolysable C fraction
N_t	total nitrogen content
DM	Dry matter
FYM	Farmyard manure
UAN	Urea ammonium nitrate
St	Straw
AS	Ammonium sulfate

References

1. Capriel, P. Trends in organic carbon and nitrogen contents in agricultural soils in Bavaria (South Germany) between 1986 and 2007. *Eur. J. Soil Sci.* **2013**, *64*, 445–454. [CrossRef]
2. Loges, R.; Bunne, I.; Reinsch, T.; Malisch, C.; Kluß, C.; Herrmann, A.; Taube, F. Forage production in rotational systems generates similar yields compared to maize monocultures but improves soil carbon stocks. *Eur. J. Agron.* **2018**, *97*, 11–19. [CrossRef]
3. Schnitzer, M. Organic matter, Principles and Processes. In *Encyclopedia of Soils in the Environment*, 3rd ed.; Hillel, D., Ed.; Elsevier Ltd.: Amsterdam, The Netherlands, 2005; pp. 85–93.
4. Menšík, L.; Hlisnikovský, L.; Pospíšilová, L.; Kunzová, E. The effect of application of organic manures and mineral fertilizers on the state of soil organic matter and nutrients in the long-term field experiment. *J. Soil Sediment.* **2018**, *18*, 2813–2822. [CrossRef]
5. Gregorich, E.G.; Liang, B.C.; Drury, C.F.; MacKenzie, A.F.; McGill, W.B. Elucidation of the source and turnover of water soluble and microbial biomass carbon in agricultural soils. *Soil Biol. Biochem.* **2000**, *32*, 581–587. [CrossRef]
6. Collins, H.P.; Blevins, R.L.; Gundy, L.G.; Christenson, D.R.; Dick, W.A.; Huggins, D.R.; Paul, E.A. Soil carbon dynamics in corn-based agroecosystems: Results from carbon-13 natural abundance. *Soil Sci. Soc. Am. J.* **1999**, *63*, 584–591. [CrossRef]
7. Ludwig, B.; John, B.; Ellerbrock, R.; Kaiser, M.; Flessa, H. Stabilization of maize in sandy soil in a long-term experiment. *Eur. J. Soil Sci.* **2003**, *54*, 117–126. [CrossRef]
8. Gregorich, E.G.; Ellert, B.H.; Drury, C.F.; Liang, B.C. Fertilization effects on soil organic matter turnover and corn residue C storage. *Soil Sci. Soc. Am. J.* **1996**, *60*, 472–476. [CrossRef]
9. Bettina, J.; Ludwig, B.; Flessa, H. Carbon dynamics determined by natural ^{13}C abundance in microcosm experiments with soils from long-term maize and rye monocultures. *Soil Biol. Biochem.* **2003**, *35*, 1193–1202. [CrossRef]
10. Rasse, D.P.; Mulder, J.; Moni, C.; Chenu, C. Carbon Turnover Kinetics with Depth in a French Loamy soil. *Soil Sci. Soc. Am. J.* **2006**, *70*, 2097–2105. [CrossRef]
11. Wanniarachchi, S.D.; Voroney, D.P.; Vyn, T.J.; Beyaert, R.P.; MacKenzie, A.F. Tillage effects on the dynamics of total and corn-residue derived soil organic matter in two Ontario soils. *Can. J. Soil Sci.* **1999**, *79*, 473–480. [CrossRef]
12. Flessa, H.; Ludwig, B.; Heil, B.; Merbach, W. The origin of soil organic C, dissolved organic C and respiration in long-term maize experiment in Halle, Germany, determined by ^{13}C natural abundance. *J. Soil Sci. Plant. Nutr.* **2000**, *163*, 157–163. [CrossRef]
13. Campbell, C.A.; Lafond, G.P.; Biederbeck, V.O.; Wen, G.; Schoenau, J.; Hahn, D. Seasonal trends in soil biochemical attributes: Effects of crop management on a Black Chernozem. *Can. J. Soil Sci.* **1999**, *79*, 85–97. [CrossRef]
14. Qin, R.; Stamp, P.; Richner, W. Impact of tillage on maize rooting in a Cambisol and Luvisol in Switzerland. *Soil Till. Res.* **2006**, *85*, 50–61. [CrossRef]
15. Balesdent, J.; Balabane, M. Maize root-derived soil organic carbon estimated by natural ^{13}C abundance. *Soil Biol. Biochem.* **1992**, *24*, 97–101. [CrossRef]
16. Gregorich, E.G.; Voroney, R.P.; Kachanoski, R.G. Turnover of carbon through the microbial biomass in soils with different texture. *Soil Biol. Biochem.* **1991**, *23*, 799–805. [CrossRef]
17. Ajwa, H.; Rice, C.; Sotomayor, D. Carbon and nitrogen mineralization in tallgrass prairie and agricultural soil profiles. *Soil. Sci. Soc. Am. J.* **1998**, *62*, 942–951. [CrossRef]
18. Eusterhues, K.; Rumpel, C.; Kleber, M.; Kogel-Knaber, I. Stabilisation of soil organic matter by interactions with minerals as revealed by mineral dissolution and oxidative degradation. *Org. Geochem.* **2003**, *34*, 1591–1600. [CrossRef]
19. Anonymous. Biom, Czech Biomass Association. Available online: https://czbiom.cz/blog/2019/06/06/digestat/ (accessed on 16 March 2020).
20. Anonymous. Cumulative Summaries about Soil Fund Based on Data from Cadastre of Czech Republic. Available online: https://www.cuzk.cz/Periodika-a-publikace/Statisticke-udaje/Souhrne-prehledy-pudniho-fondu/Rocenka_pudniho_fondu_2020.aspx (accessed on 16 March 2020).
21. Vácha, R.; Čechmánková, J.; Duffková, R. *Soil–Our Wealth [In Czech]*; VÚMOP: Prague, Czech Republic, 2005; 226p.

22. Food and Agriculture Organization of the United Nations. *World Reference Base for Soil Resources 2014. International Soil Classification System for Naming and Creating Legends for Soil Maps*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2015; 193p. Available online: http://www.fao.org/3/i3794en/I3794en.pdf (accessed on 1 September 2020).
23. Hauzé, V.; Tran, G.; Edouard, N.; Lebas, F. Maize Silage. *Feedipedia, a Programme by INRA, CIRAD, AFZ and FAO*. 2017. Available online: https://www.feedipedia.org/node/13883 (accessed on 31 August 2020).
24. Christensen, B.T. Wheat and barely straw decomposition under field conditions: Effect of soil type and plant cover on weight loss, nitrogen and potassium content. *Soil Biol. Biochem.* **1985**, *17*, 691–697. [CrossRef]
25. Wang, H.; Wang, L.; Zhang, Y.; Hu, Y.; Wu, J.; Fu, X.; Le, Y. The variability and causes of organic carbon retention ability of different agricultural straw types returned to soil. *Environ. Technol.* **2017**, *38*, 538–548. [CrossRef]
26. Guan, X.-K.; Wei, L.; Turner, C.N.; Ma, S.-C.; Yang, M.-D.; Wang, T.-C. Improved straw management practices promote in situ straw decomposition and nutrient release, and increase crop production. *J. Clean Prod.* **2020**, *20*, 119514. [CrossRef]
27. Li, D.; Li, Z.; Zhao, B.; Zhang, J. Relationship between the chemical structure of straw and composition of main microbial groups during the decomposition of wheat and maize straws as affected by soil texture. *Biol. Fert. Soils.* **2020**, *56*, 11–24. [CrossRef]
28. Schmidt, L.; Warnstorff, K.; Dörfel, H.; Leinweber, P.; Lange, H.; Merbach, W. The influence of fertilization and rotation on soil organic matter and plant yields in the long-term Eternal Rye trial in Halle (Saale), Germany. *J. Plant. Nutr. Soil Sci.* **2000**, *163*, 639–648. [CrossRef]
29. Liang, B.C.; MacKenzie, A.F. Changes in soil organic carbon and nitrogen after six years of corn production. *Soil Sci.* **1992**, *153*, 307–313. [CrossRef]
30. Balík, J.; Černý, J.; Kulhánek, M.; Sedlář, O. Soil carbon transformation in long-term field experiments with different fertilization treatments. *Plant. Soil Environ.* **2018**, *64*, 578–586. [CrossRef]
31. Ellerbrock, R.H.; Kaiser, M. Stability and composition of different soluble soil organic matter-evidence from δ^{13}C and FTIR signatures. *Geoderma* **2005**, *128*, 28–37. [CrossRef]
32. USDA NRCS. *Soil Survey Field and Laboratory Methods Manual*; National soil survey center: Lincoln, NE, USA, 2009; 436p.
33. International Organization for Standardization. *Soil Quality. Determination of Dry Bulk Density. Core Method: ISO 11272-2017*; International Organization for Standardization: Geneva, Switzerland, 2017.
34. Kononova, M. *M Organičeskoje Veščestvo Počvy: Jego Priroda, Svojstva i Metody Izucenija*; AN SSSR: Moscow, Russia, 1963.
35. Buzek, F.; Cejkova, B.; Gersl, M.; Jackova, I.; Lnenickova, Z.; Gerslova, E. Carbon Isotope Study of soil Amendment with Maize Fermentation Digestate. *IJPSS* **2017**, *14*, 1–11. [CrossRef]

4.4 The impact of the long-term application of mineral nitrogen and sewage sludge fertilizers on the quality of soil organic matter

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RESEARCH **Open Access**

The impact of the long-term application of mineral nitrogen and sewage sludge fertilizers on the quality of soil organic matter

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Abstract

Background: Soil fertility is substantially influenced by soil organic matter quality and quantity. Much attention has also been given to glomalin content as one of the qualitative parameters of soil fertility. Glomalin content is considered an indicator of soil fertility due to its positive correlation with soil organic carbon.

Aim: This study aim to (i) determine the influence of different fertilization systems on maize yield and soil organic matter quality parameters, (ii) ensure that changes in total glomalin content (TG) and easily extractable glomalin (EEG) content are sensitive enough to reflect changes in the SOM quality resulting from long-term fertilization, and (iii) determine whether both forms of glomalin must be determined or if the easily extractable glomalin (EEG) content is sufficient, even for a fertile chernozem.

Materials and methods: Long-term field experiments with silage maize monocultures were used to study relationships under different rates of mineral nitrogen and sewage sludge fertilization. The trials comprised 5 treatments: (i) no fertilization control (Con), (ii) and (iii) two different calcium ammonium nitrate rates (N120 and N240 at doses of 120 and 240 kg N ha^{-1} $year^{-1}$, respectively), and iv) and v) two different sewage sludge rates (S120 and S240 corresponding to mineral N doses). Topsoil (0–30 cm) analysis was performed 28 years after the onset of the experiment to determine soil organic carbon, the fractions of humic substances, potentially mineralizable carbon and dissolved organic carbon, the potential wettability index, soil aggregate stability, EEG, and total glomalin (TG).

Results: The control treatment, which did not include fertilization, showed significantly lower average yields (9.76 t DM ha^{-1} $year^{-1}$) than the fertilization treatments (on average 12.3 DM ha^{-1} $year^{-1}$). Significant differences in yields were not found between the fertilization treatments. A positive correlation between glomalin (EEG, TG) content and soil organic matter carbon content was observed. A periodic application of sewage sludge increased the content of glomalin. However, the highest quality of SOM was found in the control treatment (nonfertilized). This treatment had the highest values for the humic/fulvic acid ratio and the highest contents of humic acids and potentially mineralizable carbon. Furthermore, the Con treatment showed the highest soil aggregate stability and potential wettability index. Mineral N fertilization significantly reduced soil aggregate stability values.

Conclusions: The unfertilized control showed the highest SOM quality but the lowest yields. The data on glomalin content can be used to study soil organic matter quality. Because the EEG extraction method is easy to apply, uses

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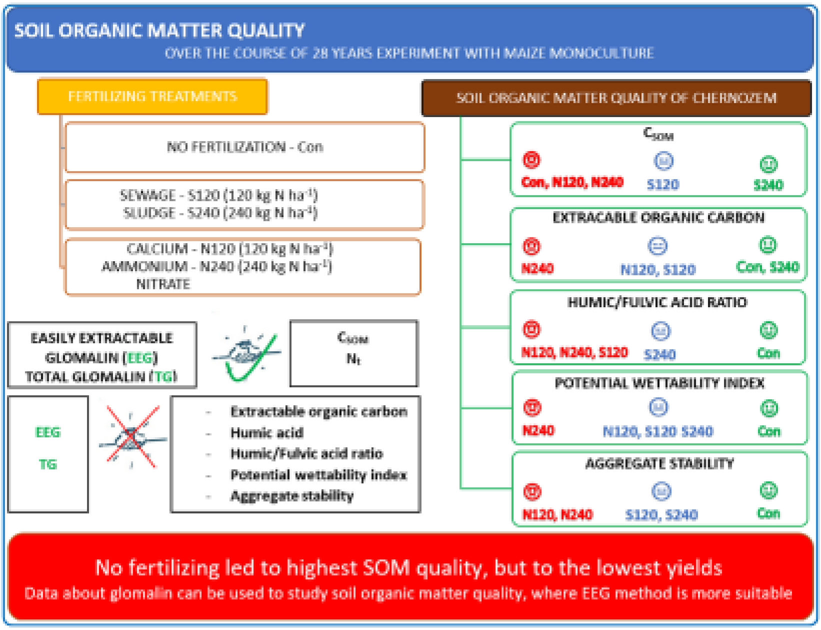
lower quantities of chemicals and consumes less time, it presents a better option than TG extraction. The sensitivity of the methods for SOM quality determination decreases with increasing SOM content.

Keywords: Maize monoculture, Potential wettability index, Aggregate stability, Glomalin

Graphical Abstract



Introduction

Soil organic matter (SOM) can be divided into stable and labile fractions. The stable fraction is represented by humic acids (C_{HA}), fulvic acids (C_{FA}) and humins (C_{HS}) [1]. The labile forms are represented by potentially mineralizable carbon (C_{HWE}) or dissolved organic carbon (C_{DOC}). The fractionation of SOM into individual forms is important for SOM quality degradability assessment [2–5].

Currently, there are a number of different methods for determining SOM quality parameters. The current aim for agricultural and even scientific practices is to find a "user friendly" method that is characterized by reliable results, a simple procedure, ease of data evaluation, low cost, rapid evaluation time and low chemical consumption. Because of this, much attention has been given to glomalin content as one of the qualitative parameters of soil fertility [6].

Glomalin is produced by arbuscular mycorrhizae and is one of the most important soil proteins. The exact molecular composition of glomalin has not yet been defined, because fractions of glomalin extracted from soils are not sufficiently clean [7, 8]. This is also a reason why the term "glomalin-related soil protein", abbreviated GRSP, is often used in the literature [9]. Wright and Upadhyaya [10] divided the forms of glomalin into easily extractable (EEG) and total glomalin (TG). Glomalin is hydrophobic and temperature stable. Substantial resistance to degradation in the soil has been described in many studies [9, 11, 12]. The slow change cycle for glomalin in the soil results from its high stability. Rillig [9] claimed that the average time for glomalin degradation in the soil is between 6 and 42 years. Harner et al. [13] estimated a similar time for a change cycle (40 years). Glomalin contains up to 85% polysaccharides that are resistant to microbiological degradation and, therefore, participate in bonding minerals and organic particles into aggregates for long periods of time [14]. Glomalin also improves C sequestration in the soil [15]. Its influence on the formation of soil aggregates is greater than that on direct plant nutrition [16]. Glomalin acts as a sticky and insoluble biofilm that glues minerals, clays, organic matter and microorganisms together [17]. Organic inputs increase the portion of macroaggregates and the mean weight diameter of aggregates as well as the GRSP fractions [18–20].

Glomalin content can be considered a good indicator of soil fertility [10, 20] due to its positive correlation with the C_{SOM} content [21, 22]. This finding supports the fact that the contribution of glomalin to the total C content in soils is greater than the contribution of microbial biomass [23]. Furthermore, the long-term application of compost and manure increased the glomalin content in a soil [19, 20]. An increase in glomalin content was also found after the application of sewage sludge [22, 24]. There are only a few scientific publications that have focused on glomalin content in soils under long-term maize monoculture, i.e., Galazka et al. [25], and some studies contained in master theses, e.g., Sekgota [26]. However, these studies evaluated other parameters or only short-term monocultures and cannot be directly compared with our results. In our previous study [27], we found a positive correlation between glomalin content and humic acid content (C_{HA}) as well as glomalin content and the humic acid and fulvic acid ratio ($C_{HA/FA}$) under long-term silage maize production on a luvisol. We also found a positive correlation with the potential wettability index (PWI).

"Diffuse reflectance infrared Fourier transform spectrometry" (DRIFTS) can also be used for SOM quality assessment. Usually, it is used to study hydrophobic and hydrophilic functional groups of SOM at wavelengths 3000–2800 cm^{-1} and 1740, 1640–1600 cm^{-1}, respectively [20, 26, 28]. SOM is also characterized by the ratio of aliphatic (C–H) and carboxylic (C–O) bonds, called the potential wettability index (PWI) [29]. Demyan et al. [30] used the DRIFTS method to monitor SOM quality in long-term trials in Bad Lauchstädt (Germany). All of the observations obtained with DRIFTS showed an influence of fertilization.

The abovementioned facts prompted the following objectives: i) determine the influence of different fertilization systems on soil organic matter quality parameters (degradable and stable C fractions including glomalin content), ii) ensure that changes in total glomalin content (TG) and easily extractable glomalin (EEG) content are sensitive enough to reflect changes in the SOM quality resulting from long-term fertilization, and iii) determine whether both forms of glomalin have to be determined or if the easily extractable glomalin content (EEG) is sufficient. even for a fertile chernozem.

Materials and methods

Site description

The study was carried out based on long-term fertilization experiments that were initiated in 1993 at the experimental stations located in Suchdol in the Czech Republic. The basic soil-climatic characteristics are given in Table 1.

Experimental design

This long-term field experiment was initiated in a randomized complete block design (4 replications) with a plot area of 46 m^2 in 1993. In general, the experiment was designed to have five treatments: i) unfertilized control (Con), ii) mineral fertilization in the form of calcium ammonium nitrate (N120), iii) mineral fertilization in the form of calcium ammonium nitrate (N240), iv) sewage sludge (S120) and iv) sewage sludge (S240). A detailed description of the individual treatments is as follows: treatments N120 and N240 received 120 and 240 kg N ha^{-1} $year^{-1}$, respectively, in the form calcium ammonium nitrate, and treatments S120 and S140 received the same dose of N in the form of sewage sludge (calculated based on the sewage sludge analysis). Maize hybrids were planted in each plot at a density of 80 thousand plants ha^{-1} (=8 plants m^{-1}). The maize was sown at the end of April/beginning of May with 70 cm between plant rows. Mineral N fertilizers were applied prior to sowing every year in spring with no additional liming or application of other nutrients. Sewage sludge (S120, S240 corresponding to mineral N doses) was applied every 3 years in autumn (October); therefore, the doses of nitrogen were 360 kg N ha^{-1} 3 $years^{-1}$ and 720 kg N ha^{-1}

Table 1 Basic description of the Suchdol experimental site at the onset of the experiment

GPS coordinates	**50°7´40´´N 14°22´33´´E**
Altitude (m above sea level)	289
Mean annual temperature (°C)	9.1
Mean annual precipitation (mm)	495
Soil type	Haplic Chernozem[a]
Soil texture	Silt loam[a]
Clay (%) (< 0.002 mm)	2.2
Silt (%) (0.002–0.05 mm)	71.8
Sand (%) (0.05–2 mm)	26.0
Bulk density (g cm^{-3})	1.42
C_{SOM} (%)	1.88[b]
pH (0.01 mol/L $CaCl_2$)	7.5
CEC ($mmol_{(+)}$ kg^{-1})	262

[a] Natural resource conservation service—United States Department of Agriculture [31]

[b] CNS analyser (see Chapter 2.3., soil analysis)

3 years^{-1} for S120 and S240, respectively. After application, the fertilizer was immediately incorporated into the soil with ploughing (25 cm depth). The sewage sludge was supplied by the same producer and was produced using the same method over the course of the entire experiment. The average dose of the sewage sludge was 10.17 t of DM ha^{-1} 3 years^{-1} and 20.34 t of DM ha^{-1} 3 years^{-1} for S120 and S240, respectively. During the experiment, the change in sewage sludge quality was insignificant. The average doses of key nutrients and the C/N ratio are presented in Table 2. The dose of sewage sludge was calculated according to the total nitrogen content determined with Kjeldahl analysis, which includes both organic and inorganic (N–NH_4^+) forms. However, N–NO_3^- and N–NO_2^- forms are not determined using this method, because in an anaerobic material, such as sewage sludge, only trace levels of nitrates and nitrites are present.

Plants were protected only against weeds. Two rows of maize aboveground biomass (18 m^2 per plot) were harvested at silage maturity (roughly 65% biomass moisture content, R4 vegetation stage) and weighed to obtain the aboveground biomass yield (BY). Dry BY was calculated based on the dry mass ratio in the subsamples.

Soil analysis

Topsoil (0–30 cm) analyses were performed with air-dried soil samples (≤ 2 mm), except C_{DOC}, collected in September 2020, after the maize harvest. For aggregate stability (WSA) assessment, the soil aggregates of diameter 2–5 mm were sieved and further analysed.

The soil organic carbon (C_{SOM}) and total nitrogen (N_t) contents of the soils and sewage sludge were determined using oxidation with a CNS analyser Elementar Vario Macro (Elementar Analysensysteme, Germany) after carbonate digestion with HCl and subsequent drying.

Fractionation of humic substances (C_{HS}) was performed according to Kononova [1] to obtain the pyrophosphate extractable fraction, which represents the sum of the carbon in humic acids (C_{HA}) and fulvic acids (C_{FA}). Briefly, C_{HA} and C_{FA} were extracted from a 5 g soil sample with a mixed solution of 0.10 mol L^{-1} NaOH and 0.10 mol L^{-1} $Na_4P_2O_7$ (1:20 w/v). The following fractions of carbon were isolated: C_{FA} was obtained from a solution that was acidified by dilute H_2SO_4 to a pH of 1.0–1.5 and left undisturbed for 24 h, and C_{HA} was obtained by the dissolution of the previously formed precipitate in a hot 0.05 mol L^{-1} NaOH solution. Before iodometric titration, the dry matter formed by the vaporisation of each sample was dissolved in a mixture of 0.067 mol L^{-1} $K_2Cr_2O_7$ and concentrated H_2SO_4 under an elevated temperature.

Table 2 Doses of applied C, N, P, and K (in kg ha^{-1} year^{-1}) and the C/N ratio of sewage sludge

Fertilizer	C	N	P	K	C/N
S120	879	120	82.0	15.6	7.32:1
S240	1758	240	164.0	31.2	

S120 sewage sludge (120 kg N ha^{-1}); *S240* sewage sludge (240 kg N ha^{-1})

For the dissolved organic carbon (C_{DOC}), the extraction agent 0.01 mol L^{-1} $CaCl_2$ was used (1:10, w/v) [32]. Analysis was performed on fresh soil samples (≤ 5 mm).

Hot water extraction was used to assess mineralizable soil organic carbon (C_{HWE}). Air dried (≤ 2 mm) soil samples were extracted with water (1:5, w/v). The suspension was boiled for 1 h [33]. The contents of C_{HWE} and C_{DOC} were determined by segmental flow analysis using infrared detection on a SKALAR SAN^{plus} SYSTEM (Skalar, Netherlands).

The potential wettability index (PWI) was determined directly in the soil samples using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). DRIFTS spectra were recorded by an infrared spectrometer (Nicolet IS10, Waltham, USA). The bands of the alkyl C–H groups—A (2948–2920 cm^{-1} and 2864–2849 cm^{-1}) were assumed to indicate the hydrophobicity, and bands of the C = O groups—B (1710 and 1640–1600 cm^{-1}) indicated hydrophilicity [28].

$$PWI = A/B \tag{1}$$

Aggregate stability (WSA) was evaluated using the WSA index and was measured using the procedure reported by Nimmo and Perkins [34]. Four grams of air-dried soil aggregates (diameter of 2–5 mm) were sieved for 3 min in distilled water (0.25-mm sieve) with a frequency of 35 cycles per minute and a vertical amplitude of 1.3 cm. The aggregates that remained on the sieve were then sieved (same frequency and amplitude) in a sodium hexametaphosphate solution (2 g L^{-1}) until only the sand particles that remained on the sieve. The aggregates that dissolved by wetting in water or hexametaphosphate solution were subsequently dried at 105 °C and weighed. The WSA index was calculated as follows:

$$WSA = Wds/(Wds + Wdw) \tag{2}$$

where Wds is the weight of aggregates dispersed in a sodium hexametaphosphate solution, and Wdw is the weight of aggregates dispersed in distilled water. An increase in the WSA value indicates an increase in soil aggregate stability.

Easily extractable glomalin (EEG) and total glomalin (TG) analysis were performed according to Wright and Upadhyaya [10]. Briefly, to the 1.00 g of air dried soil (< 2 mm) was added 8 mL of sodium citrate (20 mmol. L^{-1} of pH 7.0—EEG, 50 mmol L^{-1} pf pH 8.0—TG), followed by autoclaving at 121 °C (30 min—EEG, 60 min—TG), cooling and centrifugation at 5000 rpm (10 min—EEG, 15 min—TG). For TG, the centrifugation of the supernatant of the same sample was repeated 5 times until the supernatant no longer showed the red–brown colour that is typical of glomalin. Both forms of glomalin were determined colorimetrically using bovine albumin (BSA) as a standard for quantification and the Bradford protein assay (both from Bio-Rad, California, USA) to achieve the colour change.

Carbon sequestration efficiency (CSE) was calculated according to the following formula:

$$\mathrm{CSE}\ (\%) = ((C_{Ss} - C_S)/C_{Sa}) * 100 \tag{3}$$

where C_{Ss} is the amount of C in the topsoil of the sewage sludge treatments, C_S is the amount of C in the topsoil of the control and C_{Sa} is the total amount (for 28 years) of C applied with sewage sludge (all in kg ha^{-1}).

Statistical analysis

The results were evaluated using ANOVA statistical analysis with Tukey's test using the Statistica program (TIBCO, Paolo Alto, California, USA). Principal component analysis (PCA) was performed to evaluate the relationships between the content of glomalin (EEG, TG) and the qualitative parameters of SOM using XLSTAT (Addinsoft, New York, USA). The variables were subjected to PCA, and eigenvalues > 1, variance (%) and cumulative (%) criteria were used to define the association among variables.

Results

Yield of maize biomass

The silage maize biomass yields for the period 1997–2020 are presented in Fig. 1. The experiments were initiated in 1993, and the fields have been fertilized regularly since. Biomass yields have been monitored accurately since 1997. The average yield in the Con treatment was 9.76 t DM ha^{-1}. The nutrients in the soil at this site could not be fully utilized due to a lack of precipitation. The efficacy of nitrogen fertilization (N120 and N240) was higher than the efficacy of sewage sludge fertilization. The key reason for this difference is that the sewage sludge was applied only once every 3 years, which reduced total nitrogen utilization from sludge.

The dry matter silage maize yields are presented in Fig. 2. Only the average values from 3-year intervals are presented to make orientation easier. The year-to-year variation in the yields at this site was consistently linked mainly to the lack of rainfall during the growing season. Nevertheless, there was a certain decreasing tendency in the yields, especially since 2015.

Approximate C balance

The nitrogen dose and organic matter content in sewage sludge were reflected in the soil organic carbon content (C_{SOM}) (Table 3). The C_{SOM} content was 1.88%

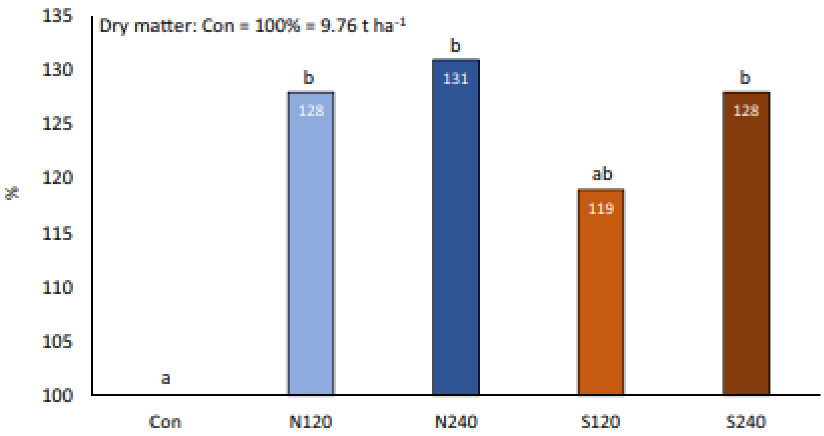


Fig. 1 Relative average silage DM yields (1997–2020). Different letters describe statistically significant differences between treatments. Tukey's HSD test ($p < 0.05$). *Con* Unfertilized control; *N120* Mineral fertilization (120 kg N ha^{-1}); *N240* Mineral fertilization (240 kg N ha^{-1}); *S120* Sewage sludge (120 kg N ha^{-1}); *S240* Sewage sludge (240 kg N $ha.^{-1}$)

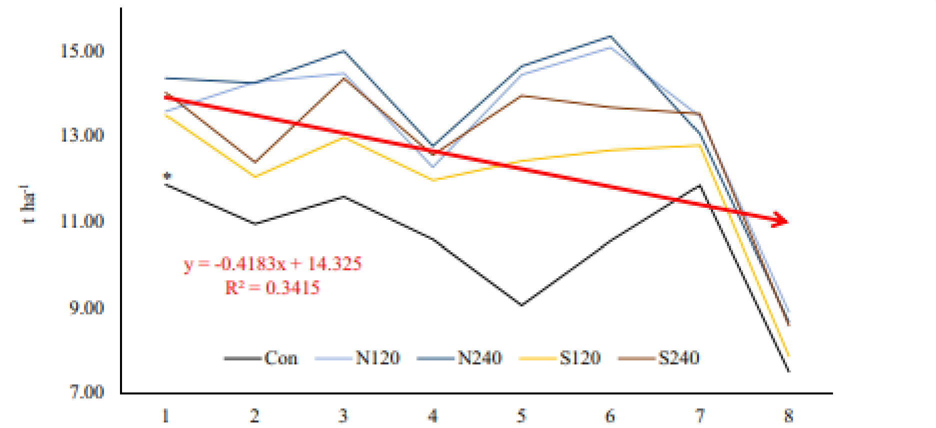


Fig. 2 Trends in maize monoculture silage dry mass yields during the experiment. The red trend line is an average of all treatments. The horizontal axis represents 3-year periods starting with 1997–1999 (1) and ending with 2018–2020 (8). *Con* Unfertilized control; *N120* Mineral fertilization (120 kg N ha^{-1}); *N240* Mineral fertilization (240 kg N ha^{-1}); *S120* Sewage sludge (120 kg N ha^{-1}); *S240* Sewage sludge (240 kg N ha^{-1}) *only the unfertilized control treatment showed significantly low yields (Tukey HSD test; p < 0.05) during all periods, and differences among the fertilization treatments were not significant

Table 3 Qualitative and quantitative soil organic matter (SOM) parameters

Parameter	Con	N120	N240	S120	S240
$C_{SOM (\%)}$	1.62^a	1.61^a	1.61^a	1.75^a	1.84^a
C_{HWE} (mg kg^{-1})	196^{cd}	165^{ab}	148^a	172^{bc}	189^c
C_{DOC} (mg kg^{-1})	26.4^c	21.1^b	15.4^a	22.4^b	29.7^d
C_{HA} (%)	0.244^c	0.162^a	0.185^{ab}	0.182^{ab}	0.222^{bc}
C_{FA} (%)	0.182^a	0.173^a	0.195^a	0.165^a	0.189^a
C_{HS} (%)	0.487^a	0.486^a	0.529^a	0.450^a	0.544^a
$C_{HA/FA}$	1.352^b	0.950^a	0.948^a	1.102^a	1.215^{ab}
N_t (%)	0.144^a	0.138^a	0.148^a	0.155^a	0.162^a
C_{SOM}/N_t	11.2^a	11.6^a	10.9^a	11.3^a	11.4^a
PWI	0.029^b	0.021^{ab}	0.020^a	0.021^{ab}	0.023^{ab}
WSA	0.509^b	0.397^a	0.387^a	0.426^{ab}	0.418^{ab}
EEG (mg kg^{-1})	983^{ab}	952^a	999^{ab}	971^{ab}	1052^b
TG (mg kg^{-1})	2091^a	2027^a	2013^a	2126^a	2145^a

Different superscript letters describe statistically significant differences between treatments. Tukey HSD test; $p < 0.05$

Con Unfertilized control; *N120* Mineral fertilization (120 kg N ha^{-1}); *N240* Mineral fertilization (240 kg N ha^{-1}); *S120* Sewage sludge (120 kg N ha^{-1}); *S240* Sewage sludge (240 kg N ha^{-1}). C_{SOM} Soil organic matter carbon; C_{HWE} Mineralizable soil organic carbon; C_{DOC} Dissolved organic carbon; C_{HA} Carbon of humic acids; C_{FA} Carbon of fulvic acids; C_{HS} Carbon of humic substances; $C_{HA/FA}$ Carbon in humic and fulvic acid ratio; N_t Total nitrogen content; C_{SOM}/N_t Soil organic matter carbon/total nitrogen ratio; *PWI* Potential wettability index; *WSA* Aggregate stability index; EEG Easily extractable glomalin; *TG* Total glomalin

at the beginning of the trial in 1993, and after 28 years, the content changed to 1.62% in the Con treatment—a reduction of 0.26%. Relatively speaking, the decrease was 13.8%. This means that there was a loss of carbon from the soil. The loss of carbon amounted to 11310 kg C ha^{-1} in the topsoil (0–30 cm). On average, the carbon loss in the Con treatment was 404 kg C ha^{-1} year^{-1}. A similar decrease was observed in the N120 and N240 treatments. The C_{SOM} content in the S120 treatment was 1.75% in 2020. This is a decrease of 0.13% in comparison with 1993. The difference between the organic carbon content (2020 minus 1993) for the S120 treatment represented losses of 5655 kg C ha^{-1}. To the total balance, we added the carbon applied with sewage sludge, i.e., 24612 kg C ha^{-1} 28 years^{-1}. The carbon losses from soil combined with sewage sludge were, therefore, 30267 kg C ha^{-1} 28 years^{-1}. Using the same calculation principle for the S240 treatment, the total carbon loss was 50964 kg C ha^{-1} 28 years^{-1}.

Soil organic matter quality parameters

Although there were no significant differences, the S120 and S240 treatments showed an increasing tendency in the C_{SOM} content related to Con. This trend was more evident in S240. The total nitrogen (N_t) content was not significantly influenced by fertilization, despite the increasing tendency for S240 (Table 3).

Intensive mineral nitrogen fertilization (N240) increased the mineralization rate of stable organic matter, which in turn caused a substantial decreasing tendency in the C_{SOM}/N_t ratio (Table 3). Soil organic matter quality was also monitored by hot water extraction and 0.01 mol L^{-1} $CaCl_2$ extraction (Table 3). The lowest values were consistently present in the N240 treatments. In the previous time period, easily degradable carbon fractions were mineralized in the N240 treatment. The lowest content of extractable carbon was observed during the most recent soil analysis (from 2020). Significant increases in the C_{HWE} and C_{DOC} contents were recorded in the S240 treatment, although the last sludge application occurred in the autumn of 2017, 3 years prior to this analysis. The C_{HWE} and C_{DOC} contents in the S240 treatment were comparable to those in the Con treatment, which demonstrated the relative stability of SOM in the Con treatment. The highest humic and fulvic acid ratios were found in the Con treatment, which was even higher than that in S240 (Table 3). None of the observed treatments produced significant differences in either humic substances content (C_{HS}) or fulvic acid content (C_{FA}).

The SOM quality was further monitored using the DRIFTS method. The potential wettability index (PWI) determines the hydrophobic and hydrophilic group ratio (Table 3). Significant differences were discovered between the Con (highest value) and N240 (lowest value) treatments. To express soil aggregate stability, the WSA index was used. The N120 and N240 treatments showed the lowest stability, with values significantly lower than those of the other treatments. This WSA method confirmed the results from the C_{HWE} and C_{DOC} extraction methods—intensive nitrogen fertilization degrades stable organic matter and, in turn, degrades soil structure. In contrast, the Con treatment had the highest soil aggregate stability, with values surpassing that of sludge application in the S240 treatment.

One of the goals of our experiment was to evaluate how all the changes influenced the glomalin (EEG and TG) content (Table 3). A significant increase in EEG content was observed only in the S240 treatment. The treatments produced no significant differences in terms of TG content. The relationships between glomalin content and other parameters related to SOM quality are presented in Table 4. Glomalin content was significantly related only to C_{SOM} and N_t content. The correlations between C_{SOM} and other parameters (C_{SOM}/N_t; PWI; C_{HWE}; C_{DOC}; C_{HA}; $C_{HA/FA}$; WSA) were not significant (Table 4).

Table 4 Relationship between glomalin and other soil parameters

	C_{SOM}	N_t	C_{FA}
EEG	0.791^b	0.773^b	0.458^a
TG	0.818^b	0.732^b	0.357
C_{SOM}	–	0.909^b	0.391

Pearson's correlation coefficient. Correlations with other parameters (C_{SOM}/N_t; PWI; C_{HWE}; C_{DOC}; C_{HA}; $C_{HA/FA}$; WSA) were not significant

C_{SOM} Soil organic matter carbon, N_t Total nitrogen content, C_{FA} Carbon of fulvic acids, *EEG* Easily extractable glomalin, *TG* Total glomalin

[a] $p < 0.05$;

[b] $p < 0.001$

Principal component analysis (PCA) was applied to the variables mentioned in Table 5. The variable associations and number of significant components were systematically selected based on three basic criteria, i.e., eigenvalues > 1.1, loading factors ≥ 0.59 and percentage of variability > 8%.

The biplot position of the components in Fig. 3 explained 78% of the cumulative variance, where the first factor described 52% and the second described 26%. PC1, which explained 52% of the total cumulative variance, and 6.7 of the eigenvalue were highly dominated by positively and strongly associated variables, such as TG, C_{SOM}, PWI, C_{HWE}, C_{DOC}, $C_{HA/FA}$, WSA and C_{HA}. Approximately 30–32% of the variable associations in PC1 was contributed by the S240 and N120 treatments. The second PC2 explained approximately 26% of the total cumulative variance and 3.4 of the eigenvalue and was dominated by the positively and strongly associated variables EE, C_{HS} and C_{FA}, and these variables were positively influenced by the S_{240} treatment, whereas the third PC accounted for 14% of the total cumulative variance and 1.8 of the eigenvalue and was dominated by the weakly associated variables C_{SOM}/N_t and N_t. PC4 also explained 8% of the total cumulative variance and 1.1 of the eigenvalue, and no variables dominated this component.

Table 5 Principal components (PCs) or factors and their loading factor values, eigenvalues, and variabilities (%)

Parameters	PC1	PC2	PC3	PC4
EE	0.55	0.82	0.17	0.00
TG	0.82	− 0.29	0.48	− 0.06
C_{SOM}	0.86	0.07	0.40	− 0.31
$C_{SOM/Nt}$	− 0.03	− 0.59	0.60	0.54
N_t	0.59	0.34	0.59	− 0.43
PWI	0.90	− 0.31	− 0.25	0.17
C_{HWE}	0.90	− 0.31	− 0.25	0.17
C_{DOC}	0.94	− 0.18	0.18	0.22
C_{HS}	0.36	0.83	0.14	0.41
C_{FA}	0.04	0.97	− 0.09	0.23
C_{HA}	0.78	0.42	− 0.45	0.08
$C_{HA/FA}$	0.90	− 0.31	− 0.25	0.17
WSA	0.78	− 0.19	− 0.45	− 0.40
Eigenvalue	6.7	3.4	1.8	1.1
Variability (%)	52	26	14	8

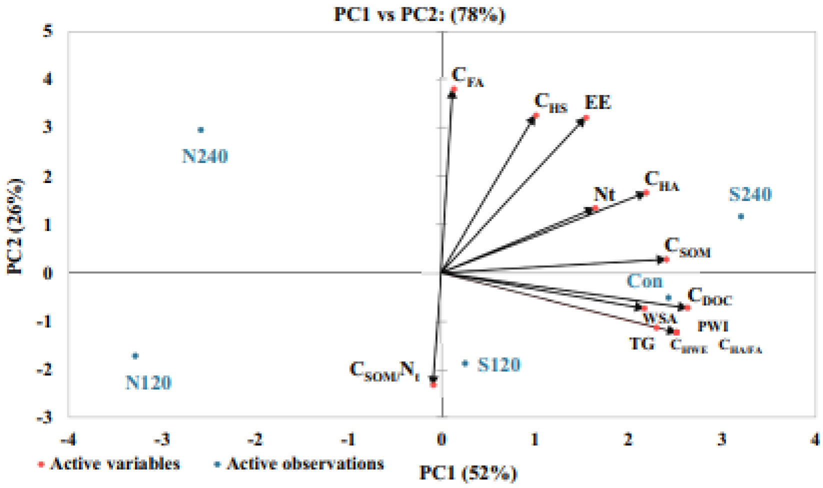


Fig. 3 Biplot position of variables determined by the first two principal components (PC1 vs. PC2). The red and blue dots represent active variables and observations, respectively

Discussion

The benefits of silage maize monoculture experiments belong to a small set of variable factors that could influence changes in the quality and quantity of soil organic matter. It is generally accepted that maize belongs to a group of crops that is substantially dependent on arbuscular mycorrhizae and, at the same time, promotes their development [35]. Another variable was the difference in the quality of the applied fertilizers (mineral N/organic). Sewage sludge was included as a treatment, because its production is consistently increasing with the increasing world population, and 37% is currently being utilized on farming lands in EU countries [36].

Aboveground biomass yields are closely correlated with the quantity of roots and stubble, which influences the soil organic matter content. The number of roots in the topsoil was not monitored in the current experiment. However, in our previous study on a luvisol [37], the yearly production of root biomass carbon was 394 kg ha^{-1}, while the fertilized treatments had 1.9 × more, approximately 749 kg ha^{-1}. The carbon content in stubble was 208 kg C ha^{-1} $year^{-1}$ in the control treatment and 1.5 × higher in the fertilized treatment (312 kg C ha^{-1} $year^{-1}$). The content in stubble + root biomass was 1061 kg C ha^{-1} (312 + 749). If we base the following calculation on results from this study, we can assume the chernozem reached approximately 10% higher values. In the fertilized treatments, stubble + root biomass (topsoil only) supplied 1100–1200 kg C ha^{-1} $year^{-1}$. The quantity of organic matter supplied to the soil by sewage sludge in the S120 and S240 treatments was 879 and 1758 kg C ha^{-1} $year^{-1}$, respectively (Table 2). The annual addition of C was lower than that with stubble + root biomass in the S120 treatment. This may be the reason for the lack of a significant increase in C_{SOM} content in the S treatments in comparison with the Con treatments. The other reason is the fact that organic matter quality in sewage sludge is not high in comparison with farmyard manure, and it does not contribute much to the SOM content. The relatively low C/N ratio (7.32:1) could also promote fast mineralization of the organic matter in sewage sludge. The carbon sequestration efficiency from both sewage sludge treatments was calculated to estimate their effect on carbon accumulation. The S240 treatment showed lower CSE (19.0%) than S120 (22.5%). The mentioned balances and data on CSE clearly show that the organic matter present in sewage sludge is not stable. Intensive nitrogen fertilization (N240) caused an increase in aboveground biomass, which caused an increase in root biomass. Root biomass has a lower C/N ratio than Con and is mineralized faster [37]. In addition, the mineralization rate of soil organic matter is greater due to the "priming effect".

The fulvic acid content was influenced by neither the intensity nor the dose of fertilizer. The humic acid content was highest in the Con treatment. The humic/fulvic acid ratio is an important parameter for the determination of soil organic matter quality [1]. The highest quality in terms of the $C_{HA/FA}$ ratio was present in the Con treatment. The changes in soil organic matter quality (in terms of C_{HA}, C_{FA}, C_{HS}, and $C_{HA/FA}$) were determined to be relatively small.

In our previous work, in which we used the same levels of fertilization on a luvisol, much larger differences in the C_{SOM} qualitative parameters were observed among treatments [27], which was caused by significantly lower organic matter content and quality (for example, in the control treatment: C_{SOM}—0.98%; C_{HA}—0.078%; $C_{HA/FA}$—0.565; C_{SOM}/N_t—10.2). For comparison, the following values were measured for the current work on the chernozem: C_{SOM}—1.62%; C_{HA}—0.244%; $C_{HA/FA}$—1.352; C_{SOM}/N_t—11.2. This is the most likely reason for their nonsignificant relationships with glomalin (both EEG and TG) content in this experiment. Another factor could be the stability of glomalin in soils; it can persist for up to several decades [9], and therefore, the influence of even long-term fertilization can appear very slowly.

A significant increase in EEG content was observed in the S240 treatment after the application of an extremely high dose of sewage sludge. Sandeep et al. [24] and Balík et al. [22] published similar results resulting from sewage sludge application.

The significant increase in EEG content in the S240 treatment was probably caused by increased root biomass and, therefore, increased glomalin production. Sewage sludge could not be confirmed to be a direct source of glomalin. The method used by Wright and Uppadhyaya [10] was designed to extract glomalin from soil and not from organic fertilizers. The presence of arbuscular mycorrhizae and glomalin was not expected in the sewage sludge, because it was obtained directly from the producer without intermediate storage.

Cross-reactivity of the Bradford assay with humic acids, polyphenolic compounds, sugars, and lipids can interfere with GRSP determination [39]. The aforementioned organic compounds can lead to misestimations of GRSP contents [21]. In particular, the treatments fertilized with sewage sludge probably increased the production of organic molecules in the soil (lipids, carbohydrates and others), which may have led to interferences during glomalin analysis.

In our experiments, mineral nitrogen fertilization did not influence glomalin content, contrary to the conclusions of other studies that reported a positive influence of N fertilization on glomalin content [22, 39]. A probable reason is that intensive mineral N fertilization leads to the degradation of stable organic matter and lowers the stability of soil aggregates. This results in soil structure deterioration, which could later decrease the abundance of arbuscular mycorrhizae and subsequently glomalin production.

Increased degradation of SOM was present during the experiment due to intensive mineral nitrogen fertilization (N120, S120). This is why the N240 treatments produced the lowest contents of C_{HWE} and C_{DOC}. This fact was not reflected in the EEG and TG contents and their correlation with C_{HWE} and C_{DOC}. These results indirectly confirmed that glomalin is relatively stable during mineralization. Glomalin persists for longer periods of time than the original organic matter present in bulk soil [40].

In this study, only a significantly positive correlation of glomalin with the C_{SOM} and N_t parameters was confirmed. Relationships between glomalin content and other soil organic matter quality parameters (C_{HA}, $C_{HA/FA}$, PWI) were not found, in contrast to our previous study with a luvisol [27]. A difference between the sensitivity of both the EEG and TG methods in relation to C_{SOM} content and other qualitative parameters of SOM was not found. Therefore, the higher usability of the EEG method was supported by its ease of implementation, as mentioned in the introduction section.

The presented results seem to suggest that soil glomalin content determination can be used to estimate the quality of soil organic matter, but with limited indicative value, especially for soils with naturally high fertility within a relatively short time horizon (for example, 10 years). This method is sensitive for estimating site characteristics. The influence of soil type can be demonstrated by comparing the glomalin content of the chernozem and luvisol: the EEG content in the Con treatment in this work (chernozem) was 983 mg kg^{-1}, while the content of EEG in the Con treatment of the luvisol was 578 mg kg^{-1}. Furthermore, the EEG/TG ratio was 0.47 in the chernozem, while in our previous study with a luvisol, it was only 0.29 [27].

The potential wettability index (PWI), which includes the ratio of aliphatic (C–H) and carboxyl (C–O) bonds, can be used to describe soil organic matter quality [29]. The Con treatment showed the highest PWI values; this was in accordance with the fact that this treatment also showed the highest $C_{HA/FA}$ ratio, which represented the highest SOM quality. The higher PWI values in the Con treatment also indicated higher stability of the soil aggregates. Statistically, the lowest values of PWI were present in N240, which also corresponded with the lowest soil aggregate stability (WSA). The S240 treatment showed a slight increase in the PWI value. Adani and Tambone [41] also reported a higher content of the aliphatic carbon fractions in humic acids, even with a lower sewage sludge dose of 1 t ha^{-1} $year^{-1}$ over a duration of 10 years. The correlations between EEG and PWI as well as the correlations between C_{SOM} and PWI presented in our earlier study [27] were not confirmed in this study.

The Con treatment showed the highest soil aggregate stability. The sewage sludge treatments led to an increasing trend in stability as well, which was also confirmed in several other studies [42]. The values of PWI and WSA in our experiment showed a nonsignificant correlation with the C_{SOM} content. This might be caused by the aforementioned fact that the current experiment was conducted on a fertile chernozem.

Conclusions

In general, the highest quality of SOM was found in the unfertilized control treatment. However, this treatment showed significantly lower yields. Because of this, other fertilizing strategies should be tested to obtain higher yields together with higher SOM sustainability.

The data on glomalin content can be used to study soil organic matter quality. EEG extraction is more advantageous than TG extraction, because the procedure and data evaluation are easier and it has lower costs, requires less time and consumes fewer chemicals relative to TG analysis.

The results suggest that with increasing SOM quantity, there was a decrease in sensitivity for all the methods used for SOM quality determination. Nonetheless, further studies focused on different crops, soil and climate conditions are necessary to prove this.

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Author contributions
JB and MK—conceptualization; JC and OS—formal analysis and methodology; JB—writing original draft; PS, SP and ADA—laboratory and statistical analysis. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated and presented in this study as well as source data are available at the corresponding author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

1. Kononova MM. soil organic matter: nature, properties and methods of study. Moscow: Academy of Sciences of Soviet Union; 1963.
2. Humus PA, Conservation S. In: Piccolo A, editor. Humic substances in terrestrial ecosystems. Amsterdam: Elsevier Science; 1996. p. 225–64.
3. Piccolo A. The nature of soil organic matter and innovative soil managements to fight global changes and maintain agricultural productivity. In: Piccolo A, editor. Carbon sequestration in agricultural Soils. Heidelberg: Springer; 2012. p. 1–19.
4. Olk DC, Bloom PR, Perdue EM, McKnight DM, Chen YA, Farenhorst N, Senesi YP, Chin P, Schmitt-Kopplin N, Hertkorn N, Harir M. Environmental and agricultural relevance of humic fractions extracted by alkali from soils and natural waters. J Environ Qual. 2019;48:217–32. https://doi.org/10.2134/jeq2019.02.0041.
5. Savarese C, Drosos M, Spaccini R, Cozzolino V, Piccolo A. Molecular characterization of soil organic matter and its extractable humic fraction from long-term field experiments under different cropping systems. Geoderma. 2021;383:114700. https://doi.org/10.1016/j.geoderma.2020.114700.
6. Bedini S, Avio S, Sbrana C, Rurnini A, Migliorini P, Vazzana C, Giovannetti M. Mycorrhizal activity and diversity in a long-term organic Mediterranean agroecosystem. Biol Fertil Soils. 2013;49:781–90. https://doi.org/10.1007/s00374-012-0770-6.
7. Schindler FV, Mercer EJ, Rice JA. Chemical characteristics of glomalin-related soil protein (GRSP) extracted from soils of varying organic matter content. Soil Biol Biochem. 2007;39:320–9. https://doi.org/10.1016/j.soilbio.2006.08.017.
8. Agnihotri R, Bharti A, Ramesh A, Prakash A, Sharma MP. Glomalin related protein and C16:1ω5 PLFA associated with AM fungi as potential signatures for assessing the soil C sequestration under contrasting soil management practices. Eur J Soil Biol. 2021;103: 103286. https://doi.org/10.1016/j.ejsobi.2021.103286.
9. Rillig MC. Arbuscular mycorrhizae, glomalin, and soil aggregation. Can J Soil Sci. 2004;84:355–63. https://doi.org/10.4141/S04-003.
10. Wright SF, Upadhyaya A. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant Soil. 1998;198:97–107. https://doi.org/10.1023/A:1004347701584.
11. Wright SF, Nichols KA, Schmidt SF. Comparison of efficacy of three extractants to solubilize glomalin on hyphae and in soil. Chemosphere. 2006;64:1219–24. https://doi.org/10.1016/j.chemosphere.2005.11.041.
12. Gadkar V, Rillig MC. The arbuscular mycorrhizal fungal protein glomalin is a putative homolog of heat shock protein 60. FEMS Microbiol Lett. 2006;263:93–101. https://doi.org/10.1111/j.1574-6968.2006.00412.x.
13. Harner MJ, Ramsey PW, Rillig MC. Protein accumulation and distribution in floodplain soils and river foam. Ecol lett. 2004;7:829–36. https://doi.org/10.1111/j.1461-0248.2004.00638.x.
14. Gunina A, Kuzyakov Y. Sugars in soil and sweets for microorganisms: review of origin, content, composition and fate. Soil Biol Biochem. 2015;90:87–100. https://doi.org/10.1016/j.soilbio.2015.07.021.
15. Singh AK, Zhu X, Che C, Wu J, Yang B, Zakari S, Jiang XJ, Singh N, Lie W. The role of glomalin in mitigation of multiple soil degradation problems. Crit Rev Environ Sci Technol. 2020;52:1604–38. https://doi.org/10.1080/10643389.2020.1862561.
16. Rillig MC, Wright SF, Eviner VT. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant Soil. 2002;238:325–33. https://doi.org/10.1023/A:1014483303813.
17. Irving TB, Alptekin B, Kleven B, Ané JM. A critical review of 25 years of glomalin research: a better mechanical understanding and robust quantification techniques required. New Phytol. 2021;232:1572–81. https://doi.org/10.1111/nph.17713.
18. Zhang X, Wu X, Zhang S, Xing Y, Wang R, Liang W. Organic amendment effects on aggregate-associated organic C, microbial biomass C and glomalin in agricultural soils. CATENA. 2014;123:188–94. https://doi.org/10.1016/j.catena.2014.08.011.

19. Řezáčová V, Czakó A, Stehlík M, Mayerová M, Šimon T, Smatanová M, Madaras M. Organic fertilization improves soil aggregation through increases in abundance of eubacteria and products of arbuscular mycorrhizal fungi. Sci Rep. 2021;11:12548. https://doi.org/10.1038/s41598-021-91653-x.
20. Dai J, Hu JL, Lin XG, Yang A, Wang R, Zhang JB, Wong MH. Arbuscular mycorrhizal fungal diversity, external mycelium length, and glomalin-related soil protein content in response to long-term fertilizer management. J Soils Sediments. 2013;13:1–11. https://doi.org/10.1007/s11368-012-0576-z.
21. Li X, Han S, Luo XS, Chen WL, Huang QY. Arbuscular mycorrhizal-like fungi and glomalin-related soil protein drive the distributions of carbon and nitrogen in a large scale. J Soil Sediments. 2020;20:963–72. https://doi.org/10.1007/s11368-019-02421-4.
22. Balík J, Sedlář O, Kulhánek M, Černý J, Smatanová M, Suran P. Effect of organic fertilisers on glomalin content and soil organic matter quality. Plant Soil Environ. 2020;66:590–7. https://doi.org/10.17221/385/2020-PSE.
23. Rillig MC, Wright SF, Nichols KA, Schmidt WF, Torn MS. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. Plant Soil. 2001;233:167–77. https://doi.org/10.1023/A:1010364221169.
24. Sandeep S, Manjaiah KM, Singh PS, Singh AK. Soil carbon fractions under maize-wheat system: effect of tillage and nutrient management. Environ Monit Assess. 2016;188:14. https://doi.org/10.1007/s10661-015-4995-3.
25. Galazka A, Gawryjolek K, Grzadziel J, Ksiezak J. Effect of different agricultural management practices on soil biological parameters including glomalin fraction. Plant Soil Environ. 2017;63:300–6. https://doi.org/10.17221/207/2017-PSE.
26. Sekgota WM. Arbuscular mycorrhizal fungi as a bio-indicator of soil health under agricultural management practices in South Africa. Master thesis, Faculty of Science, Department of Biochemistry and Microbiology, Rhodes University. 2018. http://vital.seals.ac.za:8080/vital/access/manager/PdfViewer/vital:30011/SOURCE1?viewPdf.
27. Balík J, Kulhánek M, Černý J, Sedlář O, Suran P, Alamnie AD. The influence of organic and mineral fertilizers on the quality of soil organic matter and glomalin content. Agronomy. 2022;13:1375. https://doi.org/10.3390/agronomy12061375.
28. Ellerbrock RH, Gerke H, Bachman J, Goebel M. Composition of organic matter fractions for explaining Wettability of three forest soils. Soil Sci Soc Am J. 2005;69:57–66. https://doi.org/10.2136/sssaj2005.0057.
29. Haas C, Gerker HH, Ellerbrock RH, Hallet PD, Horn R. Relating soil organic matter composition to soil water repellency for soil biopore surfaces different in history from two Bt horizons of a Haplic Luvisol. Ecohydrol. 2018;11:e1949. https://doi.org/10.1002/eco.1949.
30. Demyan MS, Rasche F, Schul E, Breulmann M, Muller T, Cadisch G. Use of specific peaks obtained by diffuse reflectance Fourier transform mid-infrared spectroscopy to study the composition of organic matter in a Haplic Chernozem. Eur J Soil Sci. 2012;63:189–99. https://doi.org/10.1111/j.1365-2389.2011.01420.x.
31. Soil taxonomy. United States Department of Agriculture – Natural Resources Conservation Service. https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_051232.pdf Accessed on 2 May 2022.
32. Houba VJG, Temminghoff EJM, Gaikhorst GA, van Vark W. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. Commun Soil Sci Plant Anal. 2008;31:1299–396. https://doi.org/10.1080/00103620009370514.
33. Körschens M, Albert E, Armbruster M, Barkusky D, Baumecker M, Behle-Schalk L, Bischoff R, Čergan Z, Ellmer F, Herbst F, Hoffmann S, Hofmann B, Kismanyoky T, Kubát J, Kunzová E, Lopez-Fando C, Merbach I, Merbach W, Pardor MT, Rühlmann RJ, Spiegel H, Schulz E, Tajnsek A, Toth Z, Wegener H, Zorn W. Effect of mineral and organic fertilization on crop yield, nitrogen uptake, carbon and nitrogen balances, as well as soil organic carbon content and dynamics: results from 20 European long-term field experiments of the twenty-first century. Arch Agron Soil Sci. 2013;59:1017–40. https://doi.org/10.1080/03650340.2012.704548.
34. Nimmo JR, Perkins KS. Aggregate stability and size distribution. In: Dane JH, Topp GC, editors. Methods of soil analysis: Part 4 Physical methods, 5.4. Madison: Soil Science Society of America; 2002. p. 317–27. https://doi.org/10.2136/sssabookser5.4.c14.
35. Šarapatka B, Alvarado-Solano DP, Čižmár D. Can glomalin content be used as an indicator for erosion damage to soil and related changes in organic matter characteristics and nutrients? CATENA. 2019;181:104078. https://doi.org/10.1016/j.catena.2019.104078.
36. Kayikcioglu HH, Yener H, Ongun AR, Okur B. Evaluation of soil and plant health associated with successive three-year sewage sludge field applications under semi-arid biodegradation condition. Arch Agron Soil Sci. 2019;65:1659–76. https://doi.org/10.1080/03650340.2019.1572882.
37. Balík J, Kulhánek M, Černý J, Sedlář O, Suran P. Soil organic matter degradation in long-term maize cultivation and insufficient organic fertilization. Plants. 2020;9:1217. https://doi.org/10.3390/plants9091217.
38. Zou YN, Srivastava AK, Wu QS, Huang YM. Glomalin-related soil protein and water relations in mycorrhizal citrus (*Citrus tangerina*) during soil water deficit. Arch Agron Soil Sci. 2013;60:1103–14. https://doi.org/10.1080/03650340.2013.867950.
39. Avio L, Castaldini M, Fabiani A, Bedini S, Sbrana C, Turrini A, Giovannetti M. Impact of nitrogen fertilization and soil tillage on arbuscular mycorrhizal fungal communities in a Mediterranean agroecosystem. Soil Biol Biochem. 2013;67:285–94. https://doi.org/10.1016/j.soilbio.2013.09.005.
40. Dorodnikov M, Kuzyakov Y, Fangmeier A, Wesenberg GBL. C and N in soil organic matter density fractions under elevated atmospheric CO2: turnover vs. stabilization. Soil Biol Biochem. 2011;43:579–89. https://doi.org/10.1016/j.soilbio.2010.11.026.
41. Adani F, Tambone F. Long-term effect of sewage sludge application on soil humic acids. Chemosphere. 2005;60:1214–21. https://doi.org/10.1016/j.chemosphere.2005.02.031.
42. Ojeda G, Alcañiz JM, Le Bissonnais Y. Differences in aggregate stability due to various sewage sludge treatments on a Mediterranean calcareous soil. Agric Ecosyyst Environ. 2008;125:48–56. https://doi.org/10.1016/j.agee.2007.11.005.

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4.5 Long-Term Application of Manure and Different Mineral Fertilization in Relation to the Soil Organic Matter Quality of Luvisols

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Long-Term Application of Manure and Different Mineral Fertilization in Relation to the Soil Organic Matter Quality of Luvisols

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Abstract: Long-term field experiments were conducted on luvisol at five sites in the Czech Republic (42–48-year duration). The average total organic carbon content in the soil varied between 9.0 and 14.0 g kg^{-1}. In these trials, seven crops were rotated in the following order: clover, winter wheat, early potato, winter wheat, spring barley, potato, and spring barley with interseeded clover. Five treatments were studied: unfertilized treatment (Con), farmyard manure (F), and combinations of farmyard manure with three mineral fertilization levels (F+M1, F+M2, F+M3). Plant residues were not incorporated into the soil. An amount of 40 t ha^{-1} of farmyard manure fresh matter was applied twice during crop rotation. Intensive mineral fertilizer (F+M3) increased the average value of the carbon sequestration efficiency (CSE) by 12.9% and up to 26.3%. Combining organic and mineral fertilizers at moderate and higher intensities increased the soil organic matter quantity and quality compared to the unfertilized or manure treatment. Data on the glomalin content can be used to study the organic matter quality. We determined a strong correlation between the total glomalin content and the soil organic matter carbon, fulvic acid content, humic acid content, extractable carbon content, and dissolved organic carbon content, as well as the potential wettability index and aromaticity index.

Keywords: humic substances fraction; glomalin; potential wettability index; aromaticity index; E4/E6 ratio

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1. Introduction

The quantity and quality of soil organic matter (SOM) are two of the basic pillars of soil fertility. Many factors influence the quality and quantity of SOM, such as: crop rotation, agrotechnical approach (till or no-till system), irrigation, etc. Other considerations include the quality and intensity of mineral and organic fertilizers. At present, there are several different methods for determining the SOM quality parameters. The fractionation of SOM into individual forms is important for the SOM quality degradability [1–3]. SOM can be divided into stable and labile fractions. The stable fraction is represented by humic acids (C_{HA}), fulvic acids (C_{FA}), and humins [4]. The labile forms are represented by potentially mineralizable carbon (C_{HWC}) [5] or dissolved organic carbon (C_{DOC}) [6,7].

Glomalin has been widely studied as an indicator of soil organic matter quality [8]. Glomalin is produced by arbuscular mycorrhiza and is one of the most important soil proteins. The accurate molecular composition of glomalin has not been defined because the glomalin fraction extracted from soil is often not sufficiently clean [9,10]. The glomalin content in soil increased with long-term compost [11,12] and manure [12,13] applications. An increase in the glomalin content was also determined after applying sewage sludge [14,15] and straw alongside mineral fertilizer [16]. Relatively significant correlations between the quality of the soil parameters and the soil organic matter, or some fraction of soil organic matter, have been found, particularly in the C_{SOM}, humic and fulvic acids, C/N ratio, and glomalin [17]. Owing to the positive correlation between the glomalin and C_{SOM} content [15,18], glomalin can be considered a good indicator of soil fertility [11,19]. In our previous study [6], we found a positive correlation between the glomalin content and humic acid content (C_{HA}), as well as the gloma lin content and humic acid and fulvic acid ratio (C_{HA}/C_{FA}), under long-term maize production on luvisol. We also found a positive correlation with the potential wettability index (PWI).

The humus quality absorbance ratio at a 400 and 600 nm (E4/E6) ratio has been used as a standard parameter to assess the characterization of humic substances [20]. The ratio is inversely related to the condensation degree of the aromatic network in humic substances. A low E4/E6 ratio indicates a relatively high degree of aromatic constituent condensation, whereas a high ratio reflects a low degree of aromatic condensation and the presence of relatively large proportions of aliphatic structures [21]. After farmyard manure application, an increased humic acid content with higher "aromaticity" was observed, indicating favorable conditions for humification [22]. On the other hand, an increase in fulvic acid production was observed after the application of farmyard manure, leading to an increased E4/E6 ratio over other treatments [23]. Similar results were presented by Song et al. [24] and Galantini and Rossel [25]. No significant correlation between the E4/E6 values and the fractions of soil organic carbon, humification index (HI), or humification rate (HR) was found in the field long-term crop rotation experiments with different organic fertilizers (manure, straw, scab) at 10 sites across the Czech Republic [26]. Furthermore, no significant relationship between the C_{SOM} and E4/E6 was observed in the long-term experiments with maize monoculture [6].

Another method for SOM quality assessment is diffuse reflectance infrared Fourier transform spectrometry (DRIFTS). SOM is also characterized by the ratio of aliphatic C–H and carboxylic (C–O) bonds, known as the potential wettability index (PWI). Demyan et al. [27] used the DRIFTS method to monitor SOM quality in long-term trials in Bad Lauchstadt. All of their observations obtained using DRIFTS showed the influence of fertilization. They also mentioned a correlation between the PWI and C_{SOM} content. A strong correlation between the PWI and total glomalin content (TG) was estimated for luvisol [6], but was not confirmed for chernozem [7].

We calculated the aromaticity index (iAR) according to the reflectance of aliphatic and aromatic bands [28]. Increased soil organic matter mineralization and the formation of aliphatic compounds in aggregates may cause increased iAR values [28].

Our aim for this work was to (i) evaluate the changes in the SOM quality and quantity in luvisol under the influence of long-term organic and mineral fertilizers; (ii) evaluate the suitability of data on the glomalin content as an indicator of the soil organic matter quality; (iii) establish whether the easily extractable or total glomalin content is more suitable for soil organic matter quality determination. We hypothesize that glomalin content determination will be a viable method for soil organic matter quality determination and one of the two glomalin fractions (EEG or TG) will be more suitable for this purpose than the other. For this study, we selected long-term field experiments on luvisol at five sites in the Czech Republic (42–48-year duration). Most luvisols are fertile soils and suitable for a wide range of agricultural uses. Luvisol occupies 500–600 million hectares worldwide [29].

2. Materials and Methods

The Central Institute for Supervising and Testing in Agriculture established long-term farm trials between 1975 and 1981 on luvisols at five experimental sites under various climatic conditions in the Czech Republic. Table 1 presents the characteristics of the experimental sites. The average total organic carbon content in the soil varies between 9.0 and 14.0 g kg^{-1}. Within these trials, seven crops were rotated in the following order: clover, winter wheat, early potato, winter wheat, spring barley, potato, and spring barley with interseeded clover. An identical tillage system and crop varieties were used on all sites. Except for the unfertilized control, liming was performed as needed based on the pH_{CaCl_2} of the fertilizer treatment and soil texture.

Table 1. Characteristics of the experimental sites and year of experiment establishment.

Site/Parameter	Staňkov	Chrastava	Jaroměřice	Hradec Nad Svitavou	Pusté Jakartice
GPS coordinates	N 49°19′59.2716″ E 13°20′10.4388″	N 50°29′30.7638″ E 14°33′56.6634″	N 49°33′21.8052″ E 15°31′22.875″	N 49°26′6.882″ E 16°18′7.2648″	N 49°34′57.201″ E 17°34′20.6178″
Established	1981	1977	1975	1981	1979
Altitude (m.a.s.l)	370	345	425	460	290
Precipitation (mm) [1]	549	738	488	616	584
Air temperature (°C) [1]	8.3	8.0	8.2	7.4	8.3
Soil group [2]	Haplic luvisol	Haplic luvisol	Haplic luvisol	Haplic luvisol	Albeluvisol
Soil texture [2]	Silt loam	Silt loam	Silt loam	Sandy loam	Silt loam
Clay (%) (<0.002 mm)	9.4	12.2	20.5	12.6	12.7
Silt (%) (0.002–0.05 mm)	57.2	73.9	56.3	32.1	65.0
Sand (%) (0.05–2 mm)	33.4	13.9	23.2	55.3	22.3
Bulk density (g cm^{-3})	1.39	1.34	1.41	1.35	1.38
C_{SOM} in 2022 (%)	1.37	1.23	1.41	0.93	1.15
pH_{CaCl_2} (Con, F, F+M1, F+M2, F+M3) *	5.7, 6.0, 5.9, 5.9, 5.8	5.3, 6.2, 6.2, 6.2, 6.3	5.6, 6.6, 6.5, 6.3, 6.1	5.9, 6.5, 6.3, 6.3, 6.2	5.9, 6.4, 6.3, 6.2, 5.9
P in 2022 (Con, F, F+M1, F+M2, F+M3) (mg kg^{-1}) [3,*]	61, 75, 93, 97, 118	56, 60, 77, 86, 102	59, 80, 109, 127, 164	70, 76, 92, 100, 124	91, 75, 193, 97, 118
K in 2022 (Con, F, F+M1, F+M2, F+M3) (mg kg^{-1}) [3,*]	94, 99, 121, 152, 195	127, 142, 174, 202, 221	155, 179, 196, 235, 301	122, 131, 147, 175, 223	94, 99, 121, 152, 195
C input in F since the establishment (t ha^{-1})	25.7	28.2	30.8	25.7	28.2
Plot size (m^2)	58	64	52	48	58

C_{SOM}—soil organic matter carbon content; [1] long-term (30 years) annual average; [2] according to NRCS USDA [30]; [3] Mehlich 3 extractable content; * values in these rows respect the order of presented treatments; Con—control, F—farmyard manure, F+M—farmyard manure with increasing levels of mineral fertilizer.

The experiment is set up in a randomized complete block design on all sites. Blocks are replicated three times. The plot sizes on the individual sites are described in Table 1. As shown in Table 2, five treatments were studied: unfertilized treatment (Con), farmyard manure (F), and combinations of farmyard manure with three levels of mineral fertilization (F+M1, F+M2, F+M3). Plant residues were not incorporated into the soil. The amount of 40 t ha^{-1} of farmyard manure fresh matter is applied to early potatoes and potatoes, i.e., twice during a crop rotation. We calculated the carbon input for the entire duration of the experiment using dry matter of 23.0% and a carbon content of 27.9% in dry matter (values based on results of long-term site monitoring).

Table 2. Experimental design and nutrient doses applied in mineral fertilizers.

Treatment	Average Annual Nutrient Dose in Mineral Fertilizers (kg ha^{-1})		
	N	P	K
1. Con	0	0	0
2. F	0	0	0
3. F+M1	57	13	33
4. F+M2	85	26	66
5. F+M3	114	53	133

The fertilizer application rates were selected to reflect low, medium, and high intensity (M1, M2, and M3 treatments, respectively) of mineral fertilizer inputs in the Czech Republic at the beginning of the experiment. The fertilizers were applied in the following forms:

N—calcium ammonium nitrate; P—triple superphosphate; K—60% potassium salt. A constant fertilizer dose was used during the entire experiment (Table 2).

2.1. Soil Analysis

For clarity and convenience, Appendix A, Table A1 provides a list of variable abbreviations that are commonly used in this paper, including variable descriptions and units or scales.

The experimental sites were established between 1975 and 1981 with a crop rotation of seven crops (rotation mentioned above). Soil sampling was performed after the barley harvest in 2022, when the crop rotation was finished using a soil probe (30 cm depth). Fifteen soil samples were collected from every experimental plot and pooled. These samples were air-dried at 25 °C, homogenized, and sieved through a 2 mm sieve. The soil samples were later used for chemical analysis. Furthermore, a 0.4 mm soil size fraction was also prepared for the C_{SOM} determination. The soil was analyzed as follows:

The soil organic carbon (C_{SOM}) content in the air-dried samples of soils was determined through oxidation using the CNS Analyzer Elementar Vario Macro (Elementar Analysensysteme, Hanau-Frankfurt am Main, Germany).

The fractionation of humic substances (C_{HS}) was performed according to Pospíšilová et al. [31], and Kononova [4] to obtain the pyrophosphate extractable fraction, which represents the sum of carbon in humic acids (C_{HA}) and fulvic acids (C_{FA}). The C_{HA} and C_{FA} were extracted from a 5 g soil sample with a mixed solution of 0.10 mol L^{-1} NaOH (Lach-ner, s.r.o., Neratovice, Czech Republic) and 0.10 mol L^{-1} $Na_4P_2O_7$ (1:20 w/v) (Penta Chemicals Unlimited, Prague, Czech Republic). The following fractions of carbon were isolated: C_{FA} was obtained from a solution that was acidified by dilute H_2SO_4 (Lach-ner, Neratovice, Czech Republic) to a pH of 1.0–1.5 and left undisturbed for 24 h, and C_{HA} was obtained through the dissolution of the previously formed precipitate in a hot 0.05 mol L^{-1} NaOH solution. Before iodometric titration, the dry matter formed by the vaporization of each sample was dissolved in a mixture of 0.067 mol L^{-1} $K_2Cr_2O_7$ (Lach-ner, s.r.o., Neratovice, Czech Republic) and concentrated H_2SO_4 at an elevated temperature.

The humus quality (E4/E6) was analyzed according to the spectrophotometric method. The soil samples were extracted using sodium pyrophosphate (0.05 M $Na_4P_2O_7$) and measured by determining the absorbance ratio at 400 and 600 nm [32] (Lambda 25 UV/Vis (Perkin Elmer, Waltham, MA, USA).

The extractable organic carbon was determined using $CaCl_2$ and hot water extraction.

For the 0.01 mol L^{-1} $CaCl_2$ (Lach-ner, s.r.o., Neratovice, Czech Republic) extraction (C_{DOC}), the extraction agent 0.01 mol L^{-1} $CaCl_2$ was used (1:10, w/v) [33]. The C_{DOC} content was determined in the soil samples through segmental flow analysis using infrared detection on a Skalarplus System (Skalar, Breda, The Netherlands).

Hot water extraction (C_{HWC}) was used to assess the extractable soil organic carbon. The soil samples were dried at 40 °C and extracted with water (1:5, w/v). The suspension was boiled for one hour [5]. The C_{HWC} was determined through segmental flow analysis using infrared detection on a Skalarplus System (Skalar, Breda, The Netherlands).

The potential wettability index (PWI) and index of aromaticity (iAR) were determined using DRIFTs (diffuse reflectance infrared Fourier transform spectroscopy) spectra. The DRIFT spectra were recorded using the infrared spectrometer (Nicolet IS10, Waltham, MA, USA). The spectra with a range of 2.50 to 25.0 µm (4000 to 400 cm^{-1}) were used. A gold mirror was used as a background reference. Sixty-four scans with a resolution of 4.00 cm^{-1} and Kubelka–Munk units were applied. The OMNIC 9.2.41 software (Thermo Fisher Scientific Inc., Waltham, MA, USA) was applied for spectra analysis. The bands of the alkyl C–H groups (A-2948–2920 cm^{-1} and 2864–2849 cm^{-1}) were assumed to indicate the hydrophobicity, and bands of the C=O groups (B-1710 and 1640–1600 cm^{-1}) indicated hydrophilicity. The ratio of hydrophobicity and hydrophilicity was used to determine the potential wettability index [34].

$$\mathrm{PWI} = \mathrm{A}/\mathrm{B}$$

The aromaticity index was calculated according to the reflectance of aliphatic bands ranging from 3000–2800 cm^{-1} (AL) and an aromatic band at 1520 cm^{-1} (AR) [35].

$$\mathrm{iAR} = \mathrm{AL}/(\mathrm{AL} + \mathrm{AR})$$

The easily extractable glomalin (EEG) and total glomalin (TG) were determined according to Wright and Upadhyaya [26]. Briefly, 8 mL of sodium citrate (Penta Chemicals Unlimited, Prague, Czech Republic) (20 mmol L^{-1} of pH 7.0—EEG, 50 mmol L^{-1} pH 8.0—TG) was added to the 1.00 g of air dried soil (<2 mm), followed by autoclaving at 121 °C (30 min—EEG, 60 min—TG), cooling, and centrifugation at 5000 rpm (10 min—EEG, 15 min—TG). For the TG, the centrifugation of the supernatant of the same sample was repeated 5 times until the supernatant no longer showed the red-brown color that is typical of glomalin. Both forms of glomalin were determined colorimetrically using bovine albumin (BSA) as a standard for quantification and the Bradford protein assay (both from Bio-Rad, Hercules, CA, USA) to achieve the color change.

Humification indices were calculated according to Iqbal et al. [35] and Raiesi [36]:

$$\text{degree of polymerization: } \mathrm{HA} = \mathrm{C_{HA}}/\mathrm{C_{FA}} \tag{1}$$

$$\text{humification rate: } \mathrm{HR} = (\mathrm{C_{FA}} + \mathrm{C_{HA}})/\mathrm{C_{SOM}} \tag{2}$$

$$\text{humification index: } \mathrm{HI} = \mathrm{C_{HA}}/\mathrm{C_{SOM}} \tag{3}$$

where $\mathrm{C_{FA}}$ is the fulvic acid carbon, $\mathrm{C_{HA}}$ is the humic acid carbon, and $\mathrm{C_{SOM}}$ is the total organic carbon in the soil.

The carbon sequestration efficiency (CSE) was calculated as follows:

$$\mathrm{CSE}\ (\%) = ((\mathrm{C_{SOMtreatment}} - \mathrm{C_{SOMunfert}})/\mathrm{TCI}) \times 100 \tag{4}$$

$\mathrm{C_{SOMtreatment}}$ is the amount of C in the soil of the fertilized treatment. $\mathrm{C_{SOMunfert}}$ is the amount of C in the soil of the unfertilized Con treatment. TCI is the total C input (t ha^{-1}) applied in the organic fertilizers during the duration of the individual experiments [37].

2.2. Statistical Analysis

The results were assessed through ANOVA analysis and Pearson's correlation coefficient using the Statistica program ver. 12 (TIBCO, Paolo Alto, CA, USA). One-way ANOVA statistical analysis was performed with Tukey's for treatment and site effects ($p < 0.05$). Pearson's correlation coefficients were used to analyze the relationships among the studied variables. The level of significance of $p < 0.05$ or less was considered statistically significant. Principal component analysis (PCA) was performed to evaluate the relationships between the content of glomalin (EEG, TG) and the qualitative parameters of SOM using XLSTAT ver. 2022.4.5 (Addinsoft, New York, NY, USA). The variables were submitted to PCA, and eigenvalues > 1, variance (%), and cumulative (%) criteria were used to define the association among the variables.

3. Results

For the statistical evaluation, all of the observed values of the soil organic matter quality indicators from all the treatments were used (Table 3). The significant influence of the sites is visible in the presented results. There were significant differences among the monitored soil organic matter indicators. Several sites may show agreement on individual indicators, but not in general. The smallest differences between sites were recorded for the following indicators: humic substance content ($\mathrm{C_{HS}}$), easily extractable carbon ($\mathrm{C_{DOC}}$), potential wettability index (PWI), and aromaticity index (iAR). Based on the results of the soil organic matter quality indicators, we found the highest quality luvisol at the Jaroměřice site, which reached the highest $\mathrm{C_{SOM}}$, $\mathrm{C_{HWC}}$, $\mathrm{C_{HS}}$, and $\mathrm{C_{HA}}/\mathrm{C_{FA}}$ ratios. The lowest E4/E6 ratio was determined on the Jaroměřice and Pusté Jakartice sites. On the other hand, the results of the soil organic matter quality indicators showed that the luvisol from the Hradec and Svitavou sites had the lowest quality (highest E4/E6, 8.05; lowest C_{HA}/C_{FA}, 0.392).

Table 3. Carbon content and qualitative parameters of soil organic matter content at each of the experimental sites.

Site/Indicator	C_{SOM}	C_{HS}	C_{FA}	C_{HA}	**E4/E6**	C_{HWC}	C_{DOC}	N_t
	(%)	**(%)**	**(%)**	**(%)**		**(mg kg^{-1})**	**(mg kg^{-1})**	**(%)**
Staňkov	1.37 ^{c}	0.410 ^{a}	0.236 ^{c}	0.138 ^{b}	6.36 ^{b}	477 ^{b}	72.6 ^{b}	0.120 ^{b}
Chrastava	1.23 ^{b}	0.508 ^{b}	0.271 ^{d}	0.124 ^{b}	7.22 ^{c}	425 ^{ab}	52.8 ^{a}	0.117 ^{b}
Jaroměřice	1.41 ^{c}	0.520 ^{b}	0.270 ^{d}	0.170 ^{c}	5.40 ^{a}	493 ^{b}	45.8 ^{a}	0.148 ^{c}
Hradec and Svitavou	0.927 ^{a}	0.372 ^{a}	0.180 ^{b}	0.071 ^{a}	8.05 ^{d}	384 ^{a}	45.4 ^{a}	0.102 ^{a}
Pusté Jakartice	1.15 ^{b}	0.414 ^{a}	0.151 ^{a}	0.130 ^{b}	5.41 ^{a}	437 ^{ab}	55.2 ^{a}	0.125 ^{b}
Site/indicator	EEG	TG	DG	PWI	iAR	HA	HR	HI
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})			(C_{HA}/C_{FA})		
Staňkov	894 ^{c}	3320 ^{c}	2427 ^{c}	0.019 ^{b}	0.04 ^{b}	0.587 ^{b}	0.273 ^{a}	0.101 ^{b}
Chrastava	634 ^{a}	2712 ^{bc}	2077 ^{bc}	0.019 ^{b}	0.04 ^{b}	0.462 ^{a}	0.324 ^{b}	0.101 ^{b}
Jaroměřice	809 ^{b}	3078 ^{bc}	2269 ^{bc}	0.016 ^{a}	0.03 ^{a}	0.629 ^{b}	0.312 ^{b}	0.120 ^{c}
Hradec nad Svitavou	702 ^{a}	2408 ^{ab}	1705 ^{ab}	0.015 ^{a}	0.03 ^{a}	0.392 ^{a}	0.272 ^{a}	0.076 ^{a}
Pusté Jakartice	706 ^{a}	1926 ^{a}	1220 ^{a}	0.015 ^{a}	0.03 ^{a}	0.860 ^{c}	0.246 ^{a}	0.114 ^{bc}

Different letters describe statistically significant differences between sites. Tukey's HSD test, $p < 0.05$.

Table 4 presents the results describing the influence of the treatments on the soil organic matter quality indicators. We evaluated the effect of the treatments by replacing the current variable values with relative ones. The relative values were calculated as $V_{treatment}/V_{site\text{-}average}$, where $V_{treatment}$ was the value of each treatment, and $V_{site\text{-}average}$ was the average value of a particular site among all treatments. Evaluating the results in this way helped eliminate individual site characteristics while maintaining the influence of the treatment. The average value of the indicators calculated for all five sites is presented along with the relative results.

The first indicator presented is the organic carbon content (C_{SOM}). The average C_{SOM} content was 1.22%. Farmyard manure (F) fertilization and farmyard manure with mineral fertilizers (F+M) significantly increased the C_{SOM} content compared to the Con treatment. The C_{SOM} content for the intensive mineral fertilization treatment (F+M3) was significantly higher than all of the other treatments. Compared with the average value (C_{SOM}, 1.22%), it increased by 4.7%, and compared with the Con treatment, it was 5.5%.

We performed the fractionation of humic substances and evaluated the C_{HS}, C_{FA}, and C_{HA} content. These indicators showed no significant difference among treatments. Estimating these indicators allowed us to calculate the humification degree (HA), humification rate (HR), and humification index (HI). There were no significant differences among the treatments for HA, HR, and HI. However, the treatments combining manure and mineral fertilizer (F+M3) showed an obvious trend of increasing values. The combined F+M3 treatment increased the C_{SOM} content and other soil organic matter quality indicators (e.g., C_{HA}/C_{FA}). The lowest C_{SOM} values were recorded for the unfertilized Con treatment, which goes hand in hand with lower soil organic matter quality.

We estimated the C_{SOM} quality using two extraction methods: hot-water extraction (C_{HWC}) and 0.01 M L^{-1} $CaCl_2$ extraction (C_{DOC}). The intensive mineral fertilizer treatment (F+M3) recorded higher values than the Con and other treatments. These differences were significant for the C_{DOC} indicator. The C_{DOC} content was 14% higher for this treatment compared to the average and was almost 19% higher than the Con treatment. The average C_{DOC} and C_{HWC} content was 54.4 and 443 mg kg^{-1}, respectively. We also calculated the C_{HWC}/C_{SOM} and C_{DOC}/C_{SOM} ratios to provide better insight into the results. The averages for the C_{HWC}/C_{SOM} and C_{DOC}/C_{SOM} ratios were 0.037 and 0.005, respectively. Therefore,

C_{HWC} and C_{DOC} can be described as potentially mineralizable carbon and easily extractable carbon, respectively. Our statistical evaluation showed no differences among the treatments for these indicators. On the other hand, there was a certain trend of increased values for the F+M3 treatment. Further investigation is necessary to confirm the increasing trend in the less stable fraction of soil organic matter for this treatment.

Table 4. The influence of fertilization on soil carbon content and qualitative parameters of soil organic matter.

	Con	F	F+M1	F+M2	F+M3	Average = 1
relative C_{SOM} (%)	0.920 ^{a}	0.988 ^{b}	1.025 ^{bc}	1.020 ^{bc}	1.047 ^{c}	1.22%
relative C_{HS} (%)	0.983 ^{a}	0.989 ^{a}	0.959 ^{a}	1.018 ^{a}	1.050 ^{a}	0.445%
relative C_{FA} (%)	0.979 ^{a}	0.940 ^{a}	1.036 ^{a}	1.015 ^{a}	1.019 ^{a}	0.222%
relative C_{HA} (%)	0.875 ^{a}	0.981 ^{a}	0.950 ^{a}	1.061 ^{a}	1.135 ^{a}	0.127%
relative HA (C_{HA}/C_{FA})	0.890 ^{a}	1.06 ^{a}	0.922 ^{a}	1.03 ^{a}	1.09 ^{a}	0.586
relative HR (C_{HS}/C_{SOM})	1.03 ^{a}	0.956 ^{a}	0.978 ^{a}	1.02 ^{a}	1.02 ^{a}	0.286
relative HI (C_{HA}/C_{SOM})	0.954 ^{a}	0.992 ^{a}	0.926 ^{a}	1.04 ^{a}	1.09 ^{a}	0.102
relative C_{HWC} (%)	0.926 ^{a}	0.938 ^{a}	1.03 ^{a}	1.03 ^{a}	1.08 ^{a}	443 mg kg^{-1}
relative C_{DOC} (%)	0.951 ^{a}	0.914 ^{a}	1.03 ^{ab}	0.969 ^{ab}	1.14 ^{b}	54.4 mg kg^{-1}
relative C_{HWC}/C_{SOM} (%)	1.00 ^{a}	0.949 ^{a}	1.00 ^{a}	1.01 ^{a}	1.04 ^{a}	0.037
relative C_{DOC}/C_{SOM} (%)	1.03 ^{a}	0.920 ^{a}	1.01 ^{a}	0.958 ^{a}	1.09 ^{a}	0.005
relative N_t (%)	0.921 ^{a}	0.979 ^{b}	1.02 ^{ab}	1.03 ^{ab}	1.05 ^{b}	0.123%
relative C_{SOM}/N_t	0.997 ^{a}	1.01 ^{a}	1.00 ^{a}	0.994 ^{a}	1.00 ^{a}	9.97
relative EEG (%)	0.971 ^{a}	0.984 ^{a}	0.982 ^{a}	1.02 ^{a}	1.04 ^{a}	749 mg kg^{-1}
relative TG (%)	0.805 ^{a}	0.969 ^{ab}	0.950 ^{ab}	1.05 ^{bc}	1.22 ^{c}	2689 mg kg^{-1}
relative DG (%)	0.746 ^{a}	0.964 ^{ab}	0.938 ^{a}	1.06 ^{ab}	1.29 ^{b}	1940 mg kg^{-1}
relative EEG/TG (%)	1.17 ^{b}	0.993 ^{ab}	0.998 ^{ab}	0.967 ^{ab}	0.870 ^{a}	0.279
relative EEG/C_{SOM} (%)	1.06 ^{a}	0.992 ^{a}	0.955 ^{a}	1.00 ^{a}	0.992 ^{a}	0.063
relative TG/C_{SOM} (%)	0.883 ^{a}	0.984 ^{ab}	0.930 ^{a}	1.038 ^{ab}	1.16 ^{b}	0.221
relative E4/E6 (%)	0.955 ^{a}	0.974 ^{ab}	1.021 ^{b}	1.021 ^{b}	1.029 ^{b}	6.49
relative PWI (%)	0.961 ^{a}	0.969 ^{a}	0.979 ^{a}	1.00 ^{a}	1.09 ^{a}	0.017
relative iAR (%)	0.955 ^{a}	0.977 ^{a}	0.977 ^{a}	1.01 ^{a}	1.08 ^{a}	0.033
relative CSE (%)	-	0.696 ^{a}	1.02 ^{ab}	1.00 ^{ab}	1.37 ^{b}	19.2%

Different letters describe statistically significant differences among treatments. Tukey's HSD test, $p < 0.05$.

We also used the easily extractable glomalin content (EEG) and total glomalin content (TG) as indicators for evaluating the organic matter quality. The difficult extractable glomalin content (DG) was calculated as the TG–EEG content. There were no significant differences in EEG among the fertilizer treatments. On the other hand, the F+M2 and F+M3 treatments were significantly different from Con in terms of the TG indicator. The F+M3 treatment was also different from the other treatments. This increase is significant and attributed to the increase in DG. The average EEG/TG ratio was 0.279. The F+M3 treatment had the lowest values for EEG/TG, whereas the Con treatment had the highest values. Both of these differences are significant. The average value of the EEG/C_{SOM} ratio was 6.26%. There were no significant differences among the treatments in this indicator. The average value for TG/C_{SOM} was 0.221. A significant increase was recorded for the F+M3 treatment (25.6%). A significant decrease in the ratio was recorded for the Con treatment (19.5%).

An additional qualitative indicator of humic substances that characterizes the process of soil humification is the optical density ratio (E4/E6). The high values of the E4/E6 ratio correspond to a low aromaticity of humic substances and the presence of large quantities of carbohydrates, amides, and several aliphatic structures in humic substances. The average E4/E6 value was 6.49. Applying mineral fertilizer (specifically, the F+M2 and F+M3 treatments) significantly increased the values. Increased values indicate higher aliphatic structure content and lower aromatic content.

We determined the potential wettability index (PWI) and index of aromaticity (iAR) using the DRIFT spectra. The bonds of the alkyl C–H groups (2948–2920 cm^{-1} and

2864–2849 cm^{-1}) were assumed to indicate hydrophobicity and the bands of the C=O groups (1710 and 1640–1660 cm^{-1}) indicate hydrophilicity. We determined the potential wettability index using the ratio of hydrophobicity to hydrophilicity. The aromaticity index was calculated according to the reflectance of the aliphatic bands ranging between 3000 and 2800 cm^{-1} (AL) and the aromatic band at 1520 cm^{-1} (AR). We found no significant differences among the treatments using the PWI and iAR parameters. However, the F+M3 treatment showed an increasing trend.

We calculated the carbon sequestration efficiency (CSE) from the difference in C_{SOM} for the fertilized and unfertilized treatments in relation to the total applied carbon in manure (for the entire experiment) (Table 4). Applying mineral fertilizer increased the organic matter content more than pure manure. This manifested after using the same calculation as an "increase" in CSE. The average CSE value was 13.4% for the F treatment. Intensive mineral fertilizer (F+M3) increased the average value by 12.9% and up to 26.3%. Combining balanced mineral fertilizer with farmyard manure increased the soil sequestration of carbon.

We used Pearson's correlation coefficients to analyze the relationships among the studied variables (Table 5). C_{SOM} was significantly correlated with all other soil organic matter quality indicators, except HR. A strong inverse correlation was revealed between C_{SOM} and E4/E6. The E4/E6 ratio is generally regarded as a soil organic matter quality indicator that describes the humification process in soil. A strong negative correlation was indicated between E4/E6 and the following soil organic matter quality indicators: C_{HA}, degree of polymerization (C_{HA}/C_{FA}), and humification index (C_{HA}/C_{SOM})—which aligns with the aforementioned supposition. As there is a strong correlation between C_{SOM} and N_t, there is also a strong negative correlation between E4/E6 and N_t. We observed a weaker relationship with extractable carbon (C_{HWC} and C_{DOC}).

An important indicator of soil organic matter quality is the glomalin content. EEG and TG were both strongly correlated with the C_{SOM} content, along with the C_{HA} content. The C_{HA} and glomalin content are both important contributors to stable soil organic matter. A stronger relationship was observed for the correlation with the total glomalin content. The repeated extraction of the total glomalin probably causes the release of more stable proteins, which likely contribute to a strong TG correlation with C_{SOM}. We estimated a correlation of moderate strength ($p < 0.01$) between the EEG and TG content.

The DRIFT analyses (potential wettability index (PWI) and aromaticity index (iAR)) indicated that both the PWI and iAR correlate strongly with C_{SOM}, C_{HS}, C_{FA}, and C_{HA}, and with C_{WHC} and C_{DOC}. A relationship between E4/E6 and the PWI and iAR was not established, although the PWI, iAR, and E4/E6 are all strongly correlated with C_{HA}. On the other hand, we demonstrated a strong, significant relationship between ($p < 0.001$) the TG and PWI, and iAR. This positive correlation was likely caused by repeated soil sample extraction during the TG estimation as the C–H groups (of hydrophobic character) are likely released in higher quantities.

Principal component analysis (PCA) can be used to comprehensively evaluate data. We selected the principal components (PCs) using a cross-validation method based on three criteria: eigenvalue > 1.3, loading factors > 0.67, and percentage of variability > 10%, as mentioned in Table 6. Based on the biplot position described in Figure 1, PC_1 has an eigenvalue of about 9.5, which explains 73% of the total cumulative variance, and PC_1 is highly dominated by positively associated variables such as C_{HS}, C_{FA}, C_{HA}, E4/E6, C_{SOM}, C_{HWC}, C_{DOC}, N_t, EEG, TG, PWI, and iAR. Based on the contribution of the variables, C_{HA}, C_{HWC}, iAR, EEG, and TG provide 9.2%, 9.6%, 9.1%, 9.5%, and 9.7% to PC_1, respectively. The variables in this component are all negatively correlated with the C_{SOM}/N_t ratio, but are positively influenced by the F+M_2 and F+M_3 treatments. The second PC has an eigenvalue of about 1.5, which is 85% of the total cumulative variance and primarily dominated by the C_{Som}/N_t variable. This variable contributes about 29%, and F treatment positively influences 49% of the variable. By contrast, the third PC has an eigenvalue of 1.3, 94% of the total cumulative variance, with no variables dominating this principal component.

Table 5. Pearson's correlation coefficients (r) among variables.

	C_{SOM}	C_{HS}	C_{FA}	C_{HA}	HA	HR	HI	E4/E6	C_{HWC}	C_{DOC}	N_t	C_{SOM}/N_t	EEG	TG	PWI
C_{HS}	0.505 ***														
C_{FA}	0.608 ***	0.643 ***													
C_{HA}	0.805 ***	0.717 ***	0.481 ***												
HA	0.323 **	0.197	−0.363 **	0.616 ***											
HR	0.068	0.652 ***	0.725 ***	0.332 **	−0.291 *										
HI	0.429 ***	0.668 ***	0.237 *	0.872 ***	0.708 ***	0.446 ***									
E4/E6	−0.503 ***	−0.271 *	−0.004	−0.671 ***	−0.744 ***	0.074	−0.646 ***								
C_{HWC}	0.622 ***	0.303 **	0.314 **	0.555 ***	0.283 *	0.010	0.323 **	−0.302 **							
C_{DOC}	0.357 **	−0.063	0.078	0.236 *	0.210	−0.124	0.119	−0.108	0.348 **						
N_t	0.822 ***	0.513 ***	0.381 ***	0.795 ***	0.490 ***	0.001	0.520 ***	−0.622 ***	0.590 ***	0.077					
C_{SOM}/N_t	0.511 ***	0.094	0.499 ***	0.216	−0.169	0.136	−0.019	0.041	0.196	0.522 ***	−0.058				
EEG	0.497 ***	−0.007	0.177	0.406 ***	0.182	−0.076	0.192	−0.285 *	0.409 ***	0.348 **	0.358 **	0.331 **			
TG	0.534 ***	0.293 *	0.497 ***	0.420 ***	−0.046	0.212	0.196	0.018	0.268 *	0.324 **	0.325 **	0.434 ***	0.364 **		
PWI	0.547 ***	0.328 **	0.399 ***	0.358 **	0.036	0.064	0.126	0.067	0.406 ***	0.457 ***	0.270 *	0.521 ***	0.203	0.383 ***	
iAR	0.651 ***	0.438 ***	0.530 ***	0.471 ***	0.030	0.163	0.203	−0.021	0.455 ***	0.400 ***	0.377 ***	0.543 ***	0.242 *	0.455 ***	0.967 ***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n = 75.

Table 6. Principal components (PCs) and their loading factor values, eigenvalues, variabilities (%), and cumulative variance (%).

Variables	PC_1	PC_2	PC_3
C_{HS}	0.75	0.43	−0.5
C_{FA}	0.70	−0.65	−0.19
C_{HA}	0.93	0.19	0.14
E4/E6	0.86	−0.35	0.37
C_{SOM}	0.89	0.02	0.46
C_{HWC}	0.96	−0.26	0.12
C_{DOC}	0.81	−0.11	−0.09
N_t	0.92	−0.05	0.38
C_{Som}/N_t	−0.34	0.67	0.54
EEG	0.95	0.1	−0.2
TG	0.96	0.26	0.05
PWI	0.91	0.26	−0.27
iAR	0.93	0.31	−0.19
Eigenvalue	9.5	1.5	1.3
Variability (%)	73	12	10
Cumulative variance (%)	73	85	94

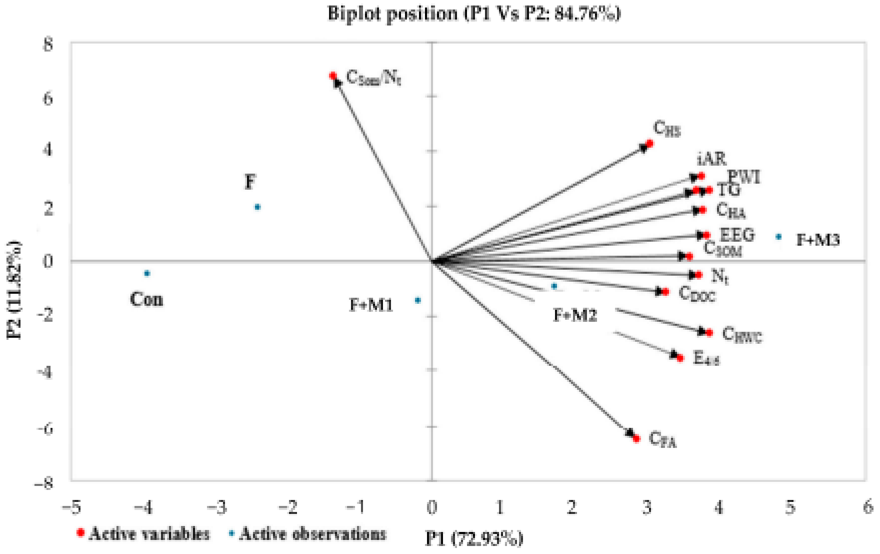

Figure 1. The biplot position of variables determined by the principal components analysis (PCA). The red and blue dots represent active observations (treatments) and variables, respectively.

4. Discussion

We calculated the carbon sequestration efficiency (CSE) from the difference in SOM for fertilized and unfertilized (Con) treatments in relation to the total applied carbon in manure. According to Wang et al. [37], carbon sequestration efficiency is primarily related to soil fertility. These values are below those reported by Sedlář et al. [26]. The aforementioned authors estimated 30.8 and 43.2% for the F and F+NPK treatments, respectively. Similar to our results, Sedlář et al. [26] described increased carbon sequestration in the soil after mineral fertilizer treatments. In our experiment, the highest C_{HA} content and C_{HA}/C_{FA} ratio were observed in the F+M3 treatment. This finding confirms the conclusions of Klik et al. [38], who stated that highly stable forms of carbon (e.g., C_{HA}) are beneficial for carbon sequestration in soil.

Based on the E4/E6 results, the Jaroměřice and Pusté Jakartice sites had the highest quality luvisol (Table 3). These sites also had the greatest degree of polymerization (C_{HA}/C_{FA}). In contrast, the highest E4/E6 values were recorded at the Hradec nad Svitavou site (8.05), which corresponds to the lowest value of C_{HA}/C_{FA} (0.392). The relationship between E4/E6 and C_{HA}/C_{FA} is site-specific [39,40]. No statistically significant differences in the C_{HA}/C_{FA} or E4/E6 ratios were found between the manure-only fertilized (F) and unfertilized (Con) treatments (Table 4). Menšík et al. [23] reported similar values, where E4/E6 produced no significant difference between fertilized and unfertilized treatments. Their experiment was also conducted in long-term luvisol field trials. Song et al. [24] and Galantitni and Rosell [25] reported a higher C_{HA}/C_{FA} ratio for the fertilized treatment than for the control group. They mentioned similar increases in E4/E6 in farmyard manure treatments while reporting on an increased fulvic acid content from farmyard manure treatment. Galantini and Rossel [25] found that higher aliphatic and phenolic -OH group contents usually developed after organic fertilizer application. Changes in the E4/E6 ratio caused by fertilization were more pronounced than changes in the carbon fractions (C_{FA}, C_{HA}, HI, HR, HA). Similarly, Gerzabek et al. [41] and Oktaba et al. [42] presented significant changes in the E4/E6 ratio caused by fertilization, despite it having no effect on the C_{HA}/C_{FA} ratio. Mineral fertilizer treatments showed a higher E4/E6 ratio than Con treatment. F+M3 treatment produced a significantly different E4/E6 ratio than Con (Table 4). A different study with similar results estimated a higher E4/E6 ratio for treatments with mineral + organic fertilizer over control or pure organic fertilizer [43]. A high E4/E6 ratio from F+M3 treatment likely corresponds to the low aromaticity of humic substances and the presence of large quantities of aliphatic structures. A strong inverse correlation appears between the E4/E6, C_{SOM}, and indicators of soil organic matter quality (C_{HA}, HA, HI, HR), which gives this method a certain perspective as a soil organic matter quality indicator (Table 5).

The quality of soil organic matter is determined by the potential wettability index (PWI), representing the ratio between aliphatic (C–H) and carboxyl (C=O) bonds. High PWI values indicate lower aggregate wettability [44]. We did not observe any significant differences in the PWI values among the treatments; however, the F+M3 treatment showed an increasing trend (Table 4). Unlike our previous results [6], farmyard manure did not significantly influence the PWI. Demyan et al. [27] observed an increase in the PWI after farmyard manure application. Organic fertilizer application increased the hydrophobic particle content and contributed to forming larger soil aggregates under these treatments. Secondary metabolites created during the decay of organic matter can be hydrophobic in character [42]. There may be several reasons for the increased PWI values under the F+M3 treatment. Mineral fertilization contributed to greater root biomass production, increased root exudate production, and an enhanced formation of stable aggregates [44]. A significant correlation between the PWI and C_{SOM}, C_{HS}, C_{FA}, C_{HA}, as well as C_{HWC} and C_{DOC}, is present (Table 5). A strong correlation between the PWI and C_{SOM} is also mentioned in other studies [6,45]. We also demonstrated a strong correlation between the TG and PWI in our experiment (Table 5). This relationship was already established in our previous study with maize monoculture on luvisol [6], but was not confirmed for chernozem [7]. Glomalin is a temperature-stable, sticky, hydrophobic glycoprotein [19]. Following simple through-chain analysis, we can conclude that if the glycoprotein content increases, hydrophobic particles and the PWI also increase. A significant correlation between the TG and PWI is in accord with this hypothesis (Table 5).

The aromaticity index (iAR) was calculated according to the reflectance of the aliphatic and aromatic bands [28]. There were no significant differences in the iAR among the treatments (Table 4). A strong correlation was established between the iAR and C_{SOM} and its indicators of quality (C_{HS}, C_{FA}, C_{HA}, C_{HWC}, and C_{DOC}). A significant relationship was also demonstrated regarding the glomalin content. These results conflict with the results of Balík et al. [7], where no significant effect of organic fertilizer application on the iAR was observed.

We established a significant correlation between the glomalin content (EEG and TG) and C_{SOM}, C_{HA}, C_{HWC}, and C_{DOC} (Table 5). Additionally, the TG was also strongly correlated with the C_{FA} content. Řezáčová et al.'s [46] conclusions were not confirmed, as they presented a tighter relationship between the EEG and C_{SOM} than the TG and C_{SOM}. Repeated extraction may cause higher correlations for the TG. With the exception of the glomalin glycoprotein, additional humic substances are released. A cross-reaction in the Bradford assay, which includes humic acids, polyphenolic compounds, sugars, and lipids, can interfere with glomalin determination [47]. The aforementioned organic compounds can also lead to misestimations of the glomalin content [18]. In our previous work, we observed a positive correlation between the glomalin content and the C_{SOM}, C_{HA}, and C_{HA}/C_{FA} ratio in long-term experiments with maize monoculture on luvisol [6]. Similarly, Vlček and Pohanka [17] established a correlation between the C_{ox}, humic and fulvic acids, C/N ratio, and glomalin content. The soil quality and type significantly influence the relationship between the glomalin content and C_{SOM} (including soil organic matter quality indicators such as C_{FA}, C_{HA}, C_{HA}/C_{FA}, C_{HWC}, and C_{DOC}). We found no significant correlations between the SOM quality and quantity indicators and the glomalin content on long-term maize monoculture with different fertilizer treatments on chernozem [7]. This result is caused by the high stability of the soil organic matter content in chernozem. The different fertilizer treatments caused no significant changes in the C_{SOM} content and quality. The glomalin content in soil has a tight relationship with the soil organic matter (SOM) content [15,18,48]. We observed no significant increase in the glomalin content after using farmyard manure in our study. The results from a long-term experiment on luvisol with crop rotation at the Červený Újezd site [22] could not be confirmed. Similarly, Bertagnoli et al. [49], Zhang et al. [13], Valarini et al. [50], Turgay et al. [12], and Dai et al. [11] observed an increase in TG after applying cattle or farmyard manure fertilizers. Combining organic and mineral fertilizers increased the TG content, especially in the F+M3 treatment. This treatment produced high biomass yield, which is connected to the highest post-harvest residues (stubble) and root biomass production, as well as a likely increase in exudate production. This was manifested as an increase in the C_{SOM} content.

The average proportion of EEG in the TG was almost 27.9%. Interestingly, the Červený Újezd experimental site estimated a similar proportion (28.8%) for luvisol [6]. Furthermore, the proportion of EEG in the TG was significantly higher in the control treatment (34.6%). The proportion of TG in the C_{SOM} is 22.1% on average; however, intense fertilizer application increases this value. For example, Comis [51] reported the proportion of TG in the C_{SOM} as 27%. Glomalin can add up to 25% of C_{SOM} [52], which is a significant proportion (22.1%). Therefore, glomalin plays an important role in soil carbon sequestration and promotes the stability of soil organic carbon through its slow degradation (and low soil turnover rate) [53].

5. Conclusions

Based on the results of long-term field experiments on luvisols at five sites in the Czech Republic, we concluded that:

(i) Combining organic and mineral fertilizers at moderate and higher intensity increases the soil organic matter quantity and quality in comparison with unfertilized or pure organic treatment. Intensive mineral fertilizer (F+M3) increased the average value of the carbon sequestration efficiency (CSE) by 12.9% and up to 26.3%.

(ii) Data on the glomalin content can be used to study the organic matter quality. We demonstrated a strong correlation between the total glomalin content (TG) and soil organic matter quality and quantity (C_{SOM}, C_{FA}, C_{HA}, C_{HWC}, C_{DOC}), as well as the potential wettability index (PWI) and aromaticity index (iAR). No significant relationship was established between the E4/E6 and glomalin content (both EEG and TG).

(iii) Neither EEG nor TG seem to be more suitable than the other for SOM quality determination.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. List of variables and their descriptions.

Abbreviation	Full Variable Description	Unit/Scale
C_{SOM}	Soil organic carbon	%
C_{HS}	Carbon humic substances	%
C_{FA}	Carbon in fulvic acid	%
C_{HA}	Carbon in humic acids	%
HA (C_{HA}/C_{FA})	Degree of polymerization	-
HR (C_{HS}/C_{SOM})	Humification rate	-
HI (C_{HA}/C_{SOM})	Humification index	-
E4/E6	Humus quality ratio (E4/E6)	-
C_{HWC}	Carbon—hot water extraction	mg kg^{-1}
C_{DOC}	Carbon—0.01 M L^{-1} $CaCl_2$	mg kg^{-1}
N_t	Total nitrogen	%
C_{SOM}/N_t	Ratio C_{SOM} and N_t	-
EEG	Easily extractable glomalin	mg kg^{-1}
TG	Total glomalin	mg kg^{-1}
DG	Difficult extractable glomalin	mg kg^{-1}
PWI	Potential wettability index	-
iAR	Aromaticity index	-
CSE	Carbon sequestration efficiency	%

References

1. Piccolo, A. The Nature of Soil Organic Matter and Innovative Soil Managements to Fight Global Changes and Maintain Agricultural Productivity. In *Carbon Sequestration in Agricultural Soils*; Piccolo, A., Ed.; Springer: Heidelberg, Germany, 2012; pp. 1–19.
2. Olk, D.C.; Bloom, P.R.; Perdue, E.M.; McKnight, D.M.; Chen, Y.A.; Farenhorst, N.; Senesi, Y.P.; Chin, P.; Schmitt-Kopplin, N.; Hertkorn, N.; et al. Environmental and Agricultural Relevance of Humic Fractions Extracted by Alkali from Soils and Natural Waters. *J. Environ. Qual.* **2019**, *48*, 217–232. [CrossRef]
3. Savarese, C.; Drosos, M.; Spaccini, R.; Cozzolino, V.; Piccolo, A. Molecular characterization of soil organic matter and its extractable humic fraction from long-term field experiments under different cropping systems. *Geoderma* **2021**, *383*, 114700. [CrossRef]
4. Kononova, M.M. *Soil Organic Matter: Nature, Properties and Methods of Study*; Academy of Sciences of Soviet Union: Moscow, Russia, 1963. (In Russian)
5. Körschens, M.; Albert, E.; Armbruster, M.; Barkusky, D.; Baumecker, M.; Behle-Schalk, L.; Bischoff, R.; Čergan, Z.; Ellmer, F.; Herbst, F. Effect of mineral and organic fertilization on crop yield, nitrogen uptake, carbon and nitrogen balances, as well as soil organic carbon content and dynamics: Results from 20 European long-term field experiments of the twenty-first century. *Arch. Agron. Soil Sci.* **2013**, *59*, 1017–1040. [CrossRef]
6. Balík, J.; Kulhánek, M.; Černý, J.; Sedlář, O.; Suran, P.; Asrade, D.A. The influence of organic and mineral fertilizers on the quality of soil organic matter and glomalin content. *Agronomy* **2022**, *12*, 1375. [CrossRef]
7. Balík, J.; Kulhánek, M.; Černý, J.; Sedlář, O.; Suran, P.; Procházková, S.; Asrade, D.A. The impact of the long-term application of mineral nitrogen and sewage sludge fertilizers on the quality of soil organic matter. *Chem. Biol. Technol. Agric.* **2022**, *9*, 86. [CrossRef]
8. Bedini, S.; Avio, L.; Sbrana, C.; Turrini, A.; Migliorini, P.; Vazzana, C.; Giovanneti, M. Mycorrhizal activity and diversity in a long-term organic Mediterranean agroecosystem. *Biol. Fertil. Soils* **2013**, *49*, 781–790. [CrossRef]
9. Schindler, F.V.; Mercer, E.J.; Ricc, A.J. Chamical characteristics of glomalin-related soil protein (GRSP) extracted from soil of varying organic matter. *Soil Biol. Biochem.* **2007**, *39*, 320–329. [CrossRef]

10. Agnihotri, R.; Bharti, A.; Ramesh, A.; Prakash, A.; Sharma, M.P. Glomalin related protein and C16:1ω5 PLFA associated with AM fungi as potential signatures for assessing the soil C sequestration under contrasting soil management practices. *Eur. J. Soil Biol.* **2021**, *103*, 103286. [CrossRef]
11. Dai, J.; Hu, J.L.; Lin, X.G.; Yang, A.; Wang, R.; Zhang, J.B.; Wong, M.H. Arbuscular mycorrhizal fungal diversity, external mycelium length, and glomalin-related soil protein content in response to long-term fertilizer management. *J. Soils Sediments* **2013**, *13*, 1–11. [CrossRef]
12. Turgay, O.C.; Buchan, D.; Moeskops, B.; De Gusseme, B.; Ortas, I.; De Neve, S. Changes in soil ergosterol content, glomalin-related soil protein, and phospholipid fatty acid profile as affected by long-term organic and chemical fertilization practices in Mediterranean Turkey. *Arid Land Res. Manag.* **2015**, *29*, 180–198. [CrossRef]
13. Zhang, X.; Wu, X.; Zhang, S.; Xing, Y.; Wang, R.; Liang, W. Organic amendment effects on aggregate-associated organic C, microbial biomass C and glomalin in agricultural soils. *Catena* **2014**, *123*, 188–194. [CrossRef]
14. Sandeep, S.; Manjaiah, K.M.; Pal, S.; Singh, A.K. Soil carbon fractions under maize-wheat system: Effect of tillage and nutrient management. *Environ. Monit. Assess.* **2016**, *188*, 14. [CrossRef] [PubMed]
15. Balík, J.; Sedlář, O.; Kulhánek, M.; Černý, J.; Smatanová, M.; Suran, P. Effect of organic fertilizers on glomalin content and soil organic matter quality. *Plant Soil Environ.* **2020**, *66*, 590–597. [CrossRef]
16. Nie, J.; Zhou, J.M.; Wang, H.Y.; Chen, X.Q.; Du, C.W. Effect of long-term rice straw return on soil glomalin, carbon and nitrogen. *Pedosphere* **2007**, *17*, 295–302. [CrossRef]
17. Vlček, V.; Pohanka, M. Glomalin-An interesting protein part of the soil organic matter. *Soil Water Res.* **2020**, *15*, 67–74. [CrossRef]
18. Li, X.; Han, S.; Luo, X.S.; Chen, W.L.; Huang, Q.Y. Arbuscular mycorrhizal-like fungi and glomalin-related soil protein drive the distributions of carbon and nitrogen in a large scale. *J. Soil Sediments* **2020**, *20*, 963–972. [CrossRef]
19. Wright, S.F.; Upadhyaya, A. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil.* **1998**, *198*, 97–107. [CrossRef]
20. Chen, Y.; Senesi, N.; Schnitzer, M. Information provided on humic substances by E4/E6 ratios. *Soil Biol. Biochem.* **1977**, *41*, 352–358. [CrossRef]
21. Quatmane, A.; Orazio, V.D.; Hafidi, H.; Senesi, N. Chemical and physico chemical characterization of humic acid like materials from compost. *Compost Sci. Util.* **2002**, *10*, 39–46. [CrossRef]
22. Mockeviciene, I.; Repsiene, R.; Amaleviciute-Volunge, K.; Karcauskiene, D.; Slepetiene, A.; Lepane, V. Effect of long-term application of organic fertilizers on improving organic matter quality in acid soil. *Arch. Agron. Soil Sci.* **2022**, *68*, 1192–1204. [CrossRef]
23. Menšík, L.; Hlisnikovský, L.; Pospíšilová, L.; Kunzová, E. The effect of application of organic manures and mineral fertilizers on the state of soil organic matter and nutrients in the long-term field experiment. *J. Soils Sediments* **2018**, *18*, 2813–2822. [CrossRef]
24. Song, X.Y.; Liu, S.T.; Liu, Q.H.; Zhang, W.J.; Hu, C.G. Carbon sequestration in soil humic substances under long-term fertilization in a wheat-maize system from North China. *J. Integr. Agric.* **2014**, *13*, 562–569. [CrossRef]
25. Galantini, J.; Rossel, R. Long-term fertilization effects on soil organic matter quality and dynamics under different production systems in semiarid Pampean soils. *Soil Tillage Res.* **2006**, *87*, 72–79. [CrossRef]
26. Sedlář, O.; Balík, J.; Černý, J.; Kulhánek, M.; Smatanová, M. Long-term application of organic fertilizers in relation to soil organic matter quality. *Agronomy* **2023**, *13*, 175. [CrossRef]
27. Demyan, M.S.; Rasche, F.; Schulz, E.; Breulmann, M.; Muller, T.; Cadisch, G. Use of specific peaks obtained by diffuse reflectance Fourier transform mid-infrared spectroscopy to study the composition of organic matter in a Haplic Chernozem. *Eur. J. Soil Sci.* **2012**, *63*, 189–199. [CrossRef]
28. Cunha, T.J.F.; Novotny, E.H.; Madari, B.E.; Martin-Neto, L.; Rezende, M.O.; Canelas, L.P.; Benitesm, V.M. Spectroscopy Characterization of Humic Acids Isolated from Amazonian Dark Earth Soils (Terra Preta De Índio). In *Amazonian Dark Earths: Wim Sombroek's Vision*, 1st ed.; Woods, W.I., Teixeira, W.G., Lehmann, J., Steiner, C., Eds.; Springer: Dordrecht, The Netherlands, 2009; pp. 363–372. ISBN 978-1-4020-9031-8.
29. Food and Agriculture Organization of the United Nations. World Reference Base for Soil Resources 2014. In *International Soil Classification System for Naming and Creating Legends for Soil Maps*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2015; Available online: http://www.fao.org/3/i3794en/I3794en.pdf (accessed on 1 September 2020).
30. United States Department of Agriculture—Natural Resources Conservation Service. Soil Taxonomy. Available online: https://www.nrcs.usda.gov/sites/default/files/2022-06/Soil%20Taxonomy.pdf (accessed on 4 October 2023).
31. Pospíšilová, L.; Vlček, V.; Hybler, V.; Hábová, M.; Jandák, J. *Standard Analytical Methods and Evaluation Criteria of Soil Physical, Agrochemical, Biological, and Hygienic Parameters*; Mendel University in Brno: Brno, Czech Republic, 2016; p. 122. (In Czech)
32. Sparks, D.L. *Methods of Soil Analysis. Part 3—Chemical Methods*; John Wiley & Sons: Hoboken, NJ, USA, 1996; p. 1390. ISBN 9780891188254.
33. Houba, V.J.G.; Temminghoff, E.J.M.; Gaikhorst, G.A.; van Vark, W. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. *Commun. Soil Sci. Plant Anal.* **2008**, *31*, 1299–1396. [CrossRef]
34. Ellerbrock, R.H.; Gerke, H.; Bachman, J.; Goebel, M. Composition of organic matter fractions for explaining Wettability of three forest soils. *Soil Sci. Soc. Am. J.* **2005**, *69*, 57–66. [CrossRef]
35. Iqbal, M.K.; Shafiq, T.; Hussain, A.; Ahmed, K. Effect of enrichment on chemical properties of MSW compost. *Bioresour. Technol.* **2010**, *101*, 5969–5977. [CrossRef]

36. Raiesi, F. The quantity and quality of soil organic matter and humic substances following dry-farming and subsequent restoration in an upland pasture. *Catena* **2021**, *202*, 105249:1–105249:11. [CrossRef]
37. Wang, S.C.; Zhao, Y.W.; Wang, J.Z.; Zhu, P.; Cui, X.; Han, X.Z.; Xu, M.G.; Lu, C.A. The efficiency of long-term straw return to sequester organic carbon in Northeast China's cropland. *J. Integr. Agric.* **2018**, *17*, 436–448. [CrossRef]
38. Klik, B.; Kulikowska, D.; Gusiatin, Z.M.; Pasieczna-Patkowska, S. Washing agents from sewage sludge: Efficiency of Cd removal from highly contaminated soils and effect on soil organic balance. *J. Soils Sediments* **2020**, *20*, 284–296. [CrossRef]
39. Maroušek, J.; Bartoš, P.; Filip, M.; Kolář, L.; Konvalina, P.; Maroušková, A.; Moudrý, J.; Peterka, J.; Šál, J.; Šoch, M.; et al. Advances in the agrochemical utilization of fermentation residues reduce the cost of purpose-grown phytomass for biogas production. *Energy Sources Part A Recovery Util. Environ. Eff.* **2020**. [CrossRef]
40. Kopecký, M.; Kolář, L.; Perná, K.; Vachalová, R.; Mráz, P.; Konvalina, P.-; Murindangabo, Y.T.; Ghorbani, M.; Menšík, L.; Dumbrovský, M. Fractionation of Soil Organic Matter into Labile and Stable Fractions. *Agronomy* **2022**, *12*, 73. [CrossRef]
41. Gerzabek, M.H.; Pichlmayer, F.; Kirchmann, H.; Haberhauer, G. The response of soil organic matter to manure amendments in a long-term experiment at Ultuna, Sweden. *Eur. J. Soil Sci.* **1997**, *48*, 273–282. [CrossRef]
42. Oktaba, L.; Odrobinska, D.; Uzarowicz, L. The impact of different land uses in urban area on humus quality. *J. Soils Sediments* **2018**, *18*, 2823–2832. [CrossRef]
43. Kutová, A.; Hetmanenko, V.; Skrylnik, I.; Paramonova, T.; Kuts, A. Effect of irrigation and fertilization on the content and composition of humus of chernozem in the vegetable-fodder crop rotation. *AgroLife Sci. J.* **2020**, *9*, 192–197.
44. Haas, C.; Gerker, H.H.; Ellerbrock, R.H.; Hallet, P.D.; Horn, R. Relating soil organic matter composition to soil water repellency for soil biopore surfaces different in history from two Bt horizons of a Haplic Luvisol. *Ecohydrology* **2018**, *11*, e1949. [CrossRef]
45. Leue, M.; Hoffman, C.; Hierold, W.; Sommer, M. In-situ multi-sensor characterization of soil cores along an erosion-deposition gradient. *Catena* **2019**, *182*, 104140. [CrossRef]
46. Řezáčová, V.; Czakó, A.; Stehlík, M.; Mayerová, M.; Šimon, T.; Smatanová, M.; Madaras, M. Organic fertilization improves soil aggregation through increases in abundance of eubacteria and products of arbuscular mycorrhizal fungi. *Sci. Rep.* **2021**, *11*, 12548. [CrossRef]
47. Avio, L.; Castaldini, M.; Fabiani, A.; Bedini, S.; Sbrana, C.; Turrini, A.; Giovannetti, M. Impact of nitrogen fertilization and soil tillage on arbuscular mycorrhizal fungal communities in a Mediterranean agroecosystem. *Soil Biol. Biochem.* **2013**, *67*, 285–294. [CrossRef]
48. Zbíral, J.; Čižmar, D.; Malý, S.; Obdržálková, E. Determination of glomalin in agriculture and forest soils by near-infrared spectroscopy. *Plant Soil Environ.* **2017**, *63*, 226–230. [CrossRef]
49. Bertagnoli, B.G.P.; Oliveira, J.F.; Barbosa, G.M.C.; Colozzi Filho, A. Poultry Litter and Liquid Swine Slurry Applications Stimulate Glomalin, Extraradicular Mycelium Production, and Aggregation in Soils. *Soil Tillage Res.* **2020**, *202*, 8. [CrossRef]
50. Valarini, P.J.; Curaqueo, G.; Seguel, A.; Manzano, K.; Rubio, R.; Cornejo, P.; Borie, F. Effect of compost application on some properties of a volcanic soil from central south Chile. *Chil. J. Agric. Res.* **2009**, *69*, 416–425. [CrossRef]
51. Comis, D. Glomalin: Hiding place for a third of the world's stored soil carbon. *Agric. Res.* **2002**, *50*, 4–7.
52. Singh, A.K.; Rai, A.; Singh, N. Effect of long-term land use systems on fractions of glomalin and soil organic carbon in the Indo-Gangetic plain. *Geoderma* **2016**, *277*, 41–50. [CrossRef]
53. Rillig, M.C.; Caldwell, B.A.; Wösten, H.A.B.; Sollins, P. Role of proteins in soil carbon and nitrogen storage: Controls on persistence. *Biogeochemistry* **2007**, *85*, 25–44. [CrossRef]

4.6 The Influence of Organic and Mineral Fertilizers on the Quality of Soil Organic Matter and Glomalin Content

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The Influence of Organic and Mineral Fertilizers on the Quality of Soil Organic Matter and Glomalin Content

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Abstract: The influence of different fertilizers (mineral/organic) on the quantity and quality of soil organic matter was monitored in long-term stationary experiments (27 years) with silage maize monoculture production on Luvisol. The main aim of this study was to investigate the relationship between easily extractable glomalin (EEG), total glomalin (TG), and parameters commonly used for the determination of soil organic matter quality, i.e., the content of humic acids (C_{HA}), fulvic acids (C_{FA}), and potential wettability index (PWI). A significant correlation was found between EEG content and C_{SOM} content, humic acid content (C_{HA}), humic acid/fulvic acid ratio (C_{HA}/C_{FA}), PWI, and index of aromaticity (IAR). Furthermore, the contents of EEG and TG correlated with soil organic carbon (C_{SOM}). Periodical application of sewage sludge and cattle slurry increased the content of glomalin in soils. From the results, it is obvious that data about glomalin content can be used to study soil organic matter quality. A more sensitive method (a method that reacts more to changes in components of soil fertility) seems to be the determination of EEG rather than TG. The factors supporting use of EEG extraction in agronomic practice are mainly the substantially shorter time of analysis than TG, C_{HA}, and C_{FA} determination and lower chemical consumption. Furthermore, the PWI method is even suitable for studying soil organic matter quality. On the other hand, the humus quality ratio (E4/E6) does not provide relevant information about soil organic matter quality.

Keywords: long-term experiments; Luvisol; cattle slurry; sewage sludge; humic substances fractionation; potential wettability index; glomalin

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1. Introduction

Glomalin-related soil protein (GRSP) is produced on arbuscular mycorrhizal fungi (AMF) hyphal walls and it is a heat-stable glycoprotein. It can remain in the soil for years and is resistant to microbial attacks. Wright and Upadhyaya [1] used the terms easily extractable glomalin (EEG) and total glomalin (TG). However, it has been demonstrated that the fraction of soil organic matter (SOM) yielded under repeated autoclaving of soil in citrate buffer contains a mixture of various proteins as well as other compounds, such as humic acids [2,3]. The procedure used for TG extraction also probably does not extract all glomalin present in a soil [4]. Unlike TG, one extraction cycle is sufficient to obtain the EEG pool. The content of both the EEG and TG is proportional to increasing AMF colonization [5–7].

GRSP binds mineral and organic particles to soil aggregates for long periods, as it contains up to 85% of polysaccharides that are resistant to microbial decomposition [8] and acts as a sticky and insoluble biofilm that glues together minerals, clays, organic matter, and microorganisms [4,9,10].

Total glomalin contents in soil have tight linkages to total soil organic matter (SOM) contents [11–13]. On the other hand, there is also evidence of a negative correlation between GRSP and SOM content [14]. A strong positive correlation between glomalin and soil fertility indicators (i.e., soil C, N, and P) has also been found [15–17]. High soil P content decreases AMF colonization [18] and the EEG content [19]. In contrast, the GRSP increases with the indicators of lower soil fertility, such as a high C:N ratio [19]. However, increasing GRSP content with increasing SOC (soil organic carbon), available P, total N, and K have also been observed [20].

Long-term application of poultry, pig, or cattle manure [21] and the application of compost [22–25] or farmyard manure [22,24–26] also increases GRSP content. Higher GRSP content was also reported after the application of sewage sludge [13,27] and straw [28,29]. A study by Nie et al. [28] further mentions that increased GRSP content in soil was found after the application of a mixture of straw and mineral fertilizer. Enhanced amounts of GRSP have also been observed after the application of different organic fertilizers, such as litter [22,24].

Many studies have demonstrated that humic acids, polyphenolic compounds, sugars, and lipids interfere with the Bradford assay [30]. The aforementioned organic compounds cross-react with the Bradford reagent, leading to the misinterpretation of the accurate GRSP content [31].

Generally, in the upper 0–10 cm soil depth, N and C contained in GRSP represent 5% of total soil N and 3.20% of total soil C_{SOM} [19], where whole GRSP compounds are representing up to 25% of total C_{SOM} in soil [15]. A positive correlation between GRSP and microbial biomass C and microbial respiration has been found in multiple soil environments [32,33]. A significant part of GRSP has long-term persistence (up to 42 years or more) in the soil medium, improving soil C sequestration [34].

GRSP production is seasonally dependent; AMF biomass peaks in the spring and summer, and consequently, GRSP content strongly increases in the summer [35]. Higher content of TG was also reported in the soil during the maize growth compared to the soil glomalin content before sowing [14].

Several recent publications have reviewed available information about the GRSP and its extraction, quantification, molecular characterization, and nomenclature in more detail [5,7,17,36,37]. Due to its positive correlation with soil organic matter carbon (C_{SOM}) content, the GRSP content is considered an indicator of changes in the quality of soil fertility [1,13,26,38,39].

This work aimed to monitor the possibility of using information about the GRSP content relative to soil organic matter quantity and even quality. Long-term experiments on Luvisol with silage maize and various mineral and organic fertilizers were used to study this issue. Maize was used in our experiment because it is one of the most produced crops across the world. The worldwide area used for maize production is about 16.8 million ha [40]. The Luvisols comprise over 500–600 million ha worldwide. Luvisols occupy mostly temperate regions, such as parts of the West Siberian Plain, East European Plains, Central Europe, the Mediterranean region, southern Australia, and the northern United States of America [41]. Luvisol represents 4.29% of the area of agriculturally used soils in the Czech Republic [42]. Most Luvisols are fertile soils and are suitable for a wide range of agricultural uses [41].

2. Materials and Methods

The study was carried out based on the long-term fertilization experiments since 1993 at the experimental stations located in Červený Újezd in the Czech Republic. Basic soil–climatic characteristics are given in Table 1.

Table 1. Basic description of the Červený Újezd experimental site at the start of the experiment.

Location	Červený Újezd	References
GPS coordinates	50°4′22″ N, 14°10′19″ E	
Altitude (m above sea level)	410	
Mean annual temperature (°C)	7.70	
Mean annual precipitation (mm)	493	
Soil type	Haplic Luvisol	NRCS USDA [43] [1]
Soil texture	Loam	NRCS USDA [43] [1]
Clay (%) (<0.002 mm)	5.40	
Silt (%) (0.002–0.05 mm)	68.1	
Sand (%) (0.05–2 mm)	26.5	
Bulk density (g cm^{-3})	1.50	
C_{SOM} (%)	1.26	CNS [2]
pH (0.01 mol/L $CaCl_2$)	6.50	ISO 10390 [44]
CEC ($mmol_{(+)}$ kg^{-1})	118	

[1] Natural Resource Conservation Service–United States Department of Agriculture. [2] CNS analyzer (Elementar Vario Macro, Elementar Analysensysteme, Hanau-Frankfurt am Main, Germany).

2.1. Experimental Design

The field experiment was conducted in a randomized complete block design with a plot area of 170 m^2. The experiments comprised four treatments: no fertilization control (Con), urea ammonium nitrate (N), cattle slurry (CS), and sewage sludge (SS). All treatments, including the control, were replicated four times. The maize hybrids used were "Malta" (1993–1996), "Torena" (1997 and 1998), "DK 254" (1999), "Compact" (2000), "Etendard" (2001–2003), "Rivaldo" (2004–2011), "RGT Indexx" (2012–2014), and "RGT Sixxtus" (2015–2019), and they were planted on each plot at a density of 80 thousand plants ha^{-1}. The maize was sown at the end of April/start of May, with 70 cm between plant rows. Since 1993, the mineral N fertilizers were applied before sowing every year in spring. Organic fertilizers sewage sludge (SS), precipitated with $FeSO_4$ and $Al_2(SO_4)_3$, and cattle slurry (CS), were applied at a different rate every year in autumn (October) and immediately incorporated into the soil with ploughing (25 cm depth). The complete fertilizing design is shown in Table 2. For the mineral nitrogen treatments, no other nutrients and liming were used from the beginning of the experiment. For the organic nitrogen treatments, the dosage of other nutrients depended on the content of nutrients in sewage sludge or cattle slurry. The dose of organic fertilizers was calculated according to the total nitrogen content determined with the Kjeldahl analysis (Table 3).

Table 2. Experimental design, applied organic and nitrogen fertilizers.

Treatment	Avg. Fresh Weight (t ha^{-1} $Year^{-1}$)	Avg. Dry Weight (t ha^{-1} $Year^{-1}$)	N (kg N ha^{-1} $Year^{-1}$)
Con	-	-	0
N	-	-	120
SS	11.2	3.39	120
CS	49.3	2.14	120

Con: control; N: urea ammonium nitrate; SS: sewage sludge; CS: cattle slurry.

Table 3. The content of C, N, P, and K in organic fertilizers.

Fertilizer	C	N	P	K	C/N
	(% Dry Weight)				
SS	25.8	3.52	2.42	0.46	7.32:1
CS	28.5	5.60	1.09	4.87	5.08:1

SS: 879 kg C ha^{-1} $year^{-1}$; CS: 610 kg C ha^{-1} $year^{-1}$; SS supplied 44% more carbon than CS.

Land leveling was not performed, to avoid overlapping treatments. The chemical protection during vegetation was performed only against weeds according to the actual situation. Pests and diseases were not eliminated due to their low appearance in the harvest plots.

Two rows of maize aboveground biomass (20 m^2 per plot) were harvested at silage maturity (roughly 65% biomass moisture content, BBCH 75 vegetation stage) and weight to obtain the aboveground biomass yield (BY). Dry BY was calculated based on the dry mass ratio in the subsamples.

2.2. Soil Analysis

Topsoil (depth of 30 cm) analyses were performed with air-dried soil samples ($\leq$2 mm) collected in September 2019, after the maize harvest.

Soil organic carbon (C_{SOM}) content in air-dried samples of soils, sewage sludge, and cattle slurry was determined using oxidation on the CNS Analyzer Elementar Vario Macro (Elementar Analysensysteme, Hanau-Frankfurt am Main, Germany).

Fractionation of humic substances (C_{HS}) was performed according to Kononova [45] to obtain the pyrophosphate extractable fraction, which represents the sum of the carbon in humic acids (C_{HA}) and fulvic acids (C_{FA}). In brief, C_{HA} and C_{FA} were extracted from a 5 g soil sample with a mixture of 0.10 mol L^{-1} NaOH and 0.10 mol L^{-1} $Na_4P_2O_7$ (1:20, v/v) solution. The carbon of humic substances C_{HS} and C_{HA} was determined using the oxidimetric titration method. The content of C_{FA} was calculated as the difference between C_{HS} and C_{HA}.

The humus quality (E4/E6) was analyzed according to the spectrophotometric method. The soil samples were extracted using sodium pyrophosphate (0.05 M $Na_4P_2O_7$) and measured by the absorbance ratio at 400 and 600 nm [46] (Lambda 25 UV/Vis (Perkin Elmer, Waltham, MA, USA).

Extractable organic carbon was determined using $CaCl_2$ and hot water extraction.

For the 0.01 mol/L $CaCl_2$ extraction (C_{DOC}), the extraction agent 0.01 mol L^{-1} $CaCl_2$ was used (1:10, w/v) [47]. The C_{DOC} content was determined in fresh soil samples by segmental flow-analysis using the infrared detection on a Skalarplus System (Skalar, Breda, The Netherlands).

Hot water extraction (C_{HWE}) was used to assess extractable soil organic carbon. Soil samples were dried at 40 °C and extracted with water (1:5, w/v). The suspension was boiled for one hour [48]. The C_{HWE} was determined by a segmental flow analysis using the infrared detection on a Skalarplus System (Skalar, Breda, The Netherlands).

The potential wettability index (PWI) and **index of aromaticity (IAR)** were determined using DRIFT (diffuse reflectance infrared Fourier transform spectroscopy) spectra. DRIFT spectra were recorded by the infrared spectrometer (Nicolet IS10, Waltham, MA, USA). The spectra with a range of 2.50 to 25.0 μm (4000 to 400 cm^{-1}) were used. The gold mirror was used as a background reference. The 64 scans with a resolution of 4.00 cm^{-1} and Kubelka–Munk units were applied. OMNIC 9.2.41 software (Thermo Fisher Scientific Inc., Waltham, MA, USA) was applied for spectra analysis. The bands of the alkyl C–H groups-A (2948–2920 cm^{-1} and 2864–2849 cm^{-1}) were assumed to indicate the hydrophobicity, and bands of the C=O groups-B (1710 and 1640–1600 cm^{-1}) indicated hydrophilicity. The ratio of hydrophobicity and hydrophilicity was used to determine the potential wettability index [49].

$$\mathrm{PWI} = \mathrm{A/B}$$

The aromaticity index was calculated according to the reflectance of aliphatic bands ranging from 3000–2800 cm^{-1} (AL) and aromatic band at 1520 cm^{-1} (AR) [50].

$$\mathrm{IAR} = \mathrm{AL/(AL + AR)}$$

Easily extractable glomalin (EEG) and **total glomalin (TG)** were performed according to Wright and Upadhyaya [38], i.e., to 1.00 g of ground dry-sieved soil, 8 mL of sodium acetate citrate (20 mmol L^{-1} of pH 7.0-EEG, 50 mmol L pf pH 8.0-TG) was added, followed with autoclaving at 121 °C (30 min-EEG, 60 min-TG), cooling down and centrifugation at 5000 rpm (10 min-EEG, 15 min-TG). In the case of the TG, the centrifugation of the supernatant of the same sample was repeated 5 times until the supernatant no longer showed the red–brown color typical for glomalin.

Mehlich 3 extraction was conducted according to Mehlich [51] to determine the available phosphorus content **(P_{M3})**. The 3.00 g soil samples were extracted with a 30 mL solution of 0.02 mol L^{-1} CH_3COOH, 0.25 mol L^{-1} NH_4NO_3, 0.015 mol L^{-1} NH_4F, 0.013 mol L^{-1} HNO_3, and 0.001 mol L^{-1} ethylenediaminetetraacetic acid (EDTA). The solution was shaken for 5 min on a horizontal shaker and subsequently filtered. Phosphorus content in extracts was measured by the ICP-OES.

The pH determination (pH_{CaCl_2}) was conducted according to ISO 10390 [44] with slight modification. A total of 5.00 g of soil sample was added to 25 mL of $CaCl_2$ solution. The slurry was shaken for 60 min and was subsequently left to rest for 60 min. After this period, pH was measured using a WTW 330i meter (Xylem Analytics, Weilheim, Germany).

Statistical analysis

The results were assessed using ANOVA statistical analysis with Tukey's test using the Statistica program (TIBCO, Paolo Alto, CA, USA). Principal component analysis (PCA) was performed to evaluate the relationships between the content of glomalin (EEG, TG) and qualitative parameters of SOM using XLSTAT (Addinsoft, New York, NY, USA). The variables were submitted to PCA, and eigenvalues > 1, variance (%), and cumulative (%) criteria were used to define the association among the variables.

List of variables

For better transparency and orientation in the text, Table 4 shows a list of used variables abbreviations, which are commonly used in the following chapters, including the variables units or scale.

Table 4. Description of variables.

Abbrev.	Full Description of Variables	Unit/Scale
C_{SOM}	Soil organic carbon	%
EEG	Easily extractable glomalin	mg kg^{-1}
T	Total glomalin	mg kg^{-1}
C_{HWE}	Carbon–hot water extraction	mg kg^{-1}
C_{DOC}	Carbon–0.01 M L^{-1} $CaCl_2$ extraction	mg kg^{-1}
C_{HA}	Carbon in humic acids	mg kg^{-1}
C_{FA}	Carbon in fulvic acids	mg kg^{-1}
C_{HS}	Carbon humic substances	mg kg^{-1}
$C_{HA/FA}$	Ratio C_{HA} and C_{FA}	-
E4/E6	Humus quality–ratio (E4/E6)	-
P_{M3}	Mehlich-3 extractable phosphorus	mg P kg^{-1}
N_t	Total nitrogen	%
C_{SOM}/N_t	Ratio C_{SOM} and N_t	-
C_{DOC}/N_{DOC}	Ratio C_{DOC} and N_{DOC}	-
PWI	Potential wettability index	-
IAR	Aromaticity index	-
pH_{CaCl_2}	Soil pH	-

3. Results

The average yield of maize biomass on Con treatment during the entire experiment (1993–2019) was 9.00 t of DM ha^{-1}. Biomass yields on SS, N, and CS treatments increased by 35, 39, and 44%, respectively (Table 5). The influence of N fertilizer and different organic fertilizers is also reflected in soil organic matter content (C_{SOM}) (Table 5). The content of C_{SOM} at the beginning of the experiment was 1.26%. After 27 years, the C_{SOM} content on Con treatment amounted only up to 0.981%, which is a decrease of 22%. The lowest C_{SOM} content was observed under mineral nitrogen fertilization (N treatment). It is generally accepted that intensive and exclusive nitrogen fertilization increases the mineralization of soil organic matter. A significantly higher C_{SOM} content was observed in the SS treatment, which was also greater than that in the CS treatment. This is also in agreement with the content of organic substances on SS in comparison with CS. SS treatment was annually supplied with 269 kg C ha^{-1} more (44% more) than CS. A greater C:N ratio on SS (7.32:1) than CS (5.08:1) could have also influenced the intensity of mineralization of fertilizer-applied organic matter and soil organic matter. An important qualitative indicator of SOC is the content of humic acids (HA) and their ratio with fulvic acids (FA). In general, the quality of organic matter is higher with increasing content of HA and decreasing content of FA. The content of C_{HA} was significantly higher after the application of SS. There was also an increasing tendency in C_{HA} content on CS treatment; however, this increase was not significant in comparison with Con and N treatments. There were no significant differences in the content of C_{FA}, but there was an increasing trend in SS. This treatment also produces significant differences in C_{HA}/C_{FA} ratio in comparison with Con in particular.

Table 5. Qualitative and qualitative parameters of soil organic matter (SOM); soil pH values and maize dry biomass yield (BY).

Parameter/Treatment	**Con**	**N**	**SS**	**CS**
C_{SOM} (%)	0.98 ^{a}	0.95 ^{a}	1.20 ^{b}	1.12 ^{ab}
EEG (mg kg^{-1})	578 ^{a}	584 ^{a}	670 ^{b}	633 ^{ab}
TG (mg kg^{-1})	1950 ^{a}	2130 ^{ab}	2370 ^{b}	2110 ^{ab}
C_{HWE} (mg kg^{-1})	186 ^{a}	172 ^{a}	243 ^{b}	240 ^{b}
C_{DOC} (mg kg^{-1})	18.5 ^{a}	22.5 ^{b}	29.1 ^{c}	23.0 ^{b}
C_{HA} (mg kg^{-1})	0.078 ^{a}	0.090 ^{ab}	0.123 ^{b}	0.110 ^{ab}
C_{FA} (mg kg^{-1})	0.138 ^{a}	0.145 ^{a}	0.158 ^{a}	0.133 ^{a}
C_{HS} (mg kg^{-1})	0.215 ^{a}	0.235 ^{a}	0.280 ^{b}	0.243 ^{ab}
$C_{HA/FA}$	0.565 ^{a}	0.629 ^{ab}	0.784 ^{b}	0.836 ^{b}
E4/E6	3.53 ^{a}	3.63 ^{a}	3.65 ^{a}	3.65 ^{a}
P_{M3} (mg kg^{-1})	142 ^{a}	102 ^{a}	366 ^{c}	304 ^{b}
N_t (%)	0.096 ^{a}	0.102 ^{a}	0.120 ^{b}	0.111 ^{ab}
C_{SOM}/N_t	10.2 ^{b}	9.31 ^{a}	9.97 ^{b}	10.1 ^{b}
C_{DOC}/N_{DOC}	0.550 ^{b}	0.161 ^{a}	1.03 ^{c}	0.954 ^{c}
PWI	0.010 ^{ab}	0.010 ^{a}	0.014 ^{b}	0.014 ^{b}
IAR	0.020 ^{a}	0.019 ^{a}	0.026 ^{b}	0.027 ^{b}
pH_{CaCl_2}	6.36 ^{ab}	5.91 ^{a}	6.25 ^{ab}	6.70 ^{b}
Maize BY (t ha^{-1})	9.00 ^{a}	12.5 ^{b}	12.2 ^{b}	13.0 ^{b}

Different letters describe statistically significant differences between treatments. Tukey's LSD test; $p < 0.05$.

Changes in quantity and quality of soil organic matter are also visibly reflected in the content of carbon extractable with hot water (C_{HWE}) and with weak extractant 0.01 M L^{-1} $CaCl_2$ (C_{DOC}). The content of C_{HWE} on both organic treatments was significantly higher than all other treatments, while the content of C_{DOC} was significantly higher only in comparison with Con. The soil organic matter quality was also investigated using the E4/E6 method with sodium pyrophosphate (0.05 mol L^{-1} $Na_4P_2O_7$) as an extractant. This method did not produce any significant differences among treatments (Table 5). The question is whether the E4/E6 method is quite suitable for evaluating soil organic matter quality.

The mineralization rate of soil organic matter is substantially influenced by the intensity and form (organic/mineral) of nitrogen fertilization. This is the reason for the inclusion of N_t and C_{SOM}/N_t ratio as well as 0.01 mol L^{-1} $CaCl_2$ (C_{DOC}/N_{DOC}) (Table 5). It is also necessary to mention that nitrogen uptake by aboveground biomass was substantially higher than applied nitrogen (120 kg N ha^{-1}).

The content of N_t was lowest in the non-fertilized Con treatment, which is following our supposition. On the other hand, the highest N_t content was observed during the

SS treatment. Considering the stability of SOM, the C/N is more important. This ratio was highest in the non-fertilized Con treatment. It is possible that SOM is in the most stable configuration against mineralization in the Con treatment. Mineral fertilization (N treatment) displayed a significantly lower C_{SOM}/N_t ratio (9.31:1). Differences in the C_{DOC}/N_{DOC} ratio are even more visible with organic fertilizers compared to Con and N treatments than C_{SOM}/N_t.

For the evaluation of soil organic matter quality, a DRIFTS method was also used. Usually, this method is used to study bands of hydrophobic alkyl C–H groups and hydrophilic C=O groups of soil organic matter at 2948–2920 cm^{-1} and 1710, 1640–1600 cm^{-1}, respectively. The ratio of hydrophobic and hydrophilic groups determines the potential wettability index (PWI). Ordinarily, PWI is directly proportional to the stability of soil aggregates and the quality of soil organic matter. It is clear that the lowest values were measured in the N treatment, while the highest values were measured in the organic fertilizer treatments (Table 5). Another studied criterion using the DRIFTS method was the aromaticity index (IAR) estimation. This index is calculated based on the reflectance of aliphatic compounds (3000–2800 cm^{-1}) and aromatic compounds (1520 cm^{-1}). Evidently, the usage of organic fertilizers increased the values of IAR. The lowest values were again produced by the N treatment.

Concerning the study of glomalin, the content of plant-available phosphorus using the extraction method Mehlich 3 was also included in the monitored parameters. In our experiments, certain treatments were annually supplied with phosphorus at rates of 82.1 kg P ha^{-1} in SS and 23.2 kg P ha^{-1} in CS. The SS treatment was significantly positive in the balance of incoming and outcoming (only plant uptake) phosphorus. On the other hand, the CS treatment had a slight, significantly negative balance while other treatments (Con and N) had a distinctly negative balance. This was subsequently manifested in the content of available phosphorus in soil. Treatments with organic fertilizers had significantly higher contents. From the values of the correlation coefficients (Table 6), it is clear that there was a significant positive correlation with EEG and an insignificant correlation with TG.

Table 6. Relationship between glomalin and other soil parameters.

	C_{SOM}	C_{HWE}	C_{DOC}	C_{HA}	C_{FA}	C_{HS}	$C_{HA/FA}$	E4/E6
EEG	0.527 *	0.424	0.393	0.590 *	0.004	0.454	0.587 *	−0.157
TG	0.521 *	0.440	0.669 **	0.558 *	0.349	0.609 *	0.405	0.420
C_{SOM}		0.872 ***	0.654 **	0.706 **	0.241	0.666 **	0.583 *	−0.131
	N_t	**P_{M3}**	**C_{SOM}/N_t**	**C_{DOC}/N_{DOC}**	**PWI**	**IAR**	**pH_{CaCl_2}**	
EEG	0.472	0.612 *	0.244	0.399	0.735 **	0.688 **	0.505 *	
TG	0.592 *	0.466	−0.100	0.394	0.333	0.335	−0.196	
C_{SOM}	0.938 ***	0.839 ***	0.356	0.752 ***	0.799 ***	0.797 ***	0.530 *	

Pearson correlation coefficient: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Based on the presented results, it is clear that this is a very diverse set of criteria from the perspective of soil organic matter quality as well as from the perspective of nutrient content (nitrogen, phosphorus). This set of criteria was consequently used for testing glomalin as an indicator of soil organic matter quality. Values of easily extractable glomalin content (EEG) showed an increase in the organic fertilizer treatment. Total glomalin content (TG) was significantly higher in the SS treatment in comparison with the control. Correlation coefficient values comparing EEG, TG, and qualitative components of SOM are presented in Table 6.

The different variables were evaluated using principal component analysis (PCA). The principal components and their association were systematically selected based on three criteria, i.e., eigenvalue > 1.1, loading factors > 0.70, and percentage of variability > 6.50%, as mentioned in Table 7. The biplot position of variables explains 93.5% (PC1 and PC2) of the total cumulative variance, where the first factor describes 72.1%, the second 21.4%, and the third 6.50% (Figure 1). PC1, which explained 72.1% of the total variance, was highly dominated by positively associated variables such as P_{M3}, EEG, IAR, N_t, PWI, C_{HWE}, C_{HS}, C_{HA}, $C_{HA/FA}$, C_{DOC}/N_{DOC}, E4/E6, C_{SOM}, C_{DOC}, and TG. The second PC2 explains about 21.4% of the variance and was positively correlated with C_{SOM}/N_t and pH_{CaCl_2} and negatively associated with C_{FA}. The third PC explains 6.5% of the total variance, and no variables dominated within the principal component. The P_{M3}, EEG, C_{SOM}, and variables are strongly associated, and the most significant variables dominated within the PC1. C_{SOM} was positively correlated with N_t ($p < 0.05$; r = 0.94), C_{HA} ($p < 0.05$; r = 0.71), PWI ($p < 0.05$; r = 0.80), C_{HWE} ($p < 0.05$; r = 0.87), and IAR ($p < 0.05$; r = 0.80).

Table 7. The principal components (PCs) or factors and their loading factor values, eigenvalues, and variabilities (%).

Parameters	**PC1**	**PC2**	**PC3**
C_{SOM}	0.981	0.074	−0.179
EEG	0.972	0.147	0.185
TG	0.800	−0.590	−0.107
C_{HWE}	0.960	0.278	−0.028
C_{DOC}	0.855	−0.509	−0.101
C_{HA}	0.980	−0.183	0.073
C_{FA}	0.397	−0.778	−0.488
C_{HS}	0.884	−0.446	−0.141
$C_{HA/FA}$	0.925	0.129	0.356
E4/E6	0.760	−0.378	0.528
P_{M3}	0.980	0.141	−0.139
N_t	0.972	−0.237	−0.001
C_{SOM}/N_t	0.259	0.822	−0.508
C_{DOC}/N_{DOC}	0.883	0.41	−0.231
PWI	0.943	0.323	0.077
IAR	0.946	0.316	0.071
pH_{CaCl_2}	0.435	0.895	0.098
Eigenvalue	12.3	3.60	1.10
Variability (%)	72.1	21.4	6.50
Cumulative variance	72.1	93.5	100

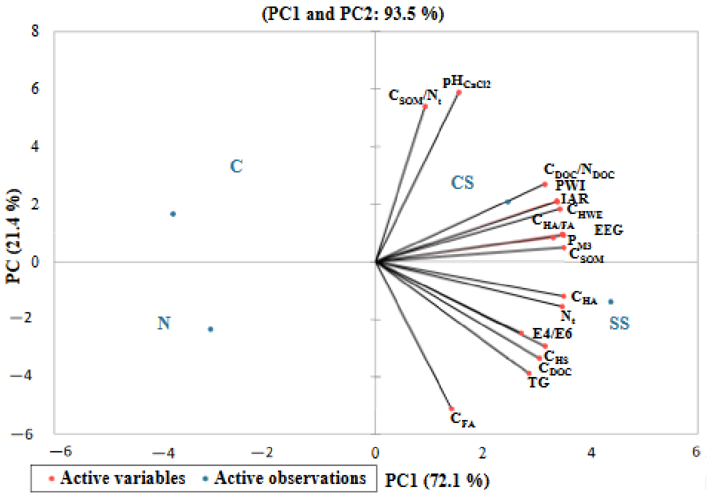

Figure 1. The biplot position of variables is determined by the first two principal components (PC1 vs. PC2); the red and blue dots represent active variables and observations, respectively.

The application of sewage sludge and cattle slurry to soil positively influenced the tested variables.

4. Discussion

The global trend of moving away from mineral fertilizers and returning to organic fertilizers creates a necessity for research of alternative and potentially available sources. Application of sewage sludge on arable soil is one of these alternatives. Sewage sludge is organic compounds and nutrients (P in particular), but it also may be a source of potentially toxic elements and substances. Therefore, extraordinary attention must be paid to their use. Production of sewage sludge is increasing in proportion to the increasing population worldwide. About 37% of produced sludge is concurrently used as fertilizer in EU countries [52]. Due to the aforementioned facts, the results of this study, focusing on soil carbon transformations, could be helpful on a greater than regional scale.

One of the benefits of our experiments is a small set of variable factors that could influence changes in the quality and quantity of soil organic matter. Long-term silage maize production can be considered a deciding factor. It is generally accepted that maize belongs to a group of crops substantially dependent on arbuscular mycorrhiza and, at the same time, promotes its development [53]. Another variable was differences in the quality of applied fertilizers (mineral/organic). Nevertheless, all fertilized treatments received 120 kg N ha^{-1} $year^{-1}$. The long duration of experiments (27 years) showed its influence on the quantity and quality of soil organic matter (Table 5). Therefore, it can be assumed that this set of different qualitative parameters was suited for monitoring changes in glomalin content in soils. It is also necessary to remember that experiments had classic tillage treatment with roughly 25 cm ploughing depth.

The following methods were used to determine the changes in soil organic matter quality and quantity: C_{SOM}, hot water extraction, 0.01 mol L $CaCl_2$ extraction, N_t assessment, and alternative determination of C_{SOM}/N_t and C_{DOC}/N_{DOC} ratios. These methods are relatively sensitive and reproducible [48,54]. Fractionation of humic substances, humic acids, and fulvic acids [45] is a very time-consuming process, with a wide variety of possible analytical inaccuracies that severely limit this method in terms of extensive soil monitoring. If performed correctly, this method is capable of producing very good results characterizing soil organic matter. Our study's results agree with Thai et al. [55] and demonstrate that the E4/E6 method is unfit for the determination of soil organic matter quality, and was therefore not used in further evaluations.

4.1. Content of Glomalin

GRSP exists in two forms: easily extractable form (EEG) and total form (TG). The determination of EEG is based on a single extraction using sodium citrate. TG is repeatedly extracted several times. On the other hand, EEG is easily extractable with relatively high solubility in water in comparison with TG [38]. The average contents of TG and EEG were 2140 mg kg^{-1} and 616 mg kg^{-1}, respectively. EEG, therefore, makes up to 29% of TG. This ratio is greater than that published in an earlier study (18%) on another experiment with crop rotation [13].

Total glomalin content (TG) in our experiments was between 1950 mg kg^{-1} and 2370 mg kg^{-1} and is in accord with the results of Agnihotri et al. [56], who state that glomalin content in regular soils lies within the interval of 2.00–15.0 mg g^{-1}. Lower TG values between 1.00–9.00 mg g^{-1} are presented in other works [14,27,56–59].

Glomalin can add up to 25% of the total C_{SOM} content of soil organic matter [15]. This proportion lies in the range of 18.8 to 22.4% in our experiments based on treatment and is in good agreement with the aforementioned authors. Glomalin contains roughly 28–48% of carbon [60]. The mean value of this interval is 36%, and using this value, we calculated carbon content in glomalin (C_{gl}) in our experiments based on treatment to be Con: 702 mg C_{gl} kg^{-1}, N: 767 mg C_{gl} kg^{-1}, SS: 853 mg C_{gl} kg^{-1}, and CS: 760 mg C_{gl} kg^{-1}. Content of C_{SOM} in the Con treatment was determined at 0.981% (9810 mg C kg^{-1}). Using further calculations, we find that proportion of glomalin carbon (C_{gl}) is 7.2% in C_{SOM} in the Con treatment. On other treatments, this proportion is N: 8.1%, SS: 7.1%, and CS: 6.9%. These results are distinctly higher than the 3.2% that was published in Lovelock et al. [19]. If 28% were to be used (instead of 36%), the resulting proportion of C_{gl} in C_{SOM} would be greater than 5.2%. Using a calculation with 36% of carbon in glomalin for easily extractable glomalin (EEG), the resulting proportion of C_{EE} in C_{SOM} would be 1.9–2.2%.

Nitrogen content in glomalin shows a relatively wide interval of 0.9–7.3% [61,62]. Treseder and Turner [63] present a substantially tighter interval of 2–4%. Using calculation with 3% of nitrogen content, the resulting nitrogen content in glomalin (N_{gl}) in soil based on treatment is Con: 59.0 mg N_{gl} kg^{-1}, N: 64.0 mg N_{gl} kg^{-1}, SS: 71.0 mg N_{gl} kg^{-1}, and CS: 63.0 mg N_{gl} kg^{-1}. Therefore, the proportion of N_{gl} in N_t is between 5.70% and 6.30% based on the treatment. These calculated values are in very good agreement with the data of Lovelock et al. [19], who presented a 5% portion of N_{gl} in N_t.

4.2. Relationship between Glomalin and SOM Parameters

Organic fertilization increases the proportion of macroaggregates in soil and the average size of these aggregates parallel with an increase of microbial biomass and the contents of C_{SOM} and GRSP [22]. The application of SS especially increased the contents of C_{SOM} and GRSP (Table 5). The relationship between C_{SOM} content, as well as TG and EEG content, is further confirmed by correlation coefficient values (Table 6). A similar positive correlation is also presented in Singh et al. [15] and Wang et al. [16]. In the conditions of the Czech Republic, the increase of GRSP content with an increase of organic matter content was also presented by Šarapatka et al. [20]. In our experiment, the strength of the relationship between C_{SOM}, TG, and EEG is almost the same. The conclusions of Řezáčová et al. [23] were not confirmed, as they presented a tighter relationship between EEG and C_{SOM} than TG and C_{SOM}.

In accordance with our results, an increase in glomalin content after the application of SS is also presented by Sandeep et al. [27] and Balík et al. [13]. Furthermore, there was no evidence suggesting that N fertilization increased glomalin content (Table 5), which conflicts with the results of previous studies [13,58,63]. The content of both GRSP forms (TG and EEG) is also in good correlation with more stable fractions of soil organic matter (C_{SOM}), that is, with the content of humic acids as opposed to the content of fulvic acids (Table 6). This indirectly confirms the hypothesis that glomalin is relatively resistant to mineralization. The persistence time of glomalin is up to 42 years, and the residence time is longer than the original organic matter in bulk soil (10–37 years) [34,64,65].

The generally accepted conclusion is that the C_{SOM}/N_t ratio characterizes the potential for mineralization of soil organic matter in a certain way. N fertilization tightens this ratio [13], which was subsequently documented in N treatment in this study. Therefore, we were interested in the relationship between the N_t content, C_{SOM}/N_t ratio, and C_{DOC}/N_{DOC} ratio on one side, and glomalin content on the other side. No significant relationship was found (Table 6). This could point towards greater stability of glomalin against mineralization than other soil organic matter. Jha et al. [66] found a positive correlation between the mineralization process (release of nitrogen from organic bonds) and EEG content. Authors further concluded that EEG content could be a certain criterion for estimating potential nitrogen mineralization from organic bonds. In our experiments, we have not confirmed this conclusion. Higher values of the correlation coefficient between TG and N_t can be caused by the fact that during repeated extraction with sodium citrate, a portion of other organic compounds, including proteins, can be released aside from glomalin [4,5].

A high content of mobile phosphorus in soil reduces colonization of roots with AMF [17] and thereby also reduces the content of EEG in soil [18]. This was not proven in our experiments, but on the other hand, it does confirm the results of Šarapatka et al. [20], who stated an increase in GRSP with an increase of available phosphorus. Cissé et al. [67] did not observe changes in GRSP content during long-term maize production and periodic phosphorus fertilization on sandy soil. The authors further mention that phosphorus fertilizer treatment had a slight trend toward increasing maize yield. Singh et al. [15] and Wang et al. [16] also observed a positive correlation between GRSP content and components of soil fertility, including phosphorus content. The disproportion of our results compared to Lovelock et al. [19] can be explained in the fact that even Con and N treatments had a good supply of available phosphorus. Maize plants were well supplied by phosphorus on all treatments, meaning there were no conditions for increased stimulation of AMF growth.

Greater content of GRSP was usually found on more acidic soils in comparison with neutral or carbonate soils. Glomalin content increased with the reduction of pH value [56]. In our experiments, an opposite effect was observed. A significant positive correlation was found between EEG content and $pH/_{CaCl_2}$ solution. Apparently, soil reaction did not have a dominant influence over glomalin content. Other factors had a decisive influence (e.g., C_{SOM}). It is important to note the influence of nutrients in organic fertilizers, whose presence leads to a significant increase in plant biomass, including root development, and, as a result, also increases glomalin production. Higher soil organic matter content also leads to the formation of bigger soil aggregates, inside which a bigger glomalin content is bound. This also reduces the intensity of mineralization.

4.3. Potential Wettability Index

The quality of soil organic matter can be described by the ratio of aliphatic (C–H) and carboxyl (C=O) bonds called the potential wettability index (PWI) [68,69]. PWI can be used (a) during the determination of stability of soil aggregates, (b) for water sorption properties of soil, and (c) for the description of soil organic matter quality. Values of PWI were in the interval of 0.010–0.014 (Table 5). Fér et al. [70] mention values of PWI for Luvisol type between 0.010–0.030, which agrees with our values. It is obvious that organic fertilization significantly increased values of PWI in comparison with the Con and N treatments. Organic fertilization, therefore, increased the fraction of hydrophobic particles and contributed to the formation of bigger soil aggregates. Adani [71] also measured an increase in aliphatic carbon fraction in humic acids after application of SS after 10 years of experiments with a dose of 1 t DM ha^{-1} $year^{-1}$. Demyan et al. [72] observed an increase in value after the application of manure. Leue et al. [73] found a positive correlation between SOM content and PWI values. Values of PWI in our experiments also showed a very strong and significant correlation with C_{SOM} content. GRSPs are temperature-stable, sticky, and hydrophobic glycoproteins [38]. Following a simple though-chain, the following conclusion can be deduced: with an increase in glycoproteins content, there is also an increase in hydrophobic particle content and an increase of PWI. A significant correlation between EEG and PWI (0.735, $p < 0.01$) is in accordance with this hypothesis. The aromaticity index (IAR) also expresses the stability of soil organic matter [55]. Treatments with organic fertilizers produce significantly higher values. Similar to PWI, IAR also correlates with EEG.

5. Conclusions

The influence of different fertilizers (mineral/organic) on the quantity and quality of soil organic matter was monitored in long-term stationary experiments with silage maize monoculture production. Two of the observed factors were the contents of easily extractable glomalin (EEG) and total glomalin (TG). The contents of EEG and TG correlated with C_{SOM}. Periodical application of sewage sludge and cattle slurry increased the content of glomalin in soils. Data about glomalin content can be used to study soil organic matter quality; however, this parameter is not singular or exceptional. A more sensitive method (a method that reacts more to changes in components of soil fertility) seems to be the determination of EEG, rather than TG, in our experiments. A significant correlation was found between the EEG content and C_{SOM} content, humic acid content (C_{HA}), C_{HA}/C_{FA} ratio, potential wettability index (PWI), and aromaticity index (IAR). Another factor supports the greater practical application of this method, and that is a substantially shorter time of analysis than TG determination. The potential wettability index method is suitable for studying soil organic matter quality. The E4/E6 method does not produce relevant soil organic matter quality results.

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References

1. Wright, S.F.; Upadhyaya, A. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Sci.* **1996**, *161*, 575–586. [CrossRef]
2. Nichols, K.A. Characterization of Glomalin, a Glycoprotein Produced by Arbuscular Mycorrhizal Fungi. Ph.D. Thesis, University of Maryland, College Park, MD, USA, 2003; p. 285.
3. Gillespie, A.W.; Farrell, R.E.; Walley, F.L.; Ross, A.R.S.; Leinweber, P.; Eckhardt, K.U.; Regier, T.Z.; Blyth, R.I.R. Glomalin-related soil protein contains non-mycorrhizal-related heat-stable proteins, lipids and humic materials. *Soil Biol. Biochem.* **2011**, *43*, 766–777. [CrossRef]
4. Irving, T.B.; Alptekin, B.; Kleven, B.; Ané, J.M. A critical review of 25 years of glomalin research: A better mechanical understanding and robust quantification techniques required. *New Phytol.* **2021**, *232*, 1572–1581. [CrossRef]
5. Holátko, J.; Brtnický, M.; Kučerík, J.; Kotianová, M.; Elbl, J.; Kintl, A.; Kynický, J.; Benada, O.; Datta, R.; Jansa, J. Glomalin—Truths, myths, and the future of this elusive soil glycoprotein. *Soil Biol. Biochem.* **2021**, *153*, 108116. [CrossRef]
6. Agnihotri, R.; Prakash, A.; Ramesh, A.; Billore, S.D.; Sharma, M.P. Coordination of crop and soil management practices built up higher mycorrhizal biomass which enhances soil carbon sequestration assessed in the rhizosphere of soybean in a longterm field trial. In Proceedings of the National Conference on Organic Waste Management for Food and Environment Security, Bhopal, India, 8–10 February 2018; p. 94.
7. Agnihotri, R.; Bharti, A.; Ramesh, A.; Prakash, A.; Sharma, M.P. Glomalin related protein and C16:1ω5 PLFA associated with AM fungi as potential signatures for assessing the soil C sequestration under contrasting soil management practices. *Eur. J. Soil Biol.* **2021**, *103*, 103286. [CrossRef]
8. Gunina, A.; Kuzyakov, Y. Sugars in soil and sweets for microorganisms: Review of origin, content, composition and fate. *Soil Biol. Biochem.* **2015**, *90*, 87–100. [CrossRef]
9. Rillig, M.C.; Wright, S.F.; Eviner, V.T. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: Comparing effects of five plant species. *Plant Soil.* **2002**, *238*, 325–333. [CrossRef]
10. Xu, P.; Liang, L.Z.; Dong, X.Y.; Shen, R.F. Effect of arbuscular mycorrhizal fungi on aggregate stability of a clay soil inoculating with two different host plants. *Acta Agric. Scand. Sect. B—Soil Plant Sci.* **2014**, *65*, 23–29. [CrossRef]
11. Zbíral, J.; Čižmar, D.; Malý, S.; Obdržálková, E. Determination of glomalin in agriculture and forest soils by near-infrared spectroscopy. *Plant Soil Environ.* **2017**, *63*, 226–230. [CrossRef]
12. Li, X.; Han, S.; Luo, X.S.; Chen, W.L.; Huang, Q.Y. Arbuscular mycorrhizal-like fungi and glomalin-related soil protein drive the distributions of carbon and nitrogen in a large scale. *J. Soil Sediments* **2020**, *20*, 963–972. [CrossRef]
13. Balík, J.; Sedlář, O.; Kulhánek, M.; Černý, J.; Smatanová, M.; Suran, P. Effect of organic fertilizers on glomalin content and soil organic matter quality. *Plant Soil Environ.* **2020**, *66*, 590–597. [CrossRef]
14. Galazka, A.; Gawryjolek, K.; Grzadziel, J.; Ksiezak, J. Effect of different agricultural management practices on soil biological parameters including glomalin fraction. *Plant Soil Environ.* **2017**, *63*, 300–306. [CrossRef]
15. Singh, A.K.; Rai, A.; Singh, N. Effect of long-term land use systems on fractions of glomalin and soil organic carbon in the Indo-Gangetic plain. *Geoderma* **2016**, *277*, 41–50. [CrossRef]
16. Wang, W.; Zhong, Z.; Wang, Q.; Wang, H.; Fu, Y.; He, X. Glomalin contributed more to carbon, nutrients in deeper soils, and differently associated with climates and soil properties in vertical profiles. *Sci. Rep.* **2017**, *7*, 13003. [CrossRef] [PubMed]

17. Singh, A.K.; Zhu, X.; Chen, C.; Wu, J.; Yang, B.; Zakari, S.; Jiang, X.J.; Singh, N.; Lie, W. The role of glomalin in mitigation of multiple soil degradation problems. *Crit. Rev. Environ. Sci. Technol.* **2020**, *52*, 1604–1638. [CrossRef]
18. Sharma, M.P.; Adholeya, A. Parameters for selecting efficient arbuscular mycorrhizal fungi for plants under microcosm conditions. *Proc. Natl. Acad. Sci. India Sect. B-Biol. Sci.* **2015**, *85*, 77–83. [CrossRef]
19. Lovelock, C.E.; Wright, S.F.; Clark, D.A.; Ruess, R.W. Soil stocks of glomalin produced by arbuscular mycorrhizal fungi across a tropical rain forest landscape. *J. Ecol.* **2004**, *92*, 278–287. [CrossRef]
20. Šarapatka, B.; Alvarado-Solano, D.P.; Čižmár, D. Can glomalin content be used as an indicator for erosion damage to soil and related changes in organic matter characteristics and nutrients? *Catena* **2019**, *181*, 104078. [CrossRef]
21. Bertagnoli, B.G.P.; Oliveira, J.F.; Barbosa, G.M.C.; Filho, A.C. Poultry litter and liquid swine slurry applications stimulate glomalin, extraradicular mycelium production, and aggregation in soils. *Soil Tillage Res.* **2020**, *202*, 104657. [CrossRef]
22. Zhang, X.; Wu, X.; Zhang, S.; Xing, Y.; Wang, R.; Liang, W. Organic amendment effects on aggregate-associated organic C, microbial biomass C and glomalin in agricultural soils. *Catena* **2014**, *123*, 188–194. [CrossRef]
23. Řezáčová, V.; Czakó, A.; Stehlík, M.; Mayerová, M.; Šimon, T.; Smatanová, M.; Madaras, M. Organic fertilization improves soil aggregation through increases in abundance of eubacteria and products of arbuscular mycorrhizal fungi. *Sci. Rep.* **2021**, *11*, 12548. [CrossRef] [PubMed]
24. Valarini, P.J.; Curaqueo, G.; Seguel, A.; Manzano, K.; Rubio, R.; Cornejo, P.; Borie, F. Effect of compost application on some properties of a volcanic soil from central south Chile. *Chil. J. Agric. Res.* **2009**, *69*, 416–425. [CrossRef]
25. Turgay, O.C.; Buchan, D.; Moeskops, B.; De Gusseme, B.; Ortas, I.; De Neve, S. Changes in soil ergosterol content, glomalin-related soil protein, and phospholipid fatty acid profile as affected by long-term organic and chemical fertilization practices in Mediterranean Turkey. *Arid Land Res. Manag.* **2015**, *29*, 180–198. [CrossRef]
26. Dai, J.; Hu, J.L.; Lin, X.G.; Yang, A.; Wang, R.; Zhang, J.B.; Wong, M.H. Arbuscular mycorrhizal fungal diversity, external mycelium length, and glomalin-related soil protein content in response to long-term fertilizer management. *J. Soils Sediments* **2013**, *13*, 1–11. [CrossRef]
27. Sandeep, S.; Manjaiah, K.M.; Pal, S.; Singh, A.K. Soil carbon fractions under maize-wheat system: Effect of tillage and nutrient management. *Environ. Monit. Assess.* **2016**, *188*, 14. [CrossRef] [PubMed]
28. Nie, J.; Zhou, J.M.; Wang, H.Y.; Chen, X.Q.; Du, C.W. Effect of long-term rice straw return on soil glomalin, carbon and nitrogen. *Pedosphere* **2007**, *17*, 295–302. [CrossRef]
29. Liang, G.; Wu, H.; Houssou, A.A.; Cai, D.; Wu, X.; Gao, L.; Wang, B.; Li, S. Soil respiration, glomalin content, and enzymatic activity response to straw application in a wheat-maize rotation system. *J. Soils Sediments* **2017**, *18*, 697–707. [CrossRef]
30. Banik, S.P.; Pal, S.; Ghorai, S.; Chowdhury, S.; Khowala, S. Interference of sugars in the Coomassie Blue G dye binding assay of proteins. *Anal. Biochem.* **2009**, *386*, 113–115. [CrossRef]
31. Jorge-Araújo, P.; Quiquampoix, H.; Matumoto-Pintro, P.T.; Staunton, S. Glomalin related soil protein in French temperate forest soils: Interference in the Bradford assay caused by co-extracted humic substances. *Eur. J. Soil Sci.* **2014**, *66*, 311–319. [CrossRef]
32. Gispert, M.; Emran, M.; Pardini, G.; Doni, S.; Ceccanti, B. The impact of land management and abandonment on soil enzymatic activity, glomalin content and aggregate stability. *Geoderma* **2013**, *202–203*, 51–61. [CrossRef]
33. Wang, Q.; Lu, H.; Chen, J.; Hong, H.; Liu, J.; Li, J.; Yan, C. Spatial distribution of glomalin-related soil protein and its relationship with sediment carbon sequestration across a mangrove forest. *Sci. Total Environ.* **2018**, *613–614*, 548–556. [CrossRef] [PubMed]
34. Rillig, M.C.; Wright, S.F.; Nichols, K.A.; Schmidt, W.F.; Torn, M.S. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant Soil* **2001**, *233*, 167–177. [CrossRef]
35. Emran, M.; Gispert, M.; Pardini, G. Patterns of soil organic carbon, glomalin and structural stability in abandoned Mediterranean terraced lands. *Eur. J. Soil Sci.* **2012**, *63*, 637–649. [CrossRef]
36. Vlček, V.; Pohanka, M. Glomalin-An interesting protein part of the soil organic matter. *Soil Water Res.* **2020**, *15*, 67–74. [CrossRef]
37. Hurisso, T.T.; Moebius-Clune, D.J.; Culman, S.W.; Moebius-Clune, B.N.; Thies, J.E.; van Es, H.M. Soil protein as a rapid soil health indicator of potentially available organic nitrogen. *Agric. Environ. Lett.* **2018**, *3*, 180006. [CrossRef]
38. Wright, S.F.; Upadhyaya, A. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil.* **1998**, *198*, 97–107. [CrossRef]
39. Xie, H.T.; Li, J.W.; Zhang, B.; Wang, L.F.; He, H.B.; Yhang, X.D. Long-term manure amendments reduced soil aggregate stability via redistribution of the glomalin-related soil protein in macroaggregates. *Sci. Rep.* **2015**, *5*, 14687. [CrossRef]
40. Hauzé, V.; Tran, G.; Edouard, N.; Lebas, F.; Maize Silage. Feedipedia, a Programme by INRA, CIRAD, AFZ and FAO. 2017. Available online: https://www.feedipedia.org/node/13883 (accessed on 31 August 2020).
41. Food and Agriculture Organization of the United Nations. *World Reference Base for Soil Resources 2014. International Soil Classification System for Naming and Creating Legends for Soil Maps*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2015; Available online: http://www.fao.org/3/i3794en/I3794en.pdf (accessed on 1 September 2020).
42. Vácha, R.; Čechmánková, J.; Duffková, R. *Soil—Our Wealth*; Profi Press: Prague, Czech Republic, 2019; p. 228. ISBN 978-80-88306-00-9. (In Czech)
43. United States Department of Agriculture–Natural Resources Conservation Service. Soil Taxonomy. Available online: https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_051232.pdf (accessed on 2 May 2022).
44. International Organization for Standardization. Soil Quality-Determination of pH. (ISO Standard No. 10390:1994). 1994. Available online: https://www.iso.org/standard/18454.html (accessed on 2 May 2022).

45. Kononova, M.M. *Soil Organic Matter: Nature, Properties and Methods of Study*; Academy of Sciences of Soviet Union: Moscow, Russia, 1963. (In Russian)
46. Sparks, D.L. *Methods of Soil Analysis. Part 3-Chemical Methods*; John Wiley & Sons: Hoboken, NJ, USA, 1996; p. 1390. ISBN 9780891188254.
47. Houba, V.J.G.; Temminghoff, E.J.M.; Gaikhorst, G.A.; van Vark, W. Soil analysis procedures using 0.01 *M* calcium chloride as extraction reagent. *Commun. Soil Sci. Plant Anal.* **2008**, *31*, 1299–1396. [CrossRef]
48. Körschens, M.; Albert, E.; Armbruster, M.; Barkusky, D.; Baumecker, M.; Behle-Schalk, L.; Bischoff, R.; Čergan, Z.; Ellmer, F.; Herbst, F.; et al. Effect of mineral and organic fertilization on crop yield, nitrogen uptake, carbon and nitrogen balances, as well as soil organic carbon content and dynamics: Results from 20 European long-term field experiments of the twenty-first century. *Arch. Agron. Soil Sci.* **2013**, *59*, 1017–1040. [CrossRef]
49. Ellerbrock, R.H.; Gerke, H.; Bachman, J.; Goebel, M. Composition of organic matter fractions for explaining Wettability of three forest soils. *Soil Sci. Soc. Am. J.* **2005**, *69*, 57–66. [CrossRef]
50. Cunha, T.J.F.; Novotny, E.H.; Madari, B.E.; Martin-Neto, L.; Re-zende, M.O.; Canelas, L.P.; Benitesm, V.M. Spectroscopy Characterization of Humic Acids Isolated from Amazonian Dark Earth Soils (Terra Preta De Índio). In *Amazonian Dark Earths: Wim Sombroek's Vision*, 1st ed.; Woods, W.I., Teixeira, W.G., Lehmann, J., Steiner, C., Eds.; Springer: Dordrecht, The Netherlands, 2009; pp. 363–372. ISBN 978-1-4020-9031-8. [CrossRef]
51. Mehlich, A. Mehlich 3 Soil Test Extractant. A Modification of the Mehlich 2 Extractant. *Commun. Soil Sci. Plant Anal.* **1984**, *15*, 1409–1416. [CrossRef]
52. Kayikcioglu, H.H.; Yener, H.; Ongun, A.R.; Okur, B. Evaluation of soil and plant health associated with successive three-year sewage sludge field applications under semi-arid biodegradation condition. *Arch. Agron. Soil Sci.* **2019**, *65*, 1659–1676. [CrossRef]
53. Plenchette, C.; Fortin, J.A.; Furlan, V. Growth Response of Several Plants Species to Mycorrhiza in a Soil of Moderate P-Fertility. *Plant Soil* **1983**, *70*, 211–217. [CrossRef]
54. Balík, J.; Černý, J.; Kulhánek, M.; Sedlář, O. Soil carbon transformation in long-term field experiments with different fertilization treatments. *Plant Soil Environ.* **2018**, *64*, 578–586. [CrossRef]
55. Thai, S.; Davídek, T.; Pavlů, L. Causes clarification of the soil aggregates stability on mulched soil. *Soil Water Res.* **2022**, *17*, 91–99. [CrossRef]
56. Agnihotri, R.; Sharma, M.P.; Prakash, A.; Ramesh, A.; Bhattacharjya, S.; Patra, A.K.; Manna, M.C.; Kurganova, I.; Kuzyakov, Y. Glycoproteins of arbuscular mycorrhiza for soil carbon sequestration: Review of mechanisms and controls. *Sci. Total Environ.* **2022**, *806*, 150571. [CrossRef] [PubMed]
57. Wuest, S.B.; Caesar-TonThat, T.C.; Wright, S.F.; Williams, J.D. Organic matter addition, N, and residue burning effects on infiltration, biological, and physical properties of an intensively tilled silt-loam soil. *Soil Tillage Res.* **2005**, *84*, 154–167. [CrossRef]
58. Luna, L.; Miralles, I.; Andrenelli, M.C.; Gispert, M.; Pellergini, S.; Vignozzi, N.; Solé-Benet, A. Restoration techniques affect soil organic carbon, glomalin and aggregate stability in degraded soils of a semiarid Mediterranean region. *Catena* **2016**, *143*, 256–264. [CrossRef]
59. Curaqueo, G.; Barea, J.M.; Acevedo, E.; Rubio, R.; Cornejo, P.; Borie, F. Effects of different tillage system on arbuscular mycorrhizal fungal propagules and physical properties in a Mediterranean agro-ecosystem in central Chile. *Soil Tillage Res.* **2011**, *113*, 11–18. [CrossRef]
60. Huang, Y.; Wang, D.; Cai, J.; Zheng, W. Review of glomalin-related soil protein and its environmental function in the rhizosphere. *Chin. J. Plant Ecol.* **2011**, *35*, 232–236. [CrossRef]
61. Sousa, C.D.; Menezes, R.S.C.; Sampaio, E.; Lima, F.D. Glomalin: Characteristics, production, limitations and contribution to soils. *Semin. Cienc. Agrar.* **2012**, *33*, 3033–3044. [CrossRef]
62. Wang, Q.; Wu, Y.; Wang, W.J.; Zhong, Z.L.; Pei, Z.X.; Ren, J.; Wang, H.M.; Zu, Y.G. Spatial variations in concentration, compositions of glomalin related soil protein in poplar plantations in northeastern China, and possible relations with soil physicochemical properties. *Sci World. J.* **2014**, *13*, 160403. [CrossRef]
63. Treseder, K.K.; Turner, K.M. Glomalin in ecosystems. *Soil Sci. Soc. Am. J.* **2007**, *71*, 1257–1266. [CrossRef]
64. Avio, L.; Castaldini, M.; Fabiani, A.; Bedini, S.; Sbrana, C.; Turrini, A.; Giovannetti, M. Impact of nitrogen fertilization and soil tillage on arbuscular mycorrhizal fungal communities in a Mediterranean agroecosystem. *Soil Biol. Biochem.* **2013**, *67*, 285–294. [CrossRef]
65. Dorodnikov, M.; Kuzyakov, Y.; Fangmeier, A.; Wesenberg, G.B.L. C and N in soil organic matter density fractions under elevated atmospheric CO_2: Turnover vs. stabilization. *Soil Biol. Biochem.* **2011**, *43*, 579–589. [CrossRef]
66. Jha, P.; Hati, K.M.; Dalal, R.C.; Dang, Y.P.; Kopittke, P.M.; McKenna, B.A.; Menzies, N.W. Effects of 50 years of no-tillage, stubble retention, and nitrogen fertilization on soil respiration, easily extractable glomalin, and nitrogen mineralization. *Agronomy* **2022**, *12*, 151. [CrossRef]
67. Cissé, G.; Essi, M.; Kedi, B.; Mollier, A.; Staunton, S. Contrasting effects of long term phosphorus fertilization on glomalin-related soil protein (GRSP). *Eur. J. Soil Biol.* **2021**, *107*, 103363. [CrossRef]
68. Leue, M.; Gerke, H.H.; Ellerbrock, R.H. Millimetre-scale distribution of organic matter composition at intact biopore and crack surfaces. *Eur. J. Soil Sci.* **2013**, *64*, 757–769. [CrossRef]
69. Haas, C.; Gerker, H.H.; Ellerbrock, R.H.; Hallet, P.D.; Horn, R. Relating soil organic matter composition to soil water repellency for soil biopore surfaces different in history from two Bt horizons of a Haplic Luvisol. *Ecohydrology* **2018**, *11*, e1949. [CrossRef]

70. Fér, M.; Leue, M.; Kodešová, R.; Gerke, H.H.; Ellerbrock, R.H. Droplet infiltration dynamics and soil wettability related to soil organic matter of soil aggregate coatings and interiors. *J. Hydrol. Hydromech.* **2016**, *64*, 111–120. [CrossRef]
71. Adani, F.; Tambone, F. Long-term effect of sewage sludge application on soil humic acids. *Chemosphere* **2005**, *60*, 1214–1221. [CrossRef] [PubMed]
72. Demyan, M.S.; Rasche, F.; Schulz, E.; Breulmann, M.; Muller, T.; Cadisch, G. Use of specific peaks obtained by diffuse reflectance Fourier transform mid-infrared spectroscopy to study the composition of organic matter in a Haplic Chernozem. *Eur. J. Soil Sci.* **2012**, *63*, 189–199. [CrossRef]
73. Leue, M.; Hoffman, C.; Hierold, W.; Sommer, M. In-situ multi-sensor characterization of soil cores along an erosion-deposition gradient. *Catena* **2019**, *182*, 104140. [CrossRef]

4.7 The relationship of soil sulfur with glomalin-related soil protein and humic substances under different mineral and organic fertilisation

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The relationship of soil sulfur with glomalin-related soil protein and humic substances under different mineral and organic fertilisation

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Abstract: In recent years, sulfur inputs into the soil have greatly diminished due to the significant decrease in SO_2 emissions. Plant nutrients, like sulfur, can be released by the mineralisation of soil organic matter (SOM), which is a complicated mixture of substances (or fractions) like glomalin-related soil protein (GRSP) and fulvic acids (FA), humic acids (HA), humic substances (HS) and others. GRSP, FA, HA, and HS content, as well as the content of mineral and organic fractions of sulfur, was determined in different mineral and organic fertiliser treatments of the long-term field experiment. Using these results, the sulfur content in GRSP was calculated based on the soil's organic matter carbon and soil's organic bound sulfur (C_{SOM}/S_{ORG}) ratio. Sulfur content in GRSP was 4.08–5.46 (easily extractable GRSP), 9.77–15.7 (difficultly extractable GRSP), and 13.9–21.1 (total GRSP) mg S/kg of soil. Overall, the application of the organic fertiliser caused an increase in S content bound to GRSP. A strong significant relationship was also observed between GRSP fractions and soil organic sulfur. A similar relationship was also observed for the HA and HS with organic sulfur.

Keywords: luvisol; fertilization; humus fractions; organic carbon; mineral sulfur

Recently, the most common avenue of sulfur inputs into the soil has diminished. This was caused by the desulfurisation of the industry in the 80s and 90s, which reduced the sulfite oxide emissions into the atmosphere and, conversely, decreased the deposition of sulfur (S) into the soil (Lehmann et al. 2008). The European Environmental Agency reported a decrease in emissions of sulfur-containing compounds by 74% from 1990 to 2011 (EEA 2015). Sulfur is present in the soil in mineral form, generally representing around 5–10% of total sulfur (S_T) (Kulhánek et al. 2018). Water-extractable sulfur (S_{H_2O}) represents around 1% of S_T. The rest is distributed among adsorbed (S_{ADS}) and co-precipitated sulfur occluded in calcium and magnesium carbonate precipitates (Tisdale et al. 1993, McLaren and Cameron 1996) that are not available to plants yet can be released (Morche 2008). The remaining 90% to 95% of sulfur is organically bounded, which is also inaccessible to plants but can also be released throughout vegetation (Boye et al. 2010). This is why the organic-bound sulfur needs to be studied closely (Scherer 2009).

Soil organic matter (SOM) is a complicated mixture of substances. The fractionation of carbon in SOM (C_{SOM}) into the fulvic acids (FA), humic acids (HA), and humic substances (HS) categories by their solubility in acids and bases (Fan et al. 2018) is one of the possible approaches to focus the debate about SOM. Together, these can make up between 20% and 80% of C_{SOM} and can be mineralised (Parsons 1989),

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releasing nutrients, although these substances are generally considered to be a stable indicator of SOM quality (Kononova 1966). This notion is also present in more recent studies, even though its accuracy is being discussed. Although much research has been done to determine the structural composition of humic substances (HS), their complexity makes the issue problematic (Zavarzina et al. 2021).

Another part of SOM received quite a lot of attention and that is glomalin content (Liu et al. 2022). Original work by Wright et al. (1996) considered it to be a specific protein produced by the arbuscular mycorrhiza fungi (AMF). Interestingly, the exact molecular structure of pure glomalin is not yet known (Liu et al. 2022), so the wider term encompassing all "glomalin-related soil protein" (GRSP) was adopted (Rillig 2004). GRSP includes heat-stable glycoproteins, humic substances (Deng et al. 2023), and polysaccharides (Gunina and Kuzyakov 2015). Pure glomalin is also considered to be a stable fraction of SOM with degradation times up to 40 years (Harner et al. 2004). There is a consensus, that GRSP consists of two different fractions. Easily extractable GRSP (EE-GRSP) and difficultly extractable GRSP (DE-GRSP) (Deng et al. 2023), their sum being, of course, total GRSP (T-GRSP). Since the SOM contains plant nutrients and is capable of releasing them (Marschner 2012), obtaining information about the nutrient content of GRSP might prove useful.

Estimating sulfur content in GRSP and fractions of SOM is problematic at best and tricky at worst. Due to the cross-reaction of dye reagents, it is not possible to measure pure glomalin content in the extracts (Rillig 2004). The GRSP extract can, however, be purified using precipitation with strong acid and dissolving with strong hydroxide followed by dialysis (Liu et al. 2021). The content of nutrients can also be determined, for example, by x-ray diffraction (XRD) (Zhong et al. 2017).

Based on the data on sulfur, GRSP, and C_{SOM} content in our experiments and literature data available on sulfur content in the aforementioned constituents of soil $C_{SOM,}$ we will attempt to (*i*) calculate the content of S in GRSP and fractions, (*ii*) establish a relationship between S and GRSP, and C_{SOM} fractions.

MATERIAL AND METHODS

Experimental design. The experiment was conducted at the long-term stationary experiment site of the Czech University of Life Sciences in Prague, located at Červený Újezd. The field trials were initiated in 1993. The experimental site characteristics at the beginning of the trials are presented in Table 1. The trials were conducted in a complete block design. There are four blocks; each block has all fertiliser treatments arranged into individual plots. This means each treatment is replicated 4 times. The area of the plot is 170 m^2. The silage maize hybrids (*Zea mays* L.) were planted each year since the start of the experiment.

Treatments. Seven fertiliser treatments were selected, namely: (1) unfertilised control (control); (2) ammonium sulfate (AS); (3) urea and ammonium nitrate (UAN); (4) UAN + wheat straw (UAN + straw); (5) sewage sludge from municipal water treatment facility (SS); (6) cattle farmyard manure (FYM); (7) cow slurry (slurry). Annual nutrient inputs from fertilisers are described in Table 2. AS and UAN fertilisers were applied in spring before sowing. Other fertilisers were applied in the autumn before tillage. Every fertiliser was applied in a single dose. No additional amendments were added to individual treatments. All organic fertilisers were immediately incorporated into the soil by ploughing. Stubble from the previous year was incorporated into the soil.

Soil sample collection. Five topsoil subsamples (0–30 cm depth) were collected in 2019 after the biomass harvest from every plot and pooled together

Table 1. Basic characteristics of the site

GPS coordinates	50°4'22"N, 4°10'19"E
Altitude (m a.s.l.)	410
Mean annual precipitation (mm)	493
Mean annual temperature (°C)	7.7
Soil type[1]	Haplic luvisol
Soil texture[1]	Silty Loam
pH_{CaCl_2}[2]	6.5
Clay (%) (< 0.002 mm)	5.4
Silt (%) (0.002–0.05 mm)	68.1
Sand (%) (0.05–2 mm)	26.5
Bulk density topsoil (g/cm^3)	1.47
Bulk density subsoil (g/cm^3)	1.50
C_{SOM} (%)[3]	1.26
Cation exchange capacity ($mmol_+$/kg)	118

[1]NRCS USDA; [2]ISO 10390 2021 (ISO 2021); [3]CNS analyser (see materials and methods); C_{SOM} – carbon in soil organic matter

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Table 2. Fertiliser treatments and annual nutrient inputs

Treatment	N	S	P	K	C/S*
	(kg nutrient/ha/year)				
Control	0	0	0	0	–
AS	120	142	0	0	–
UAN	120	0	0	0	–
UAN + straw	120 + 33.5	2.6	4.3	45.0	823
SS	120	52.4	82.1	16.0	17.0
FYM	120	16.4	32.6	129	95.7
Slurry	120	16.3	23.2	105	36.3

Control – unfertilised control; AS – ammonium sulfate; UAN – urea ammonium nitrate solution; straw – wheat straw in average dose of 5 000 kg DM (dry matter) ha/year; SS – sewage sludge in average dose of 3 101 kg DM ha/year, 30.3% DM; FYM – farmyard manure in average dose of 5 027 kg DM ha/year, 23.7% DM; slurry – slurry in the average dose of 2 280 kg DM ha/year, 5.7% DM. *Nutrient ratios in fertilisers calculated based on C/N ratio results in Balík et al. (2020) and internal results

to get one soil sample per plot. Every sample was later air dried in a forced-air oven until constant weight at 40 °C, ground and sieved for particles < 2 mm. These samples are archived until further analysis. Part of the soil sample was also passed through a 0.4 mm sieve for the CNS analysis.

Soil sulfur fractions determination. A sequential extraction method by Morche (2008) for available readily available S (S_{H_2O}) using demineralised water, and adsorbed sulfur (S_{ADS}) using NaH_2PO_4. Extraction by HCl follows the extraction of S_{ADS} to determine carbonates-occluded S, but this determination was omitted due to the low carbonate content in the investigated soil. The sum of S_{H_2O} and S_{ADS} is the bioavailable sulfur (S_{AV}).

The pseudo-total sulfur (S_T) concentration in the soil was determined by the modified ISO: 11466 1995 (ISO 1995) method using *aqua regia* extraction (Suran et al. 2023). The organic sulfur content (S_{ORG}) was calculated as the difference between the pseudo-total content (S_T) and available (S_{AV}).

Sulfur concentrations in all digests and extracts were determined using the optical emission spectroscopy with inductively coupled plasma (ICP-OES) with axial plasma configuration, Varian, VistaPro, equipped with autosampler SPS-5 (Mulgrave, Australia). The operating measurement wavelength for ICP-OES was 180.7 nm for S.

Soil carbon fraction determination. Soil organic carbon (C_{SOM}) and total nitrogen (N_T) content in air-dried samples of soils was determined using oxidation on the CNS Analyser Elementar Vario Macro (Elementar Analysensysteme, Hanau-Frankfurt am Main, Germany).

Fractionation of humic substances was performed according to Kononova (1966) to obtain the pyrophosphate extractable fraction, which represents the sum of the carbon in humic acids and fulvic acids.

Easily extractable glomalin and total glomalin were performed according to Wright and Upadhyaya (1998). Both forms of glomalin were determined colourimetrically using bovine albumin (BSA) as a standard for quantification and the Bradford protein assay to achieve the colour change. The difficultly extractable glomalin is calculated as the difference between T-GRSP and EE-GRSP.

$$\text{DE-GRSP} = \text{T-GRSP} - \text{EE-GRSP} \qquad (1)$$

Estimating the sulfur content in glomalin and fractions of carbon. Sulfur content in GRSP was estimated for individual treatments using the following formula:

$$S_{GRSP} = (GRSP \times 0.475)/(C_{SOM}/S_{ORG}) \qquad (2)$$

where: S_{GRSP} – content of sulfur in the selected glomalin fraction; GRSP – content of a GRSP fraction (either EE-GRSP, T-GRSP, or DE-GRSP). The 0.475 value is the converted content of carbon in glomalin (47.5%, Lovelock et al. (2004)). The C_{SOM}/S_{ORG} ratio is calculated for each treatment.

Details on the content of sulfur and carbon in the experiment. In this section (Table 3) is a compilation of different studies that focused on soil sulfur (Suran et al. 2022, 2023) and soil organic carbon (Balík et al. 2022) individually. Their significance can be studied in the aforementioned papers. However, their mutual relationship was not studied. The results section will present the original findings.

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Table 3. The average content of sulfur and carbon fraction in the experiment

Treatment/ parameter	S_{H_2O}	S_{ADS}	S_{AV}	S_{ORG}	S_T	EE-GRSP	T-GRSP	DE-GRSP
	(mg/kg)							
Control	6.25	4.61	10.9	146	157	578	1 954	1 376
AS	73.6	16.2	89.7	117	207	535	2 375	1 840
UAN	7.63	5.55	13.2	141	155	584	2 131	1 547
UAN + straw	8.57	5.84	14.4	163	177	624	2 511	1 888
SS	11.9	6.10	18.0	202	220	630	2 366	1 736
FYM	11.9	6.16	18.1	236	254	724	2 817	2 093
Slurry	8.08	5.24	13.3	175	189	633	2 110	1 477
	C_{SOM}	FA	HA	HS	N_T	C_{SOM}/N_T	C_{SOM}/S_{ORG}	pH_{CaCl_2}
	(%)							
Control	0.981	0.138	0.078	0.215	0.096	10.2	67.7	6.36
AS	0.926	0.178	0.083	0.260	0.098	9.45	79.3	4.69
UAN	0.951	0.145	0.090	0.235	0.102	9.31	67.7	5.91
UAN + straw	1.16	0.173	0.140	0.313	0.117	9.93	71.8	5.88
SS	1.20	0.158	0.123	0.280	0.120	10.0	59.9	6.25
FYM	1.49	0.160	0.165	0.325	0.140	10.7	63.4	6.81
Slurry	1.12	0.133	0.110	0.243	0.111	10.1	64.0	6.70

Compilation of different studies that focused on soil sulfur (Suran et al. 2022, 2023) and soil carbon (Balík et al. 2022). n = 4. $S_{AV} = S_{H_2O} + S_{ADS}$. DE-GRSP = T-GRSP – EE-GRSP. Control – unfertilised control; AS – ammonium sulfate; UAN – urea ammonium nitrate solution; straw – wheat straw; SS – sewage sludge; FYM – farmyard manure; S_{H_2O} – water-extractable sulfur; S_{ADS} – adsorbed sulfur; S_{AV} – bioavailable sulfur; S_{ORG} – organic sulfur; S_T – total sulfur; EE-GRSP – easily extractable glomalin-related soil protein; T-GRSP – total glomalin-related soil protein; DE-GRSP – difficultly extractable glomalin-related soil protein; C_{SOM} – carbon in soil organic matter; FA – fulvic acids; HA – humic acids; HS – humic substances; N_T – total nitrogen

Statistical analysis. The results were assessed by principal component analysis (PCA) to identify the most important variables and Pearson's correlation coefficient using Statistica software ver. 12 (TIBCO, Paolo Alto, USA). To simplify the results of PCA, two principal components (PC1 and PC2) were selected as they both reached cumulative variance over 70% and an eigenvalue greater than 1.0. A correlation matrix was chosen rather than a covariance one to conduct the PCA as it is more accurate for data sets with different scales and units. Pearson's correlation coefficients were used to analyse the relationships among the variables studied. The level of significance $P < 0.05$ or smaller was considered statistically significant. The results of estimated sulfur content in glomalin were analysed using the one-way analysis of variance (ANOVA) with post-hoc Tukey's *HSD* (honestly significant difference) test to expose differences among treatments at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

The most important variables were discovered using the principal component analysis (Table 4). The first principal component (PC1) was identified as a combination of S_T, C_{SOM}, E-GRSP, and HS. The second principal component (PC2) is the combination of mineral fractions of sulfur (S_{H_2O}, S_{ADS}, and S_{AV}) as well as. The influence of C_{SOM} and N_T was discussed in Balík et al. (2022) and Suran et al. (2023); therefore, it will be omitted in this communication, and the focus will be dedicated to the relationship of GRSP and C_{SOM} fractions and their interaction with sulfur fractions.

The PCA further discovered that PC1 converges with the FYM treatment, while PC2 converges the most with the AS treatment (Figure 1). The AS treatment receives the highest dose of mineral sulfur annually, and the FYM treatment receives a significant dose of farmyard manure each year (Table 2). The

Table 4. The loading factors of variables for principal components

Variable	PC1	PC2
S_{H_2O}	0.450	**−0.842**
S_{ADS}	0.420	**−0.859**
S_{AV}	0.447	**−0.846**
S_{ORG}	**−0.931**	0.028
S_T	−0.660	−0.582
pH	−0.677	0.651
C_{SOM}	**−0.975**	−0.111
EE-GRSP	**−0.828**	0.093
T-GRSP	−0.668	−0.595
DE-GRSP	−0.515	−0.657
N_T	**−0.920**	−0.213
C_{SOM}/N_T	−0.628	0.243
C_{SOM}/S_{ORG}	−0.221	0.732
FA	−0.035	−0.762
HA	**−0.898**	−0.166
HS	−0.744	−0.504
Eigenvalue	7.34	5.23
Variability (%)	45.9	32.7
Cumulative variance (%)	45.9	78.6

PC1 and PC2 represent the first and the second principal components, respectively. Bold letters correlate with their respective principal component at a value over 0.800. *n* = 28. S_{H_2O} – water-extractable sulfur; S_{ADS} – adsorbed sulfur; S_{AV} – bioavailable sulfur; S_{ORG} – organic sulfur; S_T – total sulfur; C_{SOM} – carbon in soil organic matter; EE-GRSP – easily extractable glomalin-related soil protein; T-GRSP – total glomalin-related soil protein; DE-GRSP – difficultly extractable glomalin-related soil protein; N_T – total nitrogen; FA – fulvic acids; HA – humic acids; HS – humic substances

organic matter in FYM is generally regarded as of high quality (Förster et al. 2012). Control and UAN treatment received no S or organic matter (Table 2), which explains their divergence from PC1 and PC2. Interestingly, the slurry treatment also converges with the UAN and control, although it received S and C; however, during the application, the content of water in the slurry is over 90% water (Table 2) and is susceptible to vertical movement and fast mineralisation (Islam et al. 2021). The influence of treatments over sulfur and carbon fractions was discussed in more detail in Suran et al. (2022, 2023) and Balík et al. (2022), respectively. For further evaluation, the variables comprising PC1 and PC2 were selected. However, since the entirety of GRSP and humus fractions are of interest, we will also include T-GRSP, DE-GRSP, FA, and HA in the report.

Content of S in GRSP and relationship with C_{SOM} fractions. To estimate the content of sulfur in glomalin, we made some assumptions, as no direct measurement of S in purified glomalin has not been done yet. Lovelock et al. (2004) determined that the content of N and C in pure glomalin is, on average, 4% and 47.5%, respectively. The C/N ratio of pure glomalin is therefore 11.9. Our experiment's soil C_{SOM}/N_T ratio ranges from 9.31 to 10.7. Since the C/N ratios are similar for pure glomalin and soil, we assumed the situation to be similar for C/S. Using the C_{SOM}/S_{ORG} ratio for the soil, we calculated the sulfur content in GRSP fractions (Table 5).

The $S_{T\text{-}GRSP}$ content is greater than $S_{EE\text{-}GRSP}$ as the evaluation of T-GRSP requires repeated extraction of the same sample, while EE-GRSP is only extracted once. Some significant differences among treatments were found for $S_{EE\text{-}GRSP}$. Organic fertiliser treatments generally reach higher $S_{T\text{-}GRSP}$ values than mineral ones, but the highest and most significant statistical increase was present only for FYM treatment. Literature mentions (Koide and Peoples 2013, Wu et al. 2014) that EE-GRSP is more of a direct product of AMH fungi and is the more labile form of GRSP susceptible to faster mineralisation (Cissé et al. 2021). On the other hand, the DE-GRSP is more stable and can last in the soil before mineralisation for up to 40 years (Harner et al. 2004), which means that after over 20 years of annual organic fertiliser inputs, the content of DE-GRSP increased, and so did $S_{DE\text{-}GRSP}$. The trend in $S_{EE\text{-}GRSP}$ is very similar to that in $S_{DE\text{-}GRSP}$, suggesting that both fractions react to the application of organic fertiliser similarly. The content of GRSP fractions indeed increased according to the applied fertiliser (Table 3). The slurry is comparable to the control in the $S_{T\text{-}GRSP}$ and $S_{DE\text{-}GRSP}$ (Table 5), as the content of nutrients can mineralise faster (Islam et al. 2021) and is susceptible to vertical movement due to high water content during application.

The relationship between soil sulfur fractions and GRSP fractions is described using Pearson's correlation coefficients in Table 6. GRSP fractions seem to relate more to the S_{ORG} and S_T fractions than the mineral ones. S_T and DE-GRSP increased with respect to each other based on the treatments (Table 2). The GRSP extracts are rich in carbon molecules like glycoproteins or humic acids (Gunina and Kuzyakov 2015, Deng et al. 2023) that most likely contain or-

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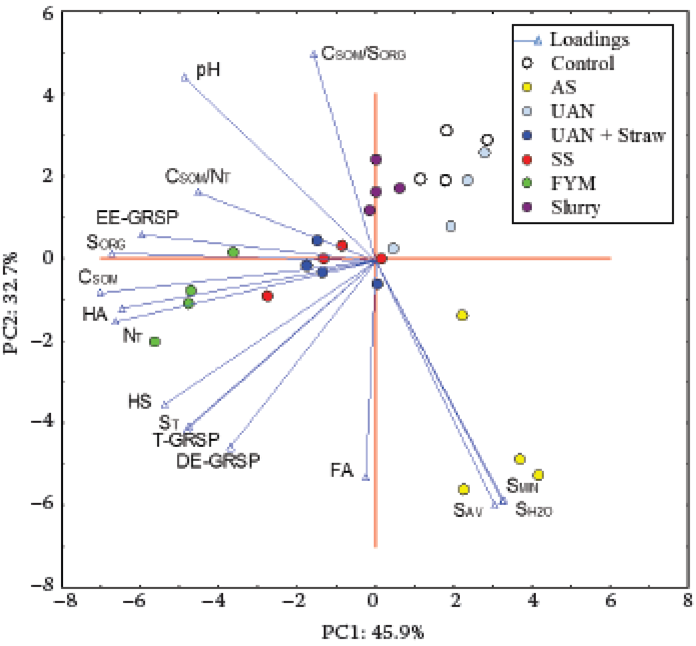

Figure 1. Loading vectors for both principal components and their relation to fertiliser treatment cases show the biplot position of PC1 *vs.* PC2. Points of different colours represent cases of different treatments. There are four cases per treatment. *n* = 28 Control – unfertilised control; AS – ammonium sulfate; UAN – urea ammonium nitrate solution; straw – wheat straw; SS – sewage sludge; FYM – farmyard manure; S_{H_2O} – water-extractable sulfur; S_{ADS} – adsorbed sulfur; S_{AV} – bioavailable sulfur; S_{ORG} – organic sulfur; S_T – total sulfur; EE-GRSP – easily extractable glomalin-related soil protein; T-GRSP – total glomalin-related soil protein; DE-GRSP – difficultly extractable glomalin-related soil protein; C_{SOM} – carbon in soil organic matter; FA – fulvic acids; HA – humic acids; HS – humic substances; N_T – total nitrogen

ganic-bound sulfur, further supporting the hypothesis that GRSP indeed contains an interesting reservoir of sulfur. On the other hand, there seems to be a weak relationship between DE-GRSP and S_{ORG}. This is most likely caused by the fact that S_{ORG} content on the AS treatment is rather low, while S_{AV} content is greatly increased (Table 3). This treatment received 142 kg S ha/year in mineral (SO_4^{2-}) form (Table 2).

A strong relationship is present for $S_{ORG,}$ HA, and HS (Table 6). Applying organic fertilisers causes a significant increase in the C_{SOM} content (Rong et al. 2016) and conversely also increases the HS content (Balík et al. 2022), as visible in Table 3. As the literature mentions, HA is, aside from other constituents, composed of sulfur (Prietzel et al. 2007, Ampong et al. 2022), and its increase in the soil also increases the content of nutrients (Li et al. 2019). Humic acids are traditionally considered to be more stable (Zavarzina et al. 2021) and could include more stable forms of S, such as S_{ORG}. Similarly, fulvic acids also contain

Table 5. The calculated sulfur content in glomalin-related soil protein (GRSP) and carbon in soil organic matter (C_{SOM}) fractions

Treatment/parameter	$S_{EE\text{-}GRSP}$	$S_{T\text{-}GRSP}$	$S_{DE\text{-}GRSP}$
		(mg S/kg soil)	
Control	4.08 ± 0.41^{ab}	13.9 ± 2.2^{a}	9.77 ± 2.0^{a}
AS	3.21 ± 0.23^{a}	14.3 ± 0.9^{a}	11.1 ± 0.8^{ab}
UAN	4.12 ± 0.37^{ab}	15.0 ± 1.4^{ab}	10.9 ± 1.1^{ab}
UAN + straw	4.15 ± 0.51^{ab}	16.7 ± 1.0^{ab}	12.5 ± 0.5^{abc}
SS	5.02 ± 0.47^{bc}	18.8 ± 1.7^{bc}	13.8 ± 1.6^{bc}
FYM	5.46 ± 0.60^{c}	21.1 ± 1.9^{c}	15.7 ± 2.0^{c}
Slurry	4.70 ± 0.39^{bc}	15.7 ± 0.4^{ab}	11.0 ± 0.6^{ab}

Different letters describe statistically significant differences using ANOVA post-hoc Tukey's *HSD* (honestly significant difference) test at $P < 0.05$. ± SD – standard deviation; control – unfertilised control; AS – ammonium sulfate; UAN – urea ammonium nitrate solution; straw – wheat straw; SS – sewage sludge; FYM – farmyard manure; $S_{EE\text{-}GRSP}$ – sulfur in easily extractable GRSP; $S_{T\text{-}GRSP}$ – sulfur in total GRSP; $S_{DE\text{-}GRSP}$ – sulfur in difficultly extractable GRSP; *n* = 4

Table 6. Pearson's correlation coefficients describe the relationship between soil sulfur and carbon fractions

Treatment/parameter	S_{H_2O}	S_{ADS}	S_{AV}	S_{ORG}	S_{ST}
EE-GRSP	–0.362	–0.339	–0.359	0.777***	0.561**
DE-GRSP	0.227	0.245	0.230	0.368	0.554**
T-GRSP	0.132	0.154	0.135	0.518**	0.644***
FA	0.490**	0.519**	0.495**	–0.071	0.283
HA	–0.272	–0.221	–0.265	0.756***	0.606***
HS	0.017	0.073	0.025	0.578**	0.628***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. $n = 28$. EE-GRSP – easily extractable glomalin-related soil protein; DE-GRSP – difficultly extractable glomalin-related soil protein; T-GRSP – total glomalin-related soil protein; FA – fulvic acids; HA – humic acids; HS – humic substances; S_{H_2O} – water-extractable sulfur; S_{ADS} – adsorbed sulfur; S_{AV} – bioavailable sulfur; S_{ORG} – organic sulfur; S_T – total sulfur

sulfur (Prietzel et al. 2007), and an increase in their content should relate to increased sulfur content in soil. This relationship was not present for S_{ORG} and FA but rather for S and FA mineral fractions. Since fulvic acids are soluble in water, some water-soluble forms of sulfur may also be co-extracted. Ammonium sulfate treatment received no organic fertiliser (Table 2) yet provided the highest FS, S_{H_2O}, S_{ADS}, and S_{AV} content even compared to the organic fertiliser treatments (Table 3).

An interesting soil sulfur pool was discovered in the glomalin-related soil protein. In general, the content of S in GRSP fractions was responsive to the fertiliser type (organic/mineral). Organic fertiliser caused an increase in the S content of GRSP fractions in soil, mainly from the treatment of farmyard manure. The relationship between soil sulfur fractions and GRSP and C_{SOM} fractions was also investigated. Significant relationships were found for S_{ORG} and S_T with GRSP and C_{SOM} fractions, as sulfur and carbon in these forms are generally considered more stable.

REFERENCES

Ampong K., Thilakaranthna M.S., Gorim L.Y. (2022): Understanding the role of humic acids on crop performance and soil health. Frontiers in Agronomy, 4: 848621.

Balík J., Kulhánek M., Černý J., Sedlář O., Suran P. (2020): Soil organic matter degradation in long-term maize cultivation and insufficient organic fertilization. Plants, 9: 1217.

Balík J., Kulhánek M., Černý J., Sedlář O., Suran P., Asrade D.A. (2022): The influence of organic and mineral fertilizers on the quality of soil organic matter and glomalin content. Agronomy, 12: 1375.

Boye K., Eriksen J., Nilsson I., Mattson L. (2010): Sulfur flow in a soil-plant system – effects of long-term treatment history and soil properties. Plant and Soil, 334: 323–334.

Cissé G., Essi M., Kedi B., Mollier A., Staunton S. (2021): Contrasting effects of long term phosphorus fertilization on glomalin related soil protein (GRSP). European Journal of Soil Biology, 107: 103363.

Deng C., Zou Y.N., Hashem A., Kuča K., Abd-Allah E.F., Wu Q.S. (2023): The visualized knowledge map and hot topic analysis of glomalin-related soil proteins in the carbon field based on Cizespace. Chemical and Biological Technologies in Agriculture, 10: 48.

EEA (2015): Sulphur Dioxide Emissions. European Environment Agency. Available at: https://www.eea.europa.eu/data-and-maps/indicators/eea-32-sulphur-dioxide-so2-emissions-1/assessment-3#tab-related-briefings (accessed on 13th December 2023)

Fan C., Song X., Chang J., Wang Y., Zhang J. (2018): Chemical compositions and copper(II) adsorption properties of sequentially extracted humic substances, including different humin fractions. Fresenius Environmental Bulletin, 27: 6485–6499.

Förster S., Welp G., Scherer H.W. (2012): Sulfur speciation in bulk soil as influenced by long-term application of mineral and organic fertilizers. Plant, Soil and Environment, 58: 316–321.

Gunina A., Kuzyakov Y. (2015): Sugars in soil and sweets for microorganisms: review of origin, content, composition and fate. Soil Biology and Biochemistry, 90: 87–100.

Harner M.J., Ramsey P.W., Rillig M.C. (2004): Protein accumulation and distribution in floodplain soils and river foam. Ecology Letters, 7: 829–836.

Islam M.R., Bilkis S., Hoque T.S., Uddin S., Jahiruddin M., Rahman M.M., Siddique A.B., Hossain M.A., Marfo T.D., Danish S., Datta R. (2021): Mineralization of farm manures and slurries under aerobic and anaerobic conditions for subsequent release of phosphorus and sulphur in soil. Sustainability, 13: 8605.

Koide R.T., Peoples M.S. (2013): Behavior of Bradford-reactive substances is consistent with predictions for glomalin. Applied Soil Ecology, 63: 8–14.

Kononova M.M. (1966): Soil Organic Matter: Nature, Properties and Methods of Study. Oxford, Pergamon Press Ltd., 45–49. ISBN: 978-0-08-011470-5

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 and Environment, 64: 455–462.

Lehmann J., Solomon D., Zhao F.J., McGrath S.P. (2008): Atmospheric SO_2 emissions since the late 1800s change organic sulfur forms in humic substance extracts of soils. Environmental Science and Technology, 42: 3550–3555.

Li Y., Fang F., Wei J., Wu X., Cui R., Li G., Then F., Tan D. (2019): Humic acid fertilizer improved soil properties and soil microbial diversity of continuous cropping peanut: a three-year experiment. Scientific Reports, 9: 12014.

Liu R.C., Gao W.Q., Srivastava A.K., Zou Y.N., Kuča K., Hashem A., Abd_Allah E.F., Wu Q.S. (2021): Differential effects of exogenous glomalin-related soil proteins on plant growth of trifoliate orange through regulating auxin changes. Frontiers in Plant Science, 12: 745402.

Liu S., Wang Q., Quian L., Zhang B., Chen X., Hong G., Shengjie W., Liu J., Yan C., Lu H. (2022): Mapping the scientific knowledge of glomalin-related soil protein with implications for carbon sequestration. Ecosystem Health and Sustainability, 8: 2085185.

Lovelock C., Wright S.F., Clark D.A., Ruess R.W. (2004): Soil stocks of glomalin produced by arbuscular mycorrhizal fungi across a tropical rain forest landscape. Journal of Ecology, 92: 278–287.

Marschner P. (2012): Marschner's Mineral Nutrition of Higher Plants. 3rd Edition. London, Elsevier, 672. ISBN: 978-0-12-384905-2

McLaren R.G., Cameron K.C. (1996): Soil, plant and fertilizer sulphur. In: McLaren R.G., Cameron K.C. (eds.): Soil Science. Auckland, Oxford University Press, 221–228. ISBN: 9780195583458

Morche L.S. (2008): S-fluxes and spatial alterations of inorganic and organic sulfur fractions in soil as well as their accumulation and depletion in the rhizosphere of agricultural crops by partial use of the radioisotope ^{35}S. [Ph.D. Thesis]. Bonn, Rheinische Friedrich-Wilhelms-Universität. (In German)

Parson J.W. (1989): Hydrolytic degradation of humic substances. In: Hayes M.H.B., McCarty P., Malcolm R.L., Swift R.S. (eds.): Humic Substances. II: In Search of Structure. New York, John Wiley and Sons, 99–120. ISBN 10: 047192279X

Prietzel J., Zhieme J., Salomé M., Knocker K. (2007): Sulfur K-edge XANES spectroscopy reveals differences in sulfur speciation of bulk soils, humic acid, fulvic acid, and particle size separates. Soil Biology and Biochemistry, 39: 877–890.

Rillig M. (2004): Arbuscular mycorrhizae, glomalin, and soil aggregation. Canadian Journal of Soil Science, 84: 355–363.

Rong Y., Su Y., Wang T., Yang Q. (2016): Effect of chemical and organic fertilization on soil carbon and nitrogen accumulation in a newly cultivated farmland. Journal of Integrative Agriculture, 15: 658–666.

Scherer W.H. (2009): Sulfur in soils. Journal of Plant Nutrition and Soil Science, 172: 326–335.

Suran P., Balík J., Kulhánek M., Sedlář O., Černý J. (2023): Influence of long-term organic fertilizers on changes in the content of various forms of sulfur in the soil under maize monoculture. Agronomy, 13: 957.

Suran P., Kulhánek M., Balík J., Černý J., Sedlář O. (2022): Evaluation of soil S pools under the 23 years of maize monoculture. Agronomy, 11: 2376.

Tisdale S.L., Nelson W.L., Beaton J.D., Havlin J.L. (1993): Soil Fertility and Fertilizers. 5th Edition. New York, Macmillan Publishing Company, 634. ISBN-10: 0024208353

Wright S.F., Franke-Snyder M., Mortin J.B., Upadhyaya A. (1996): Time-course study and partial characterization on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. Plant and Soil, 181: 193–203.

Wright S.F., Upadhyaya A. (1998): A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant and Soil, 198: 97–107.

Wu Q.S., Cao M.Q., Zou Y.N., He X.H. (2014): Direct and indirect effects of glomalin, mycorrhizal hyphae, and roots on aggregate stability in rhizosphere of trifoliate orange. Scientific Reports, 4: 5823.

Zavarzina A.F., Danchenko N.N., Demon V.V., Artemyeva Z.S., Kogut B.M. (2021): Humic substances: hypothesis and reality (a review). Soil Chemistry, 54: 1826–1854.

Zhong Z., Wang W., Wang Q., Wu Y., Wang W., Pei Z. (2017): Glomalin amount and compositional variation, and their associations with soil properties in farmland, northeastern China. Journal of Plant Nutrition and Soil Science, 180: 563–575.

NRCS USDA (1999): Soil Taxonomy. United States Department of Agriculture-Natural Resources Conservation Service. Available at: https://www.nrcs.usda.gov/sites/default/files/2022-06/Soil%20Taxonomy.pdf (accessed on 16th December 2023)

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5 Souhrnná diskuse

V následující kapitole budou diskutovány výsledky jednotlivých článků. Diskuse je rozdělena do podkapitol tak, aby odpovídala cílům práce a hypotézám. Kapitola 5.1 tedy popisuje určení rostlinám přístupné síry a bilanci síry v půdě. Kapitola 5.2 uvádí vhodnost nových metod k posuzovaní změn kvality půdní organické hmoty. Kapitola 5.3 popisuje vztah vybraných indikátorů kvality půdní organické hmoty a frakcí síry v půdě.

V publikovaných článcích (kapitola 4) jsou používány zkratky pro popis variant hnojení, popisu frakcí síry a uhlíku v půdě. Ve dvou publikovaných článcích ovšem může být stejný faktor popsán jinými zkratkami. Důvodem byl rozpor v literatuře použité pro psaní článků, vlastní zvyklost, nebo také připomínky oponentů. Například v publikaci Balík et al. (2022) se jednomu z oponentů příčilo použití zkratky SS120 pro popis varianty čistírenského kalu obsahující každoroční dávku 120 kg N.ha^{-1}.rok. Doporučil tedy použití jiné zkratky, a tak bylo použito označení S120 i přes to, že standardně používáme SS (sewage sludge) pro čistírenské kaly. Obdobně použití FYM a F pro popis chlévského hnoje ve dvou různých článcích.

V souhrnné diskuzi této práce je v následujících kapitolách každá zkratka uvedena znovu při prvním použití. Byla snaha, aby zkratky připomínaly co možná nejvíce ty, které jsou v publikovaných článcích.

5.1 Hodnocení obsahů minerálních a organických frakcí síry a bilance vstupů a výstupů síry

V této části diskuse jsou vyhodnoceny změny obsahu síry v půdě a bilance celkového obsahu síry za 23 let experimentu (1997 až 2019). Experiment byl proveden na dlouhodobém pokusu s monokulturou kukuřice a různými systémy hnojení na Červeném Újezdě. Hodnocené frakce síry jsou: síra rozpustná ve vodě (S_{H2O}), sorbovaná (S_{ADS}), rostlinám přístupná (S_{AV}), uvolněná metodou Mehlich 3 (S_{M3}), organicky vázaná (S_{ORG}) a celková (S_T). Hodnocené varianty hnojiv jsou: síran amonný (AS), DAM - dusičnan amonný s močovinou (UAN), UAN s trojitým superfosfátem a draselnou solí (UAN + PK), UAN s trojitým superfosfátem a kieseritem (UAN + PMgS), UAN a sláma (UAN + St), čistírenský kal (SS), chlévský hnůj (FYM), kejda skotu (Slurry). Jako srovnávací varianta je také v pokusu nehnojená kontrola. Pro bilance síry v půdě byla také využita varianta s úhorem. Na této variantě nejsou dodávána žádná hnojiva, ale na rozdíl od kontroly na této variantě není pěstována kukuřice.

5.1.1 Vliv hnojení sírou na výnosy a obsah síry v rostlinách

Průměrné výnosy kukuřice na siláž za celou dobu pokusu byly u všech minerálně hnojených variant statisticky srovnatelné. Výnosy u organicky hnojených variant byly také statisticky srovnatelné. Výnosy všech hnojených variant (12,1 až 13,5 t sušiny.ha^{-1}.rok^{-1}) jsou významně vyšší, než výnosy nehnojené kontroly (9,04 t. sušiny ha^{-1}.rok^{-1}). Hnojené varianty obdržely uniformní dávku N hnojiva (120 kg N.ha^{-1}rok^{-1}, UAN + St navíc dalších 33,5 kg N ve slámě). Na variantách AS, UAN + PMgS, UAN + St, Slurry, FYM a SS byla síra aplikována v hnojivu. Aplikace síry neovlivňovala výnosy biomasy kukuřice.

Vliv hnojení sírou byl prokázán na odběr síry z půdy rostlinami. Kontrola dosahovala hodnot odběrů 3,38 kg S.ha^{-1}.rok^{-1}. Všechny minerálně hnojené varianty měly významně zvýšený odběr, a to zejména AS a UAN + PMgS (9,84 a 8,31 kg S.ha^{-1}.rok^{-1}). Tyto varianty mají každoroční vstup síry z hnojiv v hodnotách 142 a 84 kg S.ha^{-1}.rok^{-1}. Na organické variantě SS byl odběr síry 7,47 kg S.ha^{-1}.rok^{-1}, což odpovídá tomu, že tato varianta dostala relativně vysokou dávku síry v hnojivu (52,4 kg S.ha^{-1}.rok-^{1}). Godlewska (2018) také uvádí významný nárůst odběru síry rostlinami kukuřice po aplikaci čistírenského kalu. Statisticky významný nárůst odběru proti kontrole zaznamenaly také varianty FYM a Slurry (5,88 a 5,71 kg S.ha^{-1}.rok^{-1}) při dávkách síry v hodnotách 16,3 a 16,4 kg S.ha^{-1}.rok^{-1}. Knights et al. (2000) také stanovili významné zvýšení odběru síry rostlinami po aplikaci FYM (odebráno 11 kg $S.ha^{-1}.rok^{-1}$) oproti kontrole (2,0 kg $S.ha^{-1}.rok^{-1}$). Velice podobné výsledky popisují také Sakal et al. (2000), kde byl zaznamenán významný nárůst odběru síry rostlinami (9,44 kg $S.ha^{-1}$) v porovnání s kontrolou (2,58 kg $S.ha^{-1}$), a to již při dávce hnojiva 40 kg $S.ha^{-1}$. V našem pokusu jsou statisticky srovnatelné odběry variant UAN, UAN + PK a UAN + St s variantami FYM a Slurry. Přitom FYM a Slurry mají vyšší vstup síry v hnojivu (16,3 a 16,4 kg $S.ha^{-1}.rok^{-1}$). Varianty UAN a UAN + PK v hnojivu síru neobsahují. Na variantě UAN + St bylo dodáno každoročně pouze 2,61 kg $S.ha^{-1}$. Je zřejmé, že u variant UAN, UAN + PK, UAN + St, FYM a Slurry jsme zaznamenali významné zvýšení odběru síry oproti kontrole v důsledku intenzivního hnojení dusíkem. Hnojení N vede ke zvýšení výnosů (Weil et Mughogho 2000, Pasley et al. 2012, Sakal et al. 2000). Vyšší výnosy vedou ke zvýšenému příjmu všech živin (Pasley et al. 2012), včetně síry (Prietzel et al. 2001). Tento závěr potvrdily také studie Weil et Mughogho (2000) a Kngihts et al. (2000).

V případě variant SS, UAN + PMgS a AS se tedy jedná o společný vliv dávky N, jako u ostatních hnojených variant a také relativně vysoké dávky síry dané hnojením. Na žádné z variant neklesl v průběhu vegetace obsah S_{AV} pod 10 $mg.kg^{-1}$, což je podle Grobler et al. (1999) hranice, pod kterou kukuřice již není schopná naplnit svůj výnosový potenciál.

5.1.2 Korelační analýza obsahu síry v rostlině s jejími frakcemi v půdě

Jedná se o souhrn dvou studií, kde byl pozorován vliv aplikovaných hnojiv na obsah síry v půdě a rostlině. První studie je zaměřená na vliv organických hnojiv (Suran et al. 2023), druhá na vliv minerálních hnojiv (Suran et al. 2021). Korelační koeficienty tedy byly vypočteny samostatně pro minerální a organická hnojiva.

Korelační analýza obsahů frakcí síry v půdě s obsahem síry v rostlině poskytuje zajímavé výsledky. V pokusu s minerálně hnojenými variantami (Suran et al. 2021) jsou korelace obsahu síry v rostlině s obsahem S_{H2O} a S_{ADS} významné ($r^2 > 0,6$; $p < 0,01$). V pokusu s organicky hnojenými variantami (Suran et al. 2023) jsou zmíněné korelační koeficienty podobné, ale méně průkazné ($r^2 > 0,6$; $p < 0,05$ pro S_{H2O}; pro S_{ADS} nevýznamný). Na druhou stranu u obou pokusů je obsah síry v rostlině velmi dobře korelován s obsahem S_{AV} (součet S_{H2O} a S_{ADS}) ($r^2 > 0,7$; $p < 0,05$). Dále byla pozorována významná korelace síry v rostlině s S_{ORG} ($r^2 = 0,564$; $p < 0,05$). Literatura uvádí, že právě frakce S_{H2O}, S_{ADS} a S_{AV} jsou pro výživu rostlin sírou nejvýznamnější (Boye et al. 2010 a Morche 2008). Zároveň tito autoři zdůrazňují význam frakce S_{ORG}, která se běžně považuje za nepřístupnou rostlinám, ale zpřístupnitelnou mineralizací organické hmoty v průběhu vegetace.

Obsah síry v rostlině koreluje s obsahem S_{M3} v půdě zpravidla nižším koeficientem, ale na stejných hladinách významnosti, v pokusech s minerálními a organickými hnojivy ($r^2 > 0,5$; $p < 0,05$). Toto extrakční činidlo obsahuje HNO_3, a je tedy pravděpodobné, že uvolní z půdního vzorku také část síry, která je rostlinám nepřístupná. Při porovnání obsahu síry v rostlinách s obsahem S_{H2O} a S_{M3}, z širokého souboru půd s kukuřicí v monokultuře a z rotace plodin, zjistili Sedlář et al. (2021), že obsah síry v rostlině nekoreluje významně s obsahem S_{H2O}, ale koreluje významně s obsahem S_{M3} (0,629; $p < 0,05$). Tyto korelace ovšem pocházejí ze vzorků odebraných v BBCH 16-18, zatímco naše studie porovnávala BBCH 75. Obsah S_{H2O} je v průběhu sezóny velice proměnlivý (Blake-Kalff et al. 2000). Dále fakt, že vzorky v publikaci Sedlář et al. (2021) byly odebrány z půd napříč celou Českou republikou, způsobuje další nárůst variability hodnot.

Metodou extrakce Mehlich 3 bylo z ornice uvolněno velmi podobné množství síry, jako obsah S_{AV}. Analýza rozptylu neukázala významné rozdíly mezi těmito metodami (Tukey´s HSD test, $p < 0,05$). Korelační koeficient těchto dvou frakcí také dosáhl významných hodnot ($r^2 > 0,9$; $p < 0,001$) v pokusu s minerálními hnojivy. Kulhánek et al. (2018) uvádějí, že obsah S_{AV} koreluje s S_{M3} ($r^2 = 0,882$, $p < 0,001$). Jedná se při tom o širší studii z různých půdních typů, systémů hnojení a rotací plodin či monokultur. Obdobné výsledky byly popsány také mimo Českou republiku (Rogers et al. 2019 a Kowalenko et al. 2014).

5.1.3 Vývoj frakcí síry v ornici v průběhu experimentu

Obecně lze konstatovat, že na všech systémech hnojení, kromě AS, došlo k významnému snížení obsahů všech minerálních frakcí síry v porovnání s obsahy při založení pokusu (rok 2019 porovnán s rokem 1997).

V průběhu pokusu došlo ke snížení obsahu S_{H2O} na 23 – 45 % původních hodnot z roku 1997 (na všech variantách). Obsah S_{H2O} je labilní a náchylný ke změnám (Morche 2008), je nejvíce ovlivněn odběrem rostlinami (Förster et al. 2012 a Knights et al. 2000), obsahem přijatelné síry (Balík et al. 2009 a Kulhánek et al. 2016), aplikovaným množstvím síry (Scherer 2009) a především snížením vstupů minerální síry z atmosféry (Lehmann et al. 2008, Yang et al. 2007, Zbíral et al. 2018). Obdobně také u frakcí S_{ADS} a S_{AV} byl zaznamenán pokles na 58 – 74 a 31 - 50 % původních hodnot. Došlo také ke snížení obsahu S_T na 73 –

94% z původních hodnot. Varianta AS je zajímavá opačným trendem. Nárůst hodnot S_{ADS} této varianty se dá vysvětlit sníženou hodnotou pH, která vede ke zvýšení sorpce síranového aniontu (Prietzel et al. 2001). Snížení pH na této variantě je reálným jevem (Suran et al. 2024). To ovšem nevysvětluje nárůst S_{H2O}. Kombinace vysoké dávky síry (142 kg $S.ha^{-1}.rok^{-1}$), se sušším obdobím po aplikaci AS, mohla vést k akumulaci minerálních frakcí S. Zvýšené hodnoty minerální frakce se pak projevily pozitivně na obsahu S_T. Hodnoty z našeho pokusu odpovídají širšímu trendu napříč různými půdami České republiky. Ve studii Balík et al. (2009) bylo zaznamenáno snížení obsahů S_{H2O}, S_{ADS}, S_{AV} a S_T mezi roky 1981 a 2008 (z původních obsahů na hodnoty 35, 61, 50 a 92 %). Dále Kulhánek et al. (2014) pozorovali obdobné změny obsahů frakcí síry (mezi roky 2014 a 1996) v rotaci plodin a různými typy hnojiv.

Na úhoru (bez produkce rostlin a bez aplikace hnojiv) jsou vidět zajímavé výsledky. Tato varianta má významný pokles v obsahu všech frakcí síry mezi lety 1997 a 2008. Následně v roce 2019 již není na této variantě pozorován tak významný pokles (nebo není pokles žádný). Je možné, že koncem devadesátých let stále docházelo k vyplavování síry z nahromaděných depozic. Vlivem snížení emisí SO_2 (CHMI 2020) ovšem nedochází k významnějším vstupům síry z atmosféry. Vyplavená síra pravděpodobně není nahrazena. V metodice publikací Suran et al. (2023) a Suran et al. (2021) je dokumentováno postupné snížení atmosférických depozic síry v našich pokusech. Na úhoru se mezi lety 2008 až 2019 vytvořila určitá rovnováha. Na kontrolní variantě (bez hnojiv ale s produkcí kukuřice) je zaznamenáno snížení obsahů síry také po roce 2008. To je dáno odběrem síry rostlinami. Obdobně Gourav et al. (2018) popisují vyšší hodnoty S_{H2O} a S_T na variantě s úhorem než na variantě kontrolní po 48 letech pěstování kukuřice na siláž v monokultuře. Na 150letém polním pokusu v Rothamstedu byl popsán vliv hnojení minerální sírou v dávkách 52 – 220 kg $S.ha^{-1}.rok^{-1}$. Bylo zjištěno, že žádná dávka hnojiva neměla vliv na zvýšení obsahu minerálního, nebo organického podílu síry v ornici (Knights et al. 2000).

Jeden z hlavních efektů aplikace organických hnojiv je udržení/zvýšení zásoby půdní organické hmoty. Balík et al. (2020) popsali změny obsahu půdního organického uhlíku (C_{SOM}) na tomto pokusu. V roce 1993 na začátku pokusu byl obsah C_{SOM} 1,26 %. V roce 2018 byl obsah C_{SOM} na kontrole, AS, UAN, UAN + St a FYM v hodnotách 0,98, 0,93, 0,95, 1,16 a 1,49 %. Pokles byl způsoben tím, že kukuřice na siláž na pozemku po sklizni zanechává velmi malé množství rostlinných zbytků (jen kořeny a strniště – více v kapitole 5.2).

Množství a kvalita organické hmoty použitého hnojiva mají samozřejmě vliv na obsahy S_{ORG} a S_T v ornici. Obsahy S_T a S_{ORG} byly vyšší na variantě FYM, než na variantě SS i přes to, že dávka síry ve variantě SS je vyšší. Chlévský hnůj má typicky vyšší poměr C/S (95,7), což mu propůjčuje výraznější stabilitu a obecně organická hmota s vyšším poměrem C/S podporuje imobilizaci síry (Förster et al. 2012). Dále aplikace FYM vedla k nárůstu obsahu C_{SOM} (Balík et al. 2020), a společně s tím také k nárůstu obsahu S_{ORG} (Suran et al. 2023). Varianta s kejdou skotu obdržela téměř stejné množství síry jako FYM a bylo na ní pozorováno snížení obsahů S_T a S_{ORG}. Kejda skotu má typicky nižší poměr C/S (Islam et al. 2021) (36,3 v našem pokusu) a je náchylnější k mineralizaci v porovnání s jinými organickými hnojivy (Barlóg et al. 2020). Nejnižší poměr C/S byl pozorován v čistírenských kalech (17,0). Takto malý poměr vede ke zvýšení mineralizace síry (Zhang et al. 2021). Podle Dewill et al. (2008) a Sommers et al. (1977) je významná část síry v čistírenských kalech také v podobě minerální, a je tedy velmi mobilní. Obecně čistírenské kaly obsahují velký podíl rychle mobilizovatelné síry ve formě síranů a sulfidů kovů (Dewil et al. 2008). V našem pokusu byl nejvyšší poměr C/S na variantě UAN + St (přes 700), což by vedlo ke zvýšené imobilizaci S (Zhang et al. 2021). Z výsledků naší studie (Suran et al. 2023) je zřejmé, že na této variantě dochází ke klesajícímu trendu obsahu S_{ORG} v ornici. Nízká dávka síry na této variantě má pravděpodobně významnější vliv než vysoký poměr C/S. Na této variantě také dochází k poklesu hodnot obsahu C_{SOM} (Balík et al. 2020). To logicky vede ke snížení obsahu organicky vázané síry. Síra se v orné půdě vyskytuje z více jak 80 % v organické formě (Suran et al. 2023).

Förster et al. (2012) studovali zásoby síry v půdě v dlouhodobém experimentu založeném v roce 1962 při rotací plodin a aplikaci různých hnojiv. Popsali nárůst frakce S_T po aplikaci FYM a SS. V našem pokusu se hodnoty S_T na těchto variantách významně nemění. Dále je z naší studie minerálně hnojených variant zřejmé, že frakce S_{AV} ztratila v průběhu experimentu 16 až 24 mg $S.kg^{-1}$, zatímco obsah S_T byl snížen o 24 až 59 mg $S.kg^{-1}$, s výjimkou varianty AS (Suran et al. 2021). To vede k závěru, že část „ztracené“ síry z S_T musela být organického původu. Na orných půdách, kde nedochází k akumulaci C_{SOM}, je jen málo možností pro imobilizaci aniontu SO_4^{2-} (Rogers et al. 2019).

Zároveň byl pozorován nárůst zastoupení S_{ORG} v obsahu S_T na všech variantách ve studii s organickými hnojivy (Suran et al. 2023). Jedná se o důsledek snížení emisí SO_2 (Zbíral et al. 2018). Snížení atmosférických depozicí síry, z hodnot v řádu stovek kg $S.ha^{-1}.rok^{-1}$ v osmdesátých letech na pouhé jednotky (Krupová et al. 2018), vedlo ke snížení obsahu minerální síry v půdě (organicky vázaná síra tedy jeví relativní nárůst zastoupení v S_T). Tento trend je reflektován napříč celou Evropou (Förster et al. 2012 a Knights et al. 2000).

5.1.4 Bilance celkového obsahu síry v experimentu

Ze vstupů (původní stav S_T v půdě, hnojiva, depozice) a výstupů (odběr rostlinami, konečný stav S_T v půdě) lze kalkulovat, zda v půdě dochází ke kumulaci, nebo ztrátám síry. Z celého půdního profilu ornice a podorničí (0 – 60 cm) je patrné, že všechny varianty zaznamenaly ztráty celkového obsahu síry. U minerálně hnojených variant a kontroly je to vysvětlitelné snížením atmosférických depozic, které byly zdrojem minerální síry v půdě (Zbíral et al. 2018). Ztráty celkového obsahu síry byly ovšem zaznamenány také na organicky hnojených variantách. Obdobný jev byl pozorován v pokusu Riley et al. (2002), kde autoři sdělují, že část síry uvolněné do půdního roztoku a vymyté do spodních vod je také z organicky vázaného podílu. Autoři dále uvádějí, že každoroční ztráty vymýváním jsou vyšší než vstupy hnojivy. Při každoročních vstupech hnojivy na variantách SS, FYM, Slurry, UAN + St, AS a UAN + PMgS v dávkách 54,2, 16,4, 16,3, 2,61, 142 a 58 kg S.ha^{-1} byly pozorovány každoroční ztráty v hodnotách 66,1, 21,4, 24,8, 8,09, 142,2 a 95,3 kg S.ha^{-1}. Je evidentní, že na všech variantách byly zaznamenány větší ztráty síry, než jejich přísun hnojivy. Celkově vysoké ztráty síry na variantách AS, UAN + PMgS a SS (3 271, 2 193 a 1 521 kg S.ha^{-1} za celých 23 let experimentu) jsou dány vysokou jednorázovou dávkou síry. Vyplavená síra může kontaminovat spodní vody (Sharma et al. 2020).

Jako opatření proti vyplavování síry se v literatuře uvádějí aplikace hnojiv se sírou v méně rozpustných formách, jako je třeba elementární síra, nebo aplikace v rámci organické hmoty (Riley et al. 2002 a Santoso et al. 1995), případně rozděleni celkové dávky do několika menších. Na druhou stranu ovšem hnojiva s pomalým uvolňováním S vedou ke snížení odběru síry rostlinami, a to zejména pokud je toto hnojivo aplikováno ve více dávkách (Santoso et al. 1995).

5.2 Prověření nových metod ve vztahu k jejich schopnosti indikovat kvalitu půdní organické hmoty

V této části je diskutován vztah indikátorů kvality půdní organické hmoty. Jedná se o výsledky dlouhodobého polního pokusu s monokulturou kukuřice na Červeném Újezdu (luvisol) (Balík et al. 2022a) na variantách hnojení UAN, SS a kejdou skotu. Ještě bude z tohoto pokusu zmíněn vývoj obsahu půdního organického uhlíku ze studie Balík et al. (2020) na variantě FYM. Dále jsou zde uvedeny výsledky ze stanoviště Suchdol (černozem) s monokulturou kukuřice hnojené dvěma různými dávkami minerálního N (N120 a N240) a SS (SS120 a SS240) (Balík et al. 2022b). V závěru jsou prezentovány výsledky z dlouhodobých pokusů ÚKZÚZ (luvisol) s rotací plodin hnojené FYM a stupňovanou dávkou NPK s FYM (FYM + NPK1; NPK2; NPK3) (Balík et al. 2023). Srovnávací varianta byla u všech pokusů nehnojená kontrola.

5.2.1 Obsah půdního organického uhlíku

Základní informací jsou změny v obsahu organického uhlíku v půdě (C_{SOM}). Byl pozorován rozsah hodnot obsahu C_{SOM} 0,93 až 1,49 % v monokultuře kukuřice na luvisolu (Balík et al. 2020 a Balík et al. 2022a). V pokusu s rotací plodin na luvisolu byl rozsah C_{SOM} pro varianty různých hnojiv 1,12 až 1,28 % (Balík et al. 2023). Obsah půdního organického uhlíku byl u obou pokusů významně ovlivněn použitými hnojivy.

V monokultuře vedla aplikace FYM ke zvýšení obsahu C_{SOM} (z 1,26 % na 1,46 %). Pozitivní vliv aplikace FYM je také uveden ve studii Menšík et al. (2018). Dále také Schmidt et al. (2000) pozorovali zvýšení obsahu C_{SOM} po aplikaci FYM (z 1,24 % na 1,64 %). V našich pokusech s rotací plodin byl na všech hnojených variantách (FYM a FYM + NPK) zaznamenán statisticky významný nárůst (obsah C_{SOM} 1,21 až 1,28 %) v porovnání s kontrolou (1,12 %) (Balík et al. 2023). Významný rozdíl byl také pozorován mezi variantou hnojenou pouze FYM (1,21 %) a variantou s FYM + NPK3 (1,28 %). Tento trend se velmi dobře shoduje se studií Mustafa et al. (2022).

Zároveň bylo prokázáno nevýznamné snížení obsahu C_{SOM} (o 8 %) na variantě UAN + St (dávka 5 tun sušiny slámy.ha^{-1}.rok^{-}) (Balík et al. 2020). Z hodnot této varianty je evidentní, že samotná aplikace slámy obilovin nemůže pozitivně ovlivnit obsah půdní organické hmoty. Běžně se uvádí, že jedním z určujících faktorů rychlosti mineralizace hnojiva je jeho poměr C/N (van der Sloot et al. 2022). Větší hodnota tohoto poměru by měla vést k nižší mineralizaci organické hmoty hnojiva (Abbsi et al. 2015). V monokultuře je poměr C/N ve slámě na variantě UAN + St roven 79,3. Při kombinaci s minerálním N na této variantě je výsledný poměr C/N roven 14,6, což ještě více podpoří mineralizaci slámy. Proto je sláma v našich pokusech velmi rychle mineralizována a její aplikace nevedla k nárůstu obsahu C_{SOM}. To je ve shodě s literaturou (Christensen 1985, Wang et al. 2017, Guan et al. 2020, Li et al. 2020a).

Významné snížení obsahu C_{SOM} je pozorovatelné na variantách UAN a kontrola v porovnání s hodnotou při založení pokusu (Balík et al. 2020). Snížení obsahu C_{SOM} pod monokulturou kukuřice bylo dokumentován také v publikacích Ludwig et al. (2003), Loges et al. (2018) a Bettina et al. (2003). Vyskytují se také studie, ve kterých je pozorován nárůst C_{SOM} v monokultuře kukuřice (Collins et al. 1999, Liang et MacKenzie 1992 a Rasse et al. 2006). V těchto studiích je ovšem kukuřice pěstována na zrno. Například Liang et MacKenzie (1992) popisují zvýšení obsahu C_{SOM} v ornici o 18 % po šestileté produkci kukuřice na zrno. V těchto pokusech ale zůstává většina nadzemní biomasy (kromě zrna) po sklizni na pozemku. V zásadě je problém produkce kukuřice na siláž v tom, že dochází k výraznému snížení zanechaných posklizňových zbytků, které by pozitivně ovlivnily půdní organickou hmotu, a to se právě týká naší studie.

Velmi specifickým je ovšem stanoviště Suchdol na černozemi (Balík et al. 2022b). V tomto pokusu je kontrola porovnána s dvěma dávkami minerálního N (N120 a N240) a dvěma dávkami čistírenského kalu (SS120 a SS240), dodávající vždy 120 a 240 kg N.ha^{-1}.rok^{-1}. V této studii byl obsah C_{SOM} v rozsahu 1,61 až 1,84 %. U varianty s vysokou dávkou čistírenského kalu (SS240) je zřetelná tendence k nárůstu obsahu C_{SOM}.

5.2.2 Glomalin-related soil protein

Glomalin-related soil protein (GRSP) existuje ve dvou formách, forma lehce extrahovatelná (EE-GRSP) a celková (T-GRSP). Z jejich rozdílu se dá stanovit obtížně extrahovatelný GRSP (DE-GRSP). Forma EE-GRSP se z půdního vzorku uvolňuje jednou extrakcí v citronanu sodném, T-GRSP využívá opakované extrakce stejného vzorku silnějším roztokem citronanu sodného. EE-GRSP má výrazně větší rozpustnost ve vodě, než T-GRSP (Wright et Upadhyaya 1998).

Na pokusech s monokulturou kukuřice na luvisolu byly průměrné obsahy EE-GRSP a T-GRSP 616 a 2 140 mg.kg^{-1} půdy (Balík et al. 2022a). Na pokusech ÚKZÚZ s rotací plodin na luvisolu jsou průměrné obsahy EE-GRSP a T-GRSP 749 a 2 689 mg.kg^{-1} (Balík et al. 2023). V pokusu na černozemi s monokulturou kukuřice byl obsah EE-GRSP a T-GRSP v průměru 991 a 2 080 mg.kg^{-1}. Černozem vykazuje vyšší podíl EE-GRSP v T-GRSP oproti luvisolům. Na černozemi je tento podíl 48 % a na luvisolech pod 30 %. Lze konstatovat, že námi stanovené obsahy GRSP odpovídají hodnotám uváděným v řadě literárních zdrojů. V zemědělsky využívaných půdách se obsahy EE-GRSP pohybují řádově ve stovkách mg.kg^{-1} (Dai et al., 2013, Turgay et al. 2015, Sandeep et al. 2016), ovšem na velmi úrodných půdách, jako jsou mollisoly, mohou obsahy EE-GRSP dosahovat také přes 3000 mg.kg^{-1} (Curaqueo et al. 2011). T-GRSP dosahuje výrazně vyšších hodnot, běžně v intervalech od 750 do 2500 mg.kg^{-1} (Dai et al., 2013, Turgay et al. 2015, Sandeep et al. 2016). Na orných půdách ČR se obsahy T-GRSP pohybují v průměru v rozmezí 1 900 až 4 200 mg.kg^{-1} půdy (Zbíral et al. 2017). Na velmi úrodných půdách dochází k nárůstu hodnot až k 9 000 mg.kg^{-1} půdy (Curaqueo et al. 2011).

5.2.2.1 Vliv monokultury a rotace plodin na obsah GRSP

V následujících odstavcích budou často porovnávány výsledky z rotace plodin s kukuřicí a monokulturou kukuřice na siláž. V našem výzkumu původně nebylo cílem porovnávat vliv rotace a monokultury. Ovšem to, že byly publikovány práce jak z monokultury, tak z rotace plodin na stejném půdním typu - luvisol - nám toto srovnání umožňuje. Studie na černozemi bude do diskuse také zahrnuta, ale přirozená kvalita půdní organické hmoty a úrodnost tohoto půdního typu produkuje výjimečné výsledky.

Wojewódzki et Cieścińska (2012) uvádějí, že obsah T-GRSP byl významně vyšší v rotaci plodin (nárůst o 50 %), než v monokultuře žita nebo brambor (v průměru 1 724 mg.kg^{-1}). V našich pracích na luvisolu je obsah GRSP vyšší v rotaci plodin než na monokultuře (nárůst o 21,6 a 25,7 % pro EE-GRSP a T-GRSP).

Wright et Upadhyaya (1998) a Holátko et al. (2021) uvádějí, že míra kolonizace a aktivita arbuskulární mikorhizy (AM) jsou klíčové pro nárůst obsahu GRSP. Podle Gosling et al. (2006) má větší diverzita rostlin v rotaci plodin pozitivní vliv na přítomnost a aktivitu AM. Bedini et al. (2007) uvádějí menší míru zastoupení AM v monokultuře kukuřice v porovnání s trvalým travním porostem. Vyšší množství zanechaných posklizňových zbytků vede také k větší kolonizaci AM (Borie et al. 2006, Roldán et al. 2007). Na druhou stranu v monokultuře kukuřice na siláž je velká část sklizené biomasy odstraněna a dochází ke snížení hmoty posklizňových zbytků (Balík et al. 2020). Obsahy obou frakcí GRSP byly na monokultuře významně vyšší při použití čistírenských kalů a kejdy skotu (Balík et al. 2022a). Je tedy evidentní, že na luvisolu bylo v monokultuře významným pozitivním jevem organické hnojení, neboť na tomto pokusu dochází k odsunu velkého množství biomasy (Balík et al. 2020) z pozemku, a to vede k nižší kolonizaci AM na variantách bez organického hnojiva (Borie et al. 2006, Roldán et al. 2007). Na druhou stranu byl na hnojených variantách v rotaci na luvisolu obsah EE-GRSP neměnný (Balík et al. 2023). Obsah T-GRSP byl výrazně vyšší na variantě FYM + NPK3 (intenzivní dávka NPK) v porovnání s FYM. Samotný FYM nebyl významně rozdílný od kontroly. V rotaci tedy nebyl pozorován až tak významný vliv hnojiv, protože zde převládal vliv vyššího množství posklizňových zbytků a větší vliv diverzity rostlin na aktivitu AM (Gosling et al. 2006, Borie et al. 2006, Roldán et al. 2007).

5.2.2.2 Obsah GRSP na luvisolu v monokultuře kukuřice a rotaci plodin v našich pokusech

Obsahy frakcí GRSP se mohou měnit v závislosti na použitém hnojivu. V monokultuře kukuřice na siláž byl prokázán významný nárůst obsahu EE-GRSP (o 15,9 %) a T-GRSP (o 21,5 %) po aplikaci čistírenského kalu (SS) v porovnání s kontrolou (Balík et al. 2022a). Obdobný vliv hnojení čistírenskými kaly byl prokázán ve studiích Sandeep et al. (2016) a Balík et al. (2020b). Také aplikace kejdy skotu v našich pokusech vedla k nárůstu obsahů EE-GRSP (o 9,51 %) a T-GRSP (o 8,2 %) v porovnaní s kontrolou. Tento nárůst hodnot je již nevýznamný. Pozitivní vliv kejdy prasat na obsah GRSP byl také popsán ve studii Bertagnoli et al. (2020). Dále z našich studií na monokultuře vyplývá, že hnojení minerálním N nezvyšovalo obsah GRSP (Balík et al. 2022a a Balík et al. 2022b) (diskutováno v kapitole 5.2.2.3).

V pokusu s rotací plodin se obsah frakcí GRSP významně nezvýšil na variantě hnojené FYM oproti nehnojené kontrole (Balík et al. 2023). Významný nárůst byl pozorován až při kombinaci FYM s vysokou dávkou minerálního NPK (FYM + NPK3). Bertagnoli et al. (2020), Zhang et al. (2014), Valarini et al. (2009), Turgay et al. (2015) a Dai et al. (2013) pozorovali nárůst T-GRSP již po aplikaci samotného FYM. V našich pokusech varianta FYM + NPK3 zaznamenala nejvyšší výnos biomasy (nepublikováno), což je spojeno s vyšším množstvím posklizňových zbytků a biomasy kořenů a jejich exudátů. To společně vedlo ke zvýšení GRSP. Tento nárůst biomasy se také projevil významným zvýšením obsahu C_{SOM}.

V literatuře nalézáme informace o tom, že, vyšší obsah GRSP je pozorován spíše na kyselých půdách než na neutrálních a zásaditých. Obsah GRSP tedy roste s klesající hodnotou pH (Agnihotri et al. 2022). V našem pokusu s monokulturou (Balík et al. 2022a) byl pozorován opačný jev. Byl zde popsán významný, pozitivní vztah mezi EE-GRSP a pH_{CaCl2} ($r^2 = 0,505$; $p < 0,05$). Půdní reakce tedy nemá jednoznačný vliv na hodnotu obsahu GRSP. Rozhodující je vliv jiných faktorů, jako například obsah C_{SOM}. Důležité je také zdůraznit vliv organických hnojiv, která vedou k růstu biomasy rostlin, včetně rozvoje kořenového systému, a následně k nárůstu obsahu GRSP. Vyšší obsah půdní organické hmoty vede také k tvorbě větších půdních agregátů, uvnitř kterých je vázán GRSP (Zhang et al. 2014), což vede ke snížení intenzity mineralizace.

Z výsledků korelací v našich studiích na luvisolech je evidentní významný vztah mezi hodnotami frakcí EE-GRSP a T-GRSP s obsahem C_{SOM}. ($r^2 = 0{,}497$ až $0{,}534$; $p < 0{,}05$ monokultura kukuřice; $p < 0{,}001$ rotace). Obdobné pozitivní korelace uvádějí také Singh et al. (2016) (všechny tři frakce GRSP, $r^2 > 0{,}9$; $p < 0{,}01$), Li et al. (2019) (T-GRSP, $r^2 = 0{,}404$; $p < 0{,}01$). Zajímavé je, že ve studii Li et al. (2019) nebyl popsán významný vztah mezi EE-GRSP a C_{SOM}. Autoři této studie předpokládají, že v jejich pokusech je snížená aktivita AM. Obsah EE-GRSP je primárně ovlivněn aktivitou AM (Koide et Peoples 2013, Wu et al. 2014). V podmínkách ČR se vztah mezi GRSP a C_{SOM} objevuje ve studii Šarapatka et al. (2019) a hodnoty korelačních koeficientů EE-GRSP a T-GRSP s C_{SOM} jsou podobné s naší studií. Řezáčová et al. (2021) ovšem uvádějí, že vztah mezi T-GRSP a C_{SOM} je mnohem silnější, než vztah mezi EE-GRSP a C_{SOM}. Opakovaná extrakce T-GRSP může vést k silnější korelaci. Při této extrakci se totiž uvolňují s molekulou glomalinu další látky, jako například část huminových kyselin, polyfenolické molekuly, cukry a lipidy, které všechny reagují s barvícím činidlem (Bradford assay) použitým pro určení obsahu GRSP ve vzorku (Avio et al. 2013). Tyto látky mohou vést k nadhodnocování výsledků obsahu GRSP (Li et al. 2020c). Díky jeho pozitivní korelaci s obsahem půdního organického uhlíku je obsah GRSP považován za indikátor změn půdní úrodnosti (Wright et Upadhyaya 1998, Xie et al. 2015, Dai et al. 2013, Li et al. 2019).

Byl také pozorován vztah obsahu GRSP k obecně uznávaným indikátorům kvality půdní organické hmoty. Obsahy EE-GRSP a T-GRSP jsou v dobré korelaci se stabilnějšími indikátory, jako jsou obsah C_{HA} ($r^2 = 0{,}590$ a $0{,}558$; $p < 0{,}05$) v monokultuře (Balík et al. 2022a). Obdobně Vlček et Pohanka (2020) pozorovali významnou korelaci mezi obsahem GRSP a obsahy C_{HA}. Dále jsou EE-GRSP a T-GRSP v malé korelaci s méně stabilními C_{FA} ($r^2 = 0{,}004$ a $0{,}349$; nevýznamné). V pokusu s rotací plodin (Balík et al. 2023) byl navíc popsán vztah GRSP k dalším indikátorům půdní organické hmoty. Byly zde stanoveny významné korelace mezi obsahem GRSP a obsahy C_{SOM}, C_{HA}, C_{HWC} a C_{DOC}.

5.2.2.3 Obsah GRSP na černozemi v monokultuře kukuřice

Na černozemi byl na variantě SS240, po aplikaci extrémně vysoké dávky čistírenského kalu (odpovídající dávce 240 kg $N.ha^{-1}.rok^{-1}$), pozorován významný nárůst EE-GRSP (o 10

%) v porovnání s variantou N120 (hnojené dávkou 120 kg $N.ha^{-1}.rok^{-1}$). To se shoduje s výše popsanými výsledky z monokultury kukuřice na luvisolu. Tento nárůst byl zřejmě způsoben výrazně vyšším růstem kořenové biomasy, a tedy zvýšenou produkcí GRSP. Lze předpokládat, že čistírenský kal nebyl přímým zdrojem GRSP. Metoda extrakce GRSP dle Wright et Upadhyaya (1998) je určena k uvolnění GRSP z půd, nikoliv z organických hnojiv. Přítomnost AM a GRSP nelze v kalech očekávat, protože kal byl dovezen přímo od producenta bez jakéhokoliv dalšího skladování. Reakce Bradfordova činidla s huminovými kyselinami, polyfenolyckými molekulami, lipidy a sacharidy (Avio et al. 2013) může vést k nadhodnocení výsledků obsahů GRSP (Li et al. 2020c). Čistírenské kaly běžně obsahují významné množství lipidů a sacharidů (Jimenez et al. 2013), a to se pravděpodobné promítne do takto hnojených variant.

Hnojení samotným minerálním N nezvyšovalo obsah GRSP, což je v rozporu se studiemi Luna et al. (2016) a Treseder et Turner (2007). Je to ale v souladu s výsledky našich pokusů na monokultuře na luvisolu (Balík et al. 2022a). Závěry studie Ma et al. (2018) do určité míry naše výsledky potvrzují. V jejich studii bylo zjištěno, že dlouhodobá aplikace minerálního N vede ke snížení diverzity a aktivity AM. Dalším důvodem může být fakt, že intenzivní minerální hnojení N vede k degradaci stabilní organické hmoty a snižuje stabilitu půdních agregátů. To vede k rozpadu půdní struktury a následnému snížení AM, a důsledkem je pokles produkce GRSP. Zvýšená degradace kvality půdní organické hmoty, daná nižšími hodnotami indexu smáčivosti (PWI) a stability půdních agregátů (WSA), byla na černozemi pozorována právě na variantách intenzivně hnojených minerálním N (N120 a N240). Když vezmeme v úvahu nízkou korelaci GRSP s C_{FA} (méně stabilní formou huminových látek - ze studie na luvisolu) a skutečnost, že obsah GRSP nebyl ovlivněn použitím samotného N, pak jsou výsledky v souladu s teorií o zvýšené odolnosti GRSP vůči mineralizaci. Doba setrvání GRSP v půdě je až 42 let a zůstává v půdě delší dobu než ostatní humifikovaná hmota (Rillig et al. 2001, Avio et al. 2013, Dorodnikov et al. 2011).

V pokusech na černozemi byl pozorován také významný vztah obsahů EE-GRSP a T-GRSP s C_{SOM} (0,791 a 0,818; $p < 0,01$). Na druhou stranu nebyly pozorovány žádné významné korelace mezi GRSP a C_{HA}, nebo C_{HA}/C_{FA}, což je v rozporu s výsledky na luvisolu. Přitom tyto parametry jsou důležité při určení změn kvality půdní organické hmoty (Kononova 1966). Změny v hodnotách C_{HA} a C_{HA}/C_{FA} ovšem nebyly na černozemi tak výrazné. Na luvisolu byly změny v obsahu C_{SOM} a jeho kvalitativních parametrech mnohem výraznější. To bylo zřejmě způsobeno významně menším obsahem a kvalitou půdní organické hmoty. Pro srovnání hodnoty na černozemi v této práci na kontrolní variantě jsou: C_{SOM} – 1,62 %; C_{HA} – 0,244 %; C_{HA}/C_{FA} – 1,352; C_{SOM}/N_T – 11,2; na luvisolu C_{SOM} – 0,98 %; C_{HA} – 0,078 %; C_{HA}/C_{FA} – 0,565; C_{SOM}/N_T – 10,2). Dalším faktorem je stabilita GRSP v půdě (zejména DE-GRSP). V tomto médiu dokáže GRSP setrvávat několik dekád (Rillig 2004) a nemusí být tedy citlivý ani k relativně dlouhodobému hnojení.

Dále je nutné zmínit, že ve všech třech pokusech byly zaznamenány významné korelace mezi obsahem obou frakcí GRSP a obsahem N_T (výjimkou je obsah EE-GRSP a N_T v monokultuře na luvisolu). V tomto vztahu je ovšem mnoho nejistot. Extrakty GRSP jsou bohaté na jiné bílkoviny obsahující dusík (Deng et al. 2023; Irving et al. 2021; Holátko et al. 2021; Gillespie et al. 2011). Extrakt GRSP se následně obarvuje Bradfordovým činidlem, které se používá pro měření obsahů široké škály proteinů (Kielkopf et al. 2020) a není specifické jen pro molekulu glomalinu.

Z našich výsledků je vidět, že obsah GRSP je ovlivněn půdním typem a systémem hospodaření (rotace vs. monokultura), jak uvádí literatura (Vlček et Pohanka 2020 a Wojewódzki et Cieścińska 2012).

5.2.3 Index smáčivosti (PWI)

Kvalita půdní organické hmoty se dá také popsat pomocí poměru C-H a C=O vazeb označovaných jako index smáčivosti (PWI) (Leue et al. 2013 a Haas et al. 2018). PWI se dá využít k: (i) určení stability půdních agregátů, (ii) posouzení retenčních vlastností půdy a (iii) popisu kvality půdní organické hmoty. Vyšší hodnota PWI ukazuje na nižší smáčivost půdních agregátů a jejich vyšší stabilitu (Haas et al. 2018).

Hodnoty PWI na monokultuře kukuřice na luvisolu (Balík et al. 2022a) byly v rozsahu 0,010 – 0,014. V pokusu s rotací plodin na luvisolu (Balík et al. 2023) byl PWI v rozsahu 0,016 – 0,019. Fér et al. (2016) zmiňují, že PWI pro luvisol je v rozsahu 0,010 – 0,030, což souhlasí s našimi hodnotami. Na monokultuře kukuřice na černozemi (Balík et al. 2022b) byly hodnoty PWI 0,020 – 0,029.

Organické hnojení (SS a kejda) na pokusu s monokulturou kukuřice na luvisolu významně zvyšuje hodnoty PWI (0,014 pro obě varianty) v porovnání s kontrolou (0,010) a samotným minerálním N (0,010). Organické hnojení zvyšuje obsah hydrofobních částic a pomáhá tvorbě větších půdních agregátů a je pravděpodobné, že zvýšení hodnot PWI je dáno právě tvorbou hydrofobních sekundární metabolitů, vzniklých v průběhu rozkladu organické hmoty (Oktaba 2018). Adani et Tambone (2005) pozorovali nárůst frakce alifatického uhlíku v huminových kyselinách po aplikaci čistírenského kalu. Demyan et al. (2012) pozorovali nárůst hodnoty PWI po aplikaci hnoje.

Na druhou stranu v rotaci plodin na luvisolu (Balík et al. 2023) nebyl pozorován žádný významný rozdíl v hodnotě PWI mezi různými variantami hnojení (včetně kontroly). Byl pozorován jen nevýznamný trend nárůstu hodnot PWI na variantě hnojené nejvyšší dávkou NPK v kombinaci s FYM (FYM + NPK3). Zvýšené hodnoty PWI na této variantě mohou být důsledkem relativně vyšší dávky minerálního hnojiva. Aplikace minerálních hnojiv vede k nárůstu biomasy kořenů, zvýšené produkci exudátů, a následně k tvorbě stabilních agregátů (Haas et al. 2018). Nevýznamné změny v tomto pokusu mohou být také vysvětleny tím, že jde o rotaci sedmi plodin. Edleusa et al. (2022) zjistili, že stabilita půdních agregátů je významně nižší na monokultuře kukuřice než v rotaci plodin. Na monokultuře na siláž mohla být stabilita půdních agregátů více ovlivněna organickým hnojením, neboť dochází k odsunu velkého množství rostlinné biomasy z pozemku po sklizni (Balík et al. 2020a). Zatímco v rotaci plodin byl větší vliv zapravených posklizňových zbytků. To potvrzují zjištění Cagna et al. (2019). V jejich pokusu byla zvýšená stabilita půdních agregátů popsána u rostlin z čeledi lipnicovitých, s hustým kořenovým systémem. V našich pokusech s rotací plodin se vyskytuje celkem sedm plodin a čtyři z nich jsou pšenice ozimá a ječmen jarní. Na monokultuře kukuřice na černozemi ovšem PWI vykazoval významné změny dle typu použitého hnojiva. Na kontrolní variantě byly zaznamenány významně vyšší hodnoty PWI v porovnání s variantou hnojenou minerálním N v dávce 240 kg $N.ha^{-1}.rok^{-1}$. Při porovnání s ostatními variantami (čistírenské kaly v dávce ekvivalentní 120 a 240 kg $N.ha^{-1}.rok^{-1}$ a minerální N v dávce 120 kg $N.ha^{-1}.rok^{-1}$) nebyly pozorovány významné rozdíly. Kontrola dosahovala také nejvyššího poměru C_{HA}/C_{FA}, což poukazuje na vysokou kvalitu půdní organické hmoty.

Na monokultuře kukuřice na luvisolu byly prokázány významné korelace hodnot PWI s obsahem C_{SOM} (0,799; $p < 0,001$). Leue et al. (2019) také popsali pozitivní korelaci mezi obsahem půdní organické hmoty a PWI. V rotaci plodin na luvisolu byla pozorována významná korelace mezi hodnotami PWI a obsahy C_{SOM} ($r^2 = 0,547$), C_{HS} ($r^2 = 0,328$), C_{FA} ($r^2 = 0,399$), C_{HA} ($r^2 = 0,358$), C_{HWE} ($r^2 = 0,406$) a C_{DOC} ($r^2 = 0,457$; $p < 0,01$). Silný vztah mezi PWI a C_{SOM} byl popsán ve sděleních Balík et al. (2022a), Leue et al. (2019). GRSP je hydrofobní glykoprotein (Wright et Upadhyaya 1998) a dá se tedy předpokládat, že zvýšený obsah GRSP by měl zvyšovat obsah hydrofobních částic, a tedy hodnoty PWI. Na obou pokusech s luvisolem byl popsán vztah GRSP k PWI. V rotaci plodin byl popsán vztah mezi

T-GRSP a PWI (0,383; $p < 0,001$). V monokultuře byla přitom silná korelace mezi EE-GRSP a PWI (0,735; $p < 0,01$). Pozitivní vztah mezi indikátory stability půdních agregátů a obsahem GRSP je popsán také ve sdělení Curaqueo et al. (2011).

5.2.4 Index aromaticity (iAR)

Půdní organická hmota je také charakterizována poměrem alifatických a aromatických sloučenin, označovaným jako index aromaticity (Cunha et al. 2009). Tento index se počítá z odraznosti alifatických pásem a aromatických pásem molekul. Vyšší hodnoty indexu aromaticity znamenají relativně nižší podíl aromatických součástí, respektive vyšší podíl alifatických složek organické hmoty ve stabilních agregátech než v agregátech nestabilních (Thai et al. 2023).

V rotaci plodin na luvisolu (Balík et al. 2023) byl rozsah iAR 0,032 až 0,036. Ve studii na monokultuře kukuřice na luvisolu (Balík et al. 2022a) byl rozsah iAR 0,019 až 0,027. V monokultuře byl popsán významný nárůst hodnoty iAR na variantách hnojených organicky (0,026 a 0,027 kejda a SS) v porovnání s minerálním N a kontrolou (0,019 a 0,020). Nárůst mineralizace organické hmoty a tvorba alifatických sloučenin v agregátech může vést k nárůstu hodnot indexu aromaticity (Cunha et al. 2009). Na druhou stranu v pokusu s rotací plodin nebyly pozorovány významné změny v tomto indexu. Pozorována je pouze nevýznamná rostoucí tendence na organicky hnojených variantách s odstupňovanou dávkou NPK. Mustafa et al. (2022) vyhodnotili dlouhodobý pokus monokultury kukuřice s dávkou NPK v kombinaci s chlévským hnojem. Autoři této studie uvádějí významný nárůst hodnot iAR. V našem pokusu s rotací byly pozorovány významné korelace iAR s následujícími parametry: C_{SOM} ($r^2 = 0,651$), C_{HS} ($r^2 = 0,438$), C_{FA} ($r^2 = 0,530$), C_{HA} ($r^2 = 0,471$), C_{HWE} ($r^2 = 0,455$), C_{DOC} ($r^2 = 0,400$) ($p < 0,001$). Zároveň iAR koreluje s obsahy EE-GRSP ($r^2 = 0,242$; $p < 0,05$) a T-GRSP ($r^2 = 0,455$; $p < 0,001$). Na monokultuře byly pozorovány významné korelace iAR s obsahy EE-GRSP ($r^2 = 0,688$; $p < 0,01$) a C_{SOM} ($r^2 = 0,799$; $p < 0,001$). Tento index a PWI vykazují velmi podobné trendy, a je tedy pravděpodobné, že vliv rotace plodin, diskutovaný výše pro PWI, má podobný vliv na hodnoty iAR,

5.3 Vztah mezi frakcemi síry v půdě a vybranými indikátory kvality půdní organické hmoty

V této části diskuze bude popsán vztah mezi půdními frakcemi síry (S_{H2O}, S_{ADS}, S_{AV}, S_{ORG} a S_T) vybranými kvalitativními indikátory (obsah frakcí GRSP a huminových látek) půdní organické hmoty na dlouhodobém pokusu s monokulturou kukuřice na siláž na stanovišti Červený Újezd.

K určení hlavních faktorů byla použita analýza hlavních faktorů (PCA). Tato analýza stanovila nejdůležitější proměnné v tomto pokusu. Prvním hlavním komponentem byla kombinace S_T, C_{SOM}, EE-GRSP a C_{HA} a tento komponent byl ovlivněn hlavně aplikací FYM. Druhý hlavní komponent byl ovlivněn nejvíce hnojením AS a zahrnuje kombinaci S_{H2O}, S_{ADS} a S_{AV} a C_{FA}. Vliv C_{SOM} a N_T byl již dříve diskutován v kapitolách 5.1 a 5.2 z dat v Suran et al. (2023) a Balík et al. (2022a). Z tohoto důvodu nebude jejich vliv diskutován zde. Zaměříme se na vztah GRSP a frakcí huminových látek ve vztahu k frakcím síry v půdě.

Každoročně je na variantě AS aplikováno velké množství minerální S, zatímco na variantě FYM je aplikováno významné množství organické hmoty v podobě chlévského hnoje, který obsahuje kvalitní organické látky (Förster et al. 2012). U kontroly a varianty s dusičnanem amonným a močovinou (UAN) nebyly žádné vstupy v podobě organické hmoty, nebo síry, což vysvětluje jejich odklon od obou hlavních komponent. Zajímavé je, že varianta hnojená kejdou skotu (Slurry) je relativně blízko minerální UAN a kontrole i přes to, že obdržela S a C. Nicméně organická hmota v kejdě je snadno mineralizovatelná (Islam et al. 2021).

Do analýzy vztahů mezi půdním organickým uhlíkem a sírou v půdě budou zahrnuty také T-GRSP, DE-GRSP, C_{FA} a C_{HA} i přes to, že podle analýzy hlavních komponent nejsou významné – důvodem je naše zaměření na tyto indikátory kvality C_{SOM}.

Vzhledem k tomu, že dosud nebylo provedeno přímé měření obsahu síry v GRSP, byla zde provedena kalkulace na základě nepřímých údajů z literatury. Lovelock et al. (2004) zjistili, že obsah N a C v čistém extraktu glomalinu je v průměru 4 a 47,5 %. Poměr C/N je tedy v molekule čistého glomalinu 11,9. V našem pokusu je poměr C_{SOM}/N_T v půdě mezi 9,31 a 10,7. Na základě podobností poměrů C/N v GRSP a půdě bylo usouzeno, že poměr C/S v glomalinu bude podobný poměru C_{SOM}/S_{ORG} v půdě. Využitím poměru C_{SOM}/S_{ORG} v půdě je možné dopočítat obsah S v GRSP.

Obsah síry v T-GRSP ($S_{T\text{-}GRSP}$) byl vyšší (13,9 – 21,1 mg S.kg^{-1} půdy), než obsah síry v EE-GRSP ($S_{EE\text{-}GRSP}$) (3,21 – 5,46 mg S.kg^{-1} půdy) díky faktu, že T-GRSP je extrahován opakovaně ze stejného vzorku, zatímco EE-GRSP je uvolňován pouze jednou. Obecně varianty hnojené organicky dosahovaly vyšších hodnot $S_{T\text{-}GRSP}$, než varianty minerální, ale nejvyšší a statisticky nejvýznamnější nárůst byl pozorován na variantě FYM. Dle literatury je EE-GRSP zřejmě přímým produktem AM (Koide et Peoples 2013, Wu et al. 2014) a podléhá rychlejší mineralizaci (Cissé et al. 2021). Na druhou stranu je DE-GRSP více odolným vůči mineralizaci v půdním prostředí s retenčním časem až kolem 40 let (Harner et al. 2004). To znamená, že po více jak 20 letech vstupů organické hmoty se obsah DE-GRSP zvýší a s ním také obsah síry v DE-GRSP ($S_{DE\text{-}GRSP}$) a to vede ke zvýšení obsahu síry v T-GRSP. Nicméně trendy ve vlivu hnojiv na $S_{EE\text{-}GRSP}$ a $S_{DE\text{-}GRSP}$ jsou podobné. Obě frakce tedy reagují velmi podobně na aplikaci organických hnojiv. Obsah frakcí GRSP se zvýšil na základě aplikovaného hnojiva. Varianta s kejdou má srovnatelné obsahy $S_{T\text{-}GRSP}$ a $S_{DE\text{-}GRSP}$ s minerálně hnojenými variantami, protože se obsah živin v kejdě skotu může velmi rychle mineralizovat (Islam et al. 2021).

Vztah mezi obsahem frakcí síry a GRSP byl hodnocen pomocí korelačních koeficientů. Všechny frakce GRSP mají významnější vztah s obsahy frakcí S_T a S_{ORG} ($r^2 > 0{,}5$; $p < 0{,}01$) než s obsahy minerálních frakcí síry. Výjimkou je vztah S_{ORG} a DE-GRSP. Slabý vztah mezi DE-GRSP a S_{ORG} je zřejmě způsoben tím, že obsah S_{ORG} na variantě s AS je relativně nízký, zatímco obsah přístupné síry (S_{AV}) je významně vyšší (Suran et al. 2021). Tato varianta obdrží každoročně 142 kg $S.ha^{-1}$ v minerální formě. Obsahy S_T a DE-GRSP se současně zvyšují v závislosti na hnojení. Extrakty GRSP jsou bohaté na molekuly obsahující uhlík, jako jsou glykoproteiny, huminové kyseliny, cukry, lipidy (Gunina et Kuzyakov 2015, Deng et al. 2023), které ve své struktuře nejspíše obsahují organicky vázanou síru a stávají se tak rezervoárem síry.

Významný vztah byl popsán také pro obsah S_{ORG} a obsahy C_{HA}, C_{HS} ($r^2 > 0{,}5$; $p < 0{,}01$). Aplikace organických hnojiv zvyšuje významně obsahy C_{SOM} (Rong et al. 2016), a tedy zvyšuje také obsah C_{HS} (Balík et al. 2022). Tento vztah se také přenesl na obsah S_T, neboť S_{ORG} tvoří přes 90 % této frakce (popsáno v kapitole 5.1). Jak je evidentní z literatury, huminové kyseliny jsou mimo jiné, také tvořeny atomy síry (Prietzel et al. 2007, Ampong et al. 2022) a zvýšení obsahu huminových kyselin v půdě vede ke zvýšení obsahu síry v půdě (Li et al. 2019). Tradičně jsou huminové kyseliny chápány jako stabilní formy uhlíku (Zavarzina et al. 2021), a mohou proto obsahovat stabilnější formy síry. Obdobné také fulvokyseliny obsahují síru (Prietzel et al. 2007) a nárůst jejich obsahu by mohl také vést k nárůstu obsahu S v půdě. Ovšem vztah mezi obsahem C_{FA} a S_{ORG} nebyl prokázán. Na druhou stranu byl prokázán vztah mezi minerálními frakcemi síry a C_{FA} ($r^2 > 0,4$; $p < 0,01$)). Je pravděpodobné, že část vodorozpustné frakce síry mohla být extrahován společně s fulvokyselinami, které jsou také rozpustné ve vodě (Zavarzina et al. 2021). Varianta AS neobdržela žádné vstupy organické hmoty, a přesto vykázala nejvyšší hodnoty C_{FA} společně s S_{H2O}, S_{ADS} a S_{AV} v porovnání s ostatními variantami.

6 Souhrnné závěry

6.1 Kritické posouzení metody Mehlich 3 jako vhodného extrakčního činidla pro stanovení rostlinám přístupné síry

- V hodnotách obsahu síry získaných metodou Mehlich 3 (S_{M3}) nebyly statisticky významné rozdíly v porovnání s obsahem rostlinám přístupné síry (S_{AV} = součet vodorozpustné a sorbované).
- Zároveň obsahy síry v extraktu Mehlich 3 dobře korelují s obsahem síry v rostlině ($r^2 > 0,5$; $p < 0,05$).
- Tento postup je obzvláště užitečný, protože touto metodou lze určit rostlinám přístupné podíly několika živin současně v rámci jediného měření. Další výhodou je fakt, že se tato metoda používá standardně při agrochemickém zkoušení zemědělských půd.

Stanovení obsahu síry v půdě metodou Mehlich 3 se dá doporučit k určení rostlinám přístupné síry, pokud je cílem současně stanovit jiné prvky. Na druhou stranu je nutné zmínit, že pokud jde pouze o určení jen rostlinám přístupného podílu síry, tak se stále jako lepší jeví metoda určení frakce S_{AV}. Tato metoda poskytuje silnější korelace s obsahem síry v rostlině ($r^2 > 0,7$; $p < 0,05$).

6.2 Bilance síry v půdě

- Obsahy vodorozpustné, sorbované a rostlinám přístupné síry se na téměř všech variantách v průměru snížily na hodnoty 34, 66 a 41 % v porovnání s obsahem na začátku experimentu (před 23 lety).
    - Na variantě se síranem amonným (AS) byl pozorován nárůst minerálních frakcí vlivem vysoké dávky minerální síry v aplikovaném hnojivu.
- Na většině variant byl pozorován pokles v obsahu celkové síry (S_T).
    - Na variantě AS nedošlo ke snížení obsahu S_T díky zvýšení obsahu minerálních frakcí síry.
    - Na variantě s chlévským hnojem (FYM) nebyl pozorován pokles obsahu S_T díky zvýšenému obsahu půdního organického uhlíku (C_{SOM}).

- Na všech variantách v pokusu s organickým hnojením došlo k nárůstu podílu obsahu organicky vázané síry (S_{ORG}) z obsahu S_T, které bylo způsobeno snížením obsahů minerálních frakcí síry.

Bilance síry, vycházející ze vstupů a výstupů síry, byla na všech hnojených variantách záporná. Největší ztráty síry byly pozorovány na variantách s čistírenskými kaly (SS), síranem amonným, dusičnanem amonným s močovinou, trojitým superfosfátem a kieseritem (UAN + PMgS). Tyto varianty při každoročních vstupech síry hnojivy v dávkách 54,2; 142,0 a 84 kg S.ha^{-1} ztratily v průměru 66,1; 142,2 a 95,3 kg S.ha^{-1}.rok^{-1}. Varianta SS ztratila 1 521 kg S.ha^{-1} za celou dobu experimentu. Na variantách AS a UAN + PMgS jsou ztráty síry způsobené vysokou jednorázovou dávkou minerální síry. Na těchto variantách byly za celou dobu experimentu kalkulovány ztráty síry 3 271 a 2 193 kg S.ha^{-1}.

6.3 Zhodnocení vybraných indikátorů kvality půdní organické hmoty

- V rotaci plodin došlo k významným změnám v obsahu celkového glomalinu (T-GRSP) na variantách s aplikovaným FYM v kombinaci se zvýšenou, nebo vysokou dávkou minerálního NPK v porovnání s kontrolou.
- Obsahy lehce extrahovatelného glomalinu (EE-GRSP) a celkového glomalinu (T-GRSP) byly v pokusech s rotací plodin vyšší o 21,6 a 25,7 % v porovnání s monokulturou kukuřice pěstované na stejném půdním typu (luvisol).
- Na monokultuře kukuřice na siláž byl stanoven významný vliv organického hnojení na nárůst obsahů T-GRSP a EE-GRSP v porovnání s kontrolou.
- Na luvisolech byly stanoveny významné vztahy mezi obsahy obou frakcí GRSP a obsahem C_{SOM} ($r^2 > 0,5$; $p < 0,05$). Dále byly stanoveny významné vztahy obou frakcí GRSP s obsahem uhlíku v huminových kyselinách (C_{HA}; $r^2 > 0,4$; $p < 0,05$).
    - V rotaci obě frakce GRSP korelovaly s obsahem rozpuštěného organické uhlíku (C_{DOC}) a uhlíkem extrahovatelným horkou vodou (C_{HWE}).
    - V monokultuře byly významné korelace T-GRSP s C_{DOC}.
- Na černozemi s monokulturou kukuřice byl stanoven vliv hnojení na obsah EE-GRSP pouze při velmi vysoké dávce čistírenského kalu (obsahující 240 kg N.ha^{-1}.rok^{-1}). Byly stanoveny významné korelace obou frakcí GRSP s obsahem C_{SOM} ($r^2 > 0,75$; $p < 0,001$).

Využití GRSP jako indikátorů změn kvality půdní organické hmoty je možné. Hodnoty GRSP jsou v dobrém vztahu s hodnotami „tradičních“ indikátorů půdní úrodnosti, ovšem tyto vztahy jsou specifické díky rozdílným půdním typům a způsobu pěstování rostlin. Je nutné zmínit, že tato problematika vyžaduje další studium na široké škále půdních typů.

- Hodnoty indexů smáčivosti (PWI) a aromaticity (iAR) se v rotaci plodin významně neměnily v závislosti na hnojení (FYM samotný a v kombinaci se stupňovanou dávkou NPK).
- Hodnoty obou indexů se významně zvýšily na monokultuře po dlouhodobé aplikaci čistírenských kalů a kejdy skotu v porovnání s dlouhodobě nehnojenou kontrolou a samotným minerálním N.
- Hodnoty PWI a iAR vykazují významné korelace s tradičními metodami (obsahem C_{SOM}, C_{HS}, C_{FA}, C_{HA}, C_{HWE} a C_{DOC}) v rotaci plodin na luvisolu.
- Díky přirozeně vysoké kvalitě půdní organické hmoty na černozemích bylo významné snížení hodnot PWI na tomto půdním typu pozorováno pouze u varianty hnojené extrémní dávkou minerálního N (240 kg.ha^{-1}rok^{-1}). Na variantách hnojených stupňovanou dávkou čistírenských kalů a menší dávkou N (120 kg.ha^{-1}rok^{-1}) nebyly zaznamenány významné změny.

Na základě zmíněných skutečností je možné konstatovat, že použití PWI a iAR k vyhodnocení změn kvality půdní organické hmoty může být vhodnou alternativou k „tradičním“ metodám. Je nutné zmínit, že tato problematika vyžaduje další studium na široké škále půdních typů.

6.4 Vztah vybraných indikátorů kvality půdní organické hmoty k frakcím síry v půdě

Byl stanoven významný vztah mezi:

- obsahem S_{ORG} a obsahy EE-GRSP a T-GRSP; obsahem S_T a T-GRSP a EE-GRSP ($r^2 > 0,5$; $p < 0,01$).
- obsahem S_{ORG} a S_T s obsahem C_{HA} ($r^2 > 0,6$; $p < 0,001$).
- obsahem S_{AV} a obsahem uhlíku ve fulvokyselinách ($r^2 = 0,495$; $p < 0,01$).

Obsah síry v EE-GRSP a T-GRSP je ovlivněn typem hnojiva (organické/minerální).

Chlévský hnůj významně zvyšuje obsah síry v GRSP (EE-GRSP a T-GRSP).

7 Publikované práce

7.1 Články ve vědeckých časopisech

Balík J., Kulhánek M., Černý J., Sedlář O., **Suran P**. 2020. Soil organic matter degradation in long-term maize cultivation and insufficient organic fertilization. Plants. 9, 1217.

Balík J., Kulhánek M., Černý J., Sedlář O., **Suran P.**, Asrade D.A. 2022a. The Influence of organic and mineral fertilizers on the quality of soil organic matter and glomalin content. Agronomy. 12, 1375.

Balík J., Kulhánek M., Černý J., Sedlář O., **Suran P.**, Procházková S., Asrade, D.A. 2022b. The impact of the long-term application of mineral nitrogen and sewage sludge fertilizers on the quality of soil organic matter. Chemical and Biological Technologies in Agriculture. 9, 86.

Balík J., **Suran P.**, Sedlář O., Černý J., Kulhánek M., Procházková S., Asrade D.A., Smatanová M. 2023. Long-term application of manure and different mineral fertilization in relation to the soil organic matter quality of luvisols. Agronomy. 13, 2678.

Suran P., Balík J., Kulhánek M., Sedlář O., Černý J. 2024. The relationship of soil sulfur with glomalin-related soil protein and humic substances under different mineral and organic fertilisation. Plant, Soil and Environement. 70, 93 – 100.

Suran P., Kulhánek M., Balík J., Černý J., Sedlář O. 2021. Evaluation of soil S pools under 23 years of maize monoculture. Agronomy. 11, 2376.

Suran P., Kulhánek M., Balík J., Černý J., Sedlář O. 2023. Influence of long-term organic fertilization on changes in the content of various forms of sulfur in the soil under maize monoculture. Agronomy. 13, 957.

7.2 Ostatní publikace

7.2.1 Články ve vědeckých časopisech – mimo rozsah práce

Balík J., Černý J., Kulhánek M., Sedlář O., **Suran P**. 2019. Balance of potassium in two long-term field experiments with different fertilization treatments. Plant, Soil and Environment. 65, 225 – 232.

Balík J., Kulhánek M., Černý J., Sedlář O., **Suran P**. 2019. Impact of organic and mineral fertilising on aluminium mobility and extractability in two temperate Cambisols. Plant, Soil and Environment. 65, 581 – 587.

Balík J., Kulhánek M., Černý J., Sedlář O., **Suran P**. 2019. Potassium fractions on soil and simple K balance in long-term fertilising experiments. Soil and Water Research. 15, 211 – 219.

Balík J., Sedlář O., Kulhánek M., Černý J., Smatanová M., **Suran P**. 2020. Effect of organic fertilisers on glomalin content and soil organic matter quality. Plant, Soil and Environment. 66, 590 – 597.

Balík J., **Suran P**., Sedlář O., Černý J., Kulhánek M., Procházková S., Asrade D.A., Smatanová M. 2023. The effect of long-term farmyard manure and mineral fertilizer application on the increase in soil organic matter quality of Cambisols. Agronomy. 13, 2960.

Kulhánek M., Černý J., Balík J., Sedlář O., **Suran P**. 2018. Potential of Mehlich 3 method for extracting plant available sulfur on the Czech agricultural soils. Plant, Soil and Environment. 64, 455 – 462.

Sedlář O., Balík J., Kulhánek M., Černý J., Matěchová M., **Suran P**. 2021. Crop sulfur status in relation to soil sulfur determined using anion exchange membranes and Mehlich 3. Journal of Plant Nutrition. 44, 1563 – 1570.

Sedlář O., Balík J., Kulhánek M., Černý J., **Suran P**. 2019. Sulphur nutrition index in relation to nitrogen uptake and quality of winter wheat grain. Chilean Journal of Agricultural Research. 79, 486 - 492.

Kulhánek M., Asrade D.A., **Suran P**., Sedlář O., Černý J., Balík J. 2023. Plant nutrition - new methods bBased on the lessons of history: a review. Plants. 12, 4150.

7.2.2 Články ve sborníku z konference

Suran P., Kulhánek M., Balík J., Černý J., Sedlář O. 2020. Vliv různých minerálních hnojiv na obsah síry v rostlinách kukuřice. In: Sborník z 26. mezinárodní konference Racionální použití hnojiv zaměřené na nové trendy ve vývoji a aplikaci hnojiv. Česká zemědělská univerzita v Praze, Praha, 157 – 160 pp.

Suran P., Kulhánek M., Balík J., Černý J., Sedlář O. 2021. Vliv různých organických hnojiv na obsah síry v rostlinách kukuřice. In Sborník z 27. mezinárodní konference Racionální použití hnojiv zaměřené na problematiku výživy hnojení fosforem. Česká zemědělská univerzita v Praze, Praha, 161 – 164 pp

Balík J., Vaněk V., Černý J., Kulhánek M., Sedlář O**., Suran P**. 2020. Význam organické hmoty v půdě (rozdělení, zdroje a úloha v půdě). In: Sborník z 25. mezinárodní konference Racionální použití hnojiv zaměřené na nové trendy ve vývoji a aplikaci hnojiv. Česká zemědělská univerzita v Praze, Praha. Česká republika, 9 - 15 pp.

7.2.3 Metodiky

Kulhánek M., Balík J., Sedlář O., Zbíral J., Smatanová M., **Suran P**. 2018. Stanovení rostlinám přístupné síry v půdě metodou Mehlich 3. Certifikovaná metodika. ČZU v Praze. Praha. 31 pp.

Sedlář O., Balík J., Kulhánek M., **Suran P**., Matěchová M. 2020. Inovace diagnostiky výživného stavu plodin fosforem a sírou. Certifikovaná metodika. ČZU v Praze. Praha. 38 pp.

8 Literatura

Aarabi F., Kusajima M., Tohge T., Konishi T., Gigolashvili, T., Takamune M., Sasazaki Y., Watanabe M., Nakashita H., Fernie A.R., Saito K., Takahashi H., Hubberten H.M., Hoefgen R., Maruyama-Nakashita A. 2016. Sulfur deficiency-induced repressor proteins optimize flucosinolate biosynthesis in plants. Science Advances. 7, e1601087.

Aarabi F., Naake T., Fernie A.R., Hoefgen R. 2020. Coordinating sulfur pools under sulfate deprivation. Trends in Plant Science. 25, 1227 – 1239.

Adani F., Tambone, F. 2005. Long-term effect of sewage sludge application on soil humic acids. Chemosphere. 60, 1214 – 1221.

Aghajanzadeh T., Hawkesford M.J., De Kok L.J. 2016. Atmospheric H_2S and SO_2 as sulfur sources for *Brassica juncea* and *Brassica rapa*: regulation of sulfur uptake and assimilation. Environmental and Experimental Botany. 124, 1.

Agnihotri R., Bharti A., Ramesh A., Prakash A., Sharma M.P. 2021. Glomalin related protein and C16:1!5 PLFA associated with AM fungi as potential signatures for assessing the soil C sequestration under contrasting soil management practices. European Journal of Soil Biology. 103, 103286.

Agnihotri R., Prakash A., Ramesh A., Billore S.D., Sharma M.P. 2018. Coordination of crop and soil management practices built up higher mycorrhizal biomass which enhances soil carbon sequestration assessed in the rhizosphere of soybean in a longterm field trial. In: Proceedings of the National Conference on Organic Waste Management for Food and Environment Security, Bhópál. Indie. 8 – 10 pp.

Agnihotri R., Sharma M.P., Prakash A., Ramesh A., Bhattacharjya S., Patra A.K., Manna M.C., Kurganova I., Kuzyakov Y. 2022. Glycoproteins of arbuscular mycorrhiza for soil carbon sequestration: Review of mechanisms and controls. Science of Total Environment. 806, 150571.

Agrawal M. 2003. Plant responses to atmospheric sulphur. In: Ahmad A. (Ed.). Sulphur in Plants. Springer. Dordrecht. 279 – 293 pp.

Ahmad G., Jan A., Arif M., Jan M.T., Khattak R.A. 2007. Influence of nitrogen and sulfur fertilization on quality of canola (*Brassica napus* L.) under rainfed conditions. Journal of Zhejiang University. 6, 731 – 737.

Allahham A., Kanno S., Zhang L., Maruyama-Nakashita A. 2020. Sulfur deficiency increases phosphate accumulation, uptake, and transport in *Arabidopsis thaliana*. International Journal of Molecular Sciences. 21, 2971.

Allison S.D. 2006. Soil minerals and humic acids alter enzyme stability: implications for ecosystem processes. Biogeochmistry. 81, 361 – 373.

Ampong K., Thilakaranthna M.S., Gorim L.Y. 2022. Understanding the role of humic acids on crop performance and soil health. Frontiers in Agronomy. 4, 848621.

Ashraf M.N., Jusheng G., Lei W., Mustafa A., Waqas A., Aziz T., Khan W.U.D., Hussain B., Farooq M., Wenju Z., Minggang X. 2021. Soil microbial biomass and extracellular enzyme–mediated mineralization potentials of carbon and nitrogen under long-term fertilization (> 30 years) in a rice–rice cropping system. Journal of Soils and Sediments. 10, 1 – 12.

Avio L., Castaldini M., Fabiani A., Bedini S., Sbrana C., Turrini A., Giovannetti M. 2013. Impact of nitrogen fertilization and soil tillage on arbuscular mycorrhizal fungal communities in a Mediterranean agroecosystem. Soil Biology and Biochemistry. 67, 285 – 294.

Awazuhara M., Fujiwara T., Hayashi H., Watanabe-Takahashi A., Takahashi H., Saito K. 2005. The function of SULTR2;1 sulfate transporter during seed development in *Arabidopsis thaliana*. Physiologia Plantarum. 125, 95 – 105.

Balík J., Kulhánek M., Černý J., Pavlíková D., Čermák, P. 2009. Differences in soil sulfur fractions due to limitation of atmospheric deposition. Plant, Soil and Environment. 55, 344 – 352

Balík J., Kulhánek M., Černý J., Sedlář O., Suran P. 2020a. Soil organic matter degradation in long-term maize cultivation and insuffient organic fertilization. Plants. 9, 1217.

Balík J., Kulhánek M., Černý J., Sedlář O., Suran P., Asrade D.A. 2022a. The Influence of Organic and Mineral Fertilizers on the Quality of Soil Organic Matter and Glomalin Content. Agronomy. 12, 1375.

Balík J., Kulhánek M., Černý J., Sedlář O., Suran P., Procházková S., Asrade D.A. 2022b. The impact of the long-term application of mineral nitrogen and sewage sludge fertilizers on the quality of soil organic matter. Chemical and Biological Technologies in Agriculture. 9, 86.

Balík J., Sedlář O., Kulhánek M., Černý J., Smatanová M., Suran P. 2020b. Effect of organic fertilisers on glomalin content and soil organic matter quality. Plant, Soil and Environment. 66, 590 – 597.

Balík J., Suran P., Sedlář O., Černý J., Kulhánek M., Procházková S., Asrade D.A., Smatanová M. 2023. Long-Term Application of Manure and Different Mineral Fertilization in Relation to the Soil Organic Matter Quality of Luvisols. Agronomy. 13, 2678.

Balík J., Vaněk V., Černý J., Kulhánek M., Sedlář O., Suran P. 2020. Význam organické hmoty v půdě (rozdělení, zdroje a úloha v půdě). In: Sborník z 25. mezinárodní konference Racionální použití hnojiv zaměřené na nové trendy ve vývoji a aplikaci hnojiv. Česká zemědělská univerzita v Praze, Praha. Česká republika. 9 - 15 pp

Balisz B., Lachacz A., Glaźewski R., Grabowski K. 2017. Labile organic carbon fractions after amendment of sandy soil with municipal sewage sludge and compost. Journal of Elementology. 22, 785 – 797.

Banger K., Kukal S.S., Toor G.S., Sudhir K., Hanumanthraju T.H. 2009. Impact of long-term additions of chemical fertilizers and farm yard manure on carbon and nitrogen sequestration under rice-cowpea cropping system in semi-arid tropics. Plant and Soil. 318, 27 – 35.

Banik S.P., Pal S., Ghorai S., Chowdhury S., Khowala S. 2009. Interference of sugars in the Coomassie Blue G dye binding assay of proteins. Analytical Biochemistry. 386, 113 – 115.

Barber S.A. 1995. Sulfur. In: Barber, S.A. (Ed.). Soil Nutrient Bioavailability. John Wiley & Sons, Inc. New York, United States. 301 – 310 pp.

Barlóg P., Hlisnikovský L., Kunzová E. 2020. Effect of digestate on soil organic carbon and plant-available nutrient content compared to cattle slurry and mineral fertilization. Agronomy. 10, 379.

Barrow NJ. 1960. A comparison of the mineralization of nitrogen and of sulfur from decomposing organic materials. Australian Journal of Agricultural Research. 11, 960 - 969.

Barrow N.J., Debnath A. 2015. Effect of phosphate status and pH on sulphate sorption and desorption. European Journal of Soil Science. 66, 286–297.

Baveye P.H.C. Wander M. 2019. The (bio)chemistry of soil humus and humic substances: why is the "New view" still considered novel after more than 80 years?" Frontiers in Environmental Science. 7, 27.

Bedini S., Avio L., Argese E., Giovannetii M. 2007. Effects of long-term land use on arbuscular mycorrhizal fungi and glomalin-related soil protein. Agriculture, Ecosystems and Environment. 120, 463 – 466.

Bedini S., Avio L., Sbrana C., Turrini A., Migliorini P., Vazzana C., Giovanneti M. 2013. Mycorrhizal activity and diversity in a long-term organic Mediterranean agroecosystem. Biology and Fertility of Soils. 49, 781 – 790.

Bender R.R., Haegele J.W., Ruffo M.L., Below F.E. 2013. Nutrient uptake, partitioning, and remobilization in modern transgenic insect-protected maize hybrids. Agronomy Journal. 105, 161 – 170

Bergholm J., Majdi H. 2001. Accumulation of nutrients in above and below ground biomass in response to ammonium sulfate addition in a Norway spruce stand in southwest Sweden. Water, Air and Soil Pollution. 130, 1049 – 1054.

Bertagnoli B.G.P., Oliveira J.F., Barbosa G.M.C., Filho A.C. 2020. Poultry litter and liquid swine slurry applications stimulate glomalin, extraradicular mycelium production, and aggregation in soils. Soil and Tillage Research. 202, 104657.

Bertrand I., Holloway R.E., Armstrong R.D., McLaughlin M.J. 2003. Chemical characteristics of phosphorus in alkaline soils from southern Australia. Australian Journal of Agricultural Research. 41, 61–76.

Bettina J., Ludwig B., Flessa H. 2003. Carbon dynamics determined by natural ^{13}C abundance in microcosm experiments with soils from long-term maize and rye monocultures. Soil Biology and Biochemistry. 35, 1193 – 1202

Biederbeck V.O. 1978. Soil organic sulfur and soil fertility. In: Schnitzer M., Khan SU. (Eds.). Soil Organic Matter. Elsevier, Amsterdam. 273-310 pp.

Bisutti I., Hilke I., Raessler M. 2004. Determination of total organic carbon: an overview of current methods. Trends in Analytical Chemistry. 2004, 23, 716–726.

Blake-Kalff M.M.A., Hawkesford M.J., Zhao F.J., McGrath SP. 2000. Diagnosing sulfur deficiency in field-grown oilseed rape (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). Plant and Soil. 225, 95 - 107.

Blum S.C., Lehmann J., Solomon D., Caires E.F., Alleoni L.R.F. 2013. Sulfur forms in organic substrates affecting S mineralization in soil. Geoderma. 200 – 201, 156 – 164.

Boguta P., Sokołowska Z., Skic K. 2017. Use of thermal analysis coupled with differential scanning calorimetry, quadrupole mass spectrometry and infrared spectroscopy (TG-

DSC-QMS-FTIR) to monitor chemical properties and thermal stability of fulvic and humic acids. PLoS ONE. 12, e0189653.

Bohn H.L., Barrow N.J., Rajan S.S.S., Parfitt L. 1986. Reactions of inorganic sulfur in soils. In: Tabatabai MA. (ed.) Sulfur in Agriculture, American Society of Agronomy, Madison, Wisconsin, United States, 233–249 pp.

Bongiorno G., Bünemann E.K., Oguejiofor C.U., Meir J., Gort F., Comans R., Mäder P., Brussaard L., de Goede R. 2019. Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe. Ecological Indicators. 99, 28 – 50.

Borie F., Rubio R., Rouanet J.L., Morales A., Borie G., Rojas C. 2006. Effects of tillage systems on soil charactectics, glomalin and mycorrhizalpropagules in a Chilean Ultisol. Soil and Tillage Research. 88, 253 – 261.

Boye K., Eriksen J., Nilsson I., Mattson L. 2010. Sulfur flow in a soil-plant system-effects of long-term treatment history and soil properties. Plant and Soil. 334, 323 – 334.

Brodie C.R., Leng M.J., Casford J.S.I., Kendrick C.P. Lloyd J.M., Yongqiang Z., Bird M.I. 2011. Evidence for bias in C and N concentrations and δ^{13}C composition of terrestrial and aquatic organic materials due to pre-analysis acid preparation methods. Chemical Geology. 282, 67 - 83.

Burton E.D., Bush R.T., Sullivan L.A. 2006. Elemental sulfur in drain sediments associated with acid sulfate soils. Applied Geochemistry. 21, 1240–1247.

Cagna C.P., Calábria Z.K.P., Guedes Filho O., Pacheco L.P,. Silva TJ.A. 2019. Structural properties of soil in maize and forage grass intercropping under no-tillage in the brazilian cerrado. Engenharia. Agrícola. 39, 512 – 517.

Canellas L.P., Canellas N.O.A., Irinev L.E.S.D., Olivares F.L., Piccolo A. 2020. Plant chemical priming by humic acids. Chemical and Biological technologies in Agriculture. 7, 12.

Carciochi W.D., Divito G.A., Fernández L.A., Echeverria H. 2017. Sulfur affects root growth and improves nitrogen recovery and internal efficiency in wheat. Journal of Plant Nutrition. 40, 1231 – 1242.

Carciochi W.D., Wyngaard N., Calvo N.I.R., Pagani A., Divito G.A., Echeverria H.E., Ciampitti I..A. 2019. Critical sulfur dilution curve and sulfur nutrition index in maize. Agronomy Journal, 111, 448 - 456.

Castellano S.D., Dick R.P. 1991. Cropping and sulfur fertilization influence on sulfur transformations in soil. Soil Science Society of America Journal. 55, 114 - 121.

Cissé G., Essi M., Kedi B., Mollier A., Staunton S. 2021. Contrasting effects of long term phosphorus fertilization on glomalin related soil protein (GRSP). European Journal of Soil Biology, 107, 103363.

Collins H.P., Blevins R.L., Gundy L.G., Christenson D.R., Dick W.A., Huggins D.R., Paul E.A. 1999. Soil carbon dynamics in corn-based agroecosystems: Results from carbon-13 natural abundance. Soil Science Society of America Jurnal. 63, 584 – 591.

Conant R.T. (Ed). 2011. Temperature and soil organic matter decomposition rates-synthesis of current knowledge and a way forward. Global Change Biology. 17, 3392 – 3404.

Cotrufo M.F., Ranalli M.G., Haddix M.L., Six J., Lugato E. 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. Nature Geoscience. 12, 989 – 994.

Cunha TJ.F., Novotny E.H., Madari B.E., Martin-Neto L., Rezende M.O., Canelas L.P., Benitesm V.M. 2009. Spectroscopy Characterization of Humic Acids Isolated from Amazonian Dark Earth Soils (Terra Preta De Índio). In: Woods W.I., Teixeira W.G., Lehmann J., Steiner C. (Ed.). Amazonian Dark Earths: Wim Sombroek's Vision. Springer: Dordrecht, Dordrecht, Netherlands, 363 – 372 pp.

Curaqueo G., Barea J.M., Acevedo E., Rubio R., Cornejo P., Borie F. 2011. Effects of different tillage system on arbuscular mycorrhizal fungal propagules and physical properties in a Mediterranean agro-ecosystem in central Chile. Soil and Tillage Research. 113, 11 – 18.

Curtin D., Syers J.K. 1990. Extractability and adsorption of sulphate in soils. Journal of Soil Science. 41, 305 – 312.

ČUZK. 2021. Souhrnné přehledy o půdním fondu z údajů katastru nemovitostí České republiky. Český úřad zeměměřický a katastrální. Dostupné online na: https://cuzk.cz/Periodika-a-publikace/Statisticke-udaje/Souhrne-prehledy-pudniho-fondu/Rocenka_pudniho_fondu_2021-(1).aspx (otevřeno 28. 2. 2022).

Dai J., Hu J., Lin X., Yang A., Wang R., Zhang J., Wong M.H. 2013. Arbuscular mycorrhizal fungal diversity, external mycelium length, and glomalin-related soil protein content in response to long-term fertilizer management. Journal of Soils and Sediments. 13, 1 – 11.

Davis M.R., Alves B.J.R., Karlen D.I., Kline K.I, Galdos M., Abulebdeh D. 2018. Review of soil organic carbon measurement protocols: A U.S. and Brazil comparison and recommendation. Sustainability. 10, 53.

de Melo B.A.G., Motta F.L., Santana M.H.A. 2016. Humic acids: Structural properties and multiple functionalities for novel technological developments. Materials Science and Engineering: C. 62, 967 – 974.

Demyan M.S., Rasche F., Schul E., Breulmann M., Muller T., Cadisch G. 2012. Use of specific peaks obtained by diffuse reflectance Fourier transform mid-infrared spectroscopy to study the composition of organic matter in a Haplic Chernozem. European Journal of Soil Science. 63, 189 – 199.

Deng C., Zou Y.N., Hashem A., Kuča K., Abd-Allah E.F., Wu Q.S. 2023. The visualized knowledge map and hot topic analysis of glomalin-related soil proteins in the carbon field based on Cizespace. Chemical and Biological Technologies in Agriculture, 10, 48.

Dewil R., Baeyens J., Roles J., Van De Steene B. 2008. Distribution of sulphur compounds in sewage sludge treatment. Environmental Engineering Science. 25, 879 – 886.

Doetterl S., Stevens A., Six J., Merckx R., van Oost K., Pinto M.C., Casanova-Katny A., Muñoz C., Boudin M., Venegas EZ., Boeckx P. 2015. Soil carbon storage controlled by interactions between geochemistry and climate. Nature Geoscience. 8, 780 – 783.

Dorodnikov M., Kuzyakov Y., Fangmeier A., Wesenberg G.B.L. 2011. C and N in soil organic matter density fractions under elevated atmospheric CO_2: Turnover vs. stabilization. Soil Biolofy and Biochemistry. 43, 579 – 589.

Driver J.D., Holben W.E. Rillig M.C. 2005. Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry. 37, 101 – 106.

Dubuis P.H., Marazzi C., Stadler E., Mauch F. 2005. Sulphur deficiency causes a reduction in antimicrobial potential and leads to increased disease susceptibilityof oilseed rape. Journal of Phytopathology. 153, 27 – 36.

Dumale J.W.A., Miyazaki T., Nishimura T., Seki K. 2009. CO_2 evolution and short-term carbon turnover in stable soil organic carbon from soils applied with fresh organic matter. Reophysical Research Letters. 36, 1 – 6.

Dwivedi S.K., Singh R.S., Dwivedi K.N. 2002. Effect of sulphur and zinc nutrition on yield and quality of maize in typic ustochrept soil of Kanpur. Journal of the Indian Society of Soil Science. 50, 70 – 74.

Edleusa P.S., Mendes L.A., Stein A.M. 2022. Corn yield in monoculture and intercropped with cover plants and aggregates stability. European Jounral of Agriculture and Food Science. 4, 426.

EEA. 2015. Sulphur dioxide emissions. European Environment Agency. Dostupné online na: https://www.eea.europa.eu/data-and-maps/indicators/eea-32-sulphur-dioxide-so2-emissions-1/assessment-3#tab-related-briefings (otevřeno 1. 3. 2022).

Ellerbrock R.H., Gerke H., Bachman J., Goebel M. 2005. Composition of organic matter fractions for explaining Wettability of three forest soils. Soil Science Society of America Journal. 69, 57–66.

Ellerbrock R.H., Gerke H., Bachman J., Goebel M. 2005. Composition of organic matter fractions for explaining wettability of three forest soils. Soil Science Society of America Journal. 69, 57 – 66.

Emran M., Gispert M., Pardini, G. 2012. Patterns of soil organic carbon, glomalin and structural stability in abandoned Mediterranean terraced lands. European Journal of Soil Science. 63, 637 – 649.

Engart M., Simpson, D., Schwikowski M., Granat L. 2017. Deposition of sulphur and nitrogen in Europe 1900–2050. Model calculations and comparison to historical observations. Chemical and Physical Meteorology. 69 (1), 1328945.

Ercoli L., Adruini I., Mariotti M., Lulli L. 2012. Management of sulphur fertiliser to improve durum wheat production and minimise S leaching. European Journal of Agronomy. 38 (1), 74 – 82.

Eriksen J. 1996. Incorporation of S into soil organic matter in the field as determined by the natural abundance of stable S isotopes. Biology and Fertility of Soils. 22, 149-155.

Eriksen J. 1997. Sulphur cycling in Danish agricultural soils: Inorganic sulphate dynamics and plant uptake. Soil Biology and Biochemistry. 29, 1379 - 1385.

Eriksen J., Mortensen J.V., Kjellerup V.K., Kristjansen O. 1995. Forms and plant availability of sulfur in cattle and pig slurry. Zeitschrift für Pflanzenernährung und Bodenkunde. 158, 113 - 116.

Eriksen J., Murphy M.D., Schnug E. 1998. Soil Sulphur Cycle. In: Schnug E., Heneklaus S. (Eds.). Sulphur in Agroecosystems. Springer, Dordrecht, 39 – 73 pp.

Etienne P., Sorin E., Maillard A., Gallardo K., Arkoun M., Guerrand J, Cruz F., Yvin J.C., Ourry A. 2018. Assessment of sulfur deficiency under field conditions by single measurements of sulfur, chloride and phosphorus in mature leaves. Plants. 7, 37.

Federici E., Massaccesi L., Pezzolla D., Fidati L., Montalbani E., Proietti P., Nasini L., Regni L., Scargetta S., Gigliotti G. 2017. Short-term modifications of soil microbial community structure and soluble organic matter chemical composition following amendment with different solid olive mill waste and their derived composts. Applied Soil Ecology. 119, 234–241.

Feinberg A., Stenke A., Peter T., Hinckley E.L.S., Driscoll C.T., Winkel L.H.E. 2021. Reductions in the deposition of sulfur and selenium to agricultural soils pose risk of future nutrient deficiencies. Communications Earth and Environment. 2, 101.

Fér M., Leue M., Kodešová R., Gerke H.H., Ellerbrock R.H. 2016. Droplet infiltration dynamics and soil wettability related to soil organic matter of soil aggregate coatings and interiors. Journal of Hydrology and Hydromechanics. 64, 111 – 120.

Fiedler S.R. Buczko U., Jurasinski G., Glatzel S. 2015. Soil respiration after tillage under different fertiliser treatments – implications for modelling and balancing. Soil and Tillage Research. 150, 30 – 42.

Fismes J., Vong P.C., Guckert A. Frossard E. 2000. Influence of sulfur on apparent N-use efficiency, yield and quality of oilseed rape (*Brassica napus* L.) grown on a calcareous soil. European Journal of Agronomy. 12, 127 – 141.

Fitzgerald J.W., Strickland T.C., Swank W.T. 1982. Metabolic fate of inorganic sulphate in soil samples from undisturbed and managed forest ecosystems. Soil Biology and Biochemistry. 14, 529-536.

Flaete N.E.S., Hollung K., Ruud L., Sogn T., Faergestad E.M., Skarpeid H.J., Magnus E.M., Uhlen A.K. 2005. Combined nitrogen and sulphur fertilisation and its effect on wheat qualityand protein composition measured by SE - FPLC and proteomics. Journal of Cereal Science. 41, 357 – 369.

Förster S., Welp G., Scherer H.W. 2012. Sulfur specification in bulk soil as influenced by long-term application of mineral and organic fertilizers. Plant Soil and Environment. 58, 316-321.

Fox G.P., Panozzo J.F., Li C.D., Lance R.C.M., Inkerman P.A., Henry R.J. 2003. Molecular basis of barley quality. Australian Journal of Agricultural Research. 54, 1081 – 1101.

Franco-Zorrilla J.M., Martin, A.C., Leyva A., Par-Ares J.P. 2005. Interaction between phosphate-starvation, sugar, and cytokinin signaling in *Arabidopsis* and the roles of cytokinin receptors CRE1/AHK4 and AHK3. Plant Physiology. 138, 847–857.

Freney J.R. 1986. Forms and reactions of organic sulfur compounds in soils. In: Tabatabai MA. (Eds.). Sulfur in Agriculture. Agronomy Monograph No. 27. ASA-CSSA-SSSA, Madison.

Freney J.R., Melville G.E., Williams C.H. 1970. The determination of carbon bonded sulphur in soils. Soil Science. 109, 310 – 318.

Fuentes M., Baigorri B., González-Gaitano G., García-Mina J. 2018. New methodology to assess the quantity and quality of humic substances in organic materials and commercial products for agriculture. Journal of Soils and Sediments. 18, 1389 – 1399.

Fuentes-Lara L.O., Medrano-Macías J., Pérez-Labrada F., Rivas-Martínez E.N., García-Enciso E.L., González-Morales S., Juárez-Maldonado A., Rincón-Sánchez F., Benavides-Mendoza A. 2019. From elemental sulfur to hydrogen sulfide in agricultural soils and plants. Molecules. 24, 2282.

Gadkar V., Rillig M.C. 2006. The arbuscular mycorrhizal fungal protein glomalin is a putative homolog of heat shock protein 60. FEMS Microbiology Letters. 263, 93 – 101.

Galazka A., Gawryjolek K., Grzadziel J., Ksiezak J. 2017. Effect of different agricultural management practices on soil biological parameters including glomalin fraction. Plant, Soil and Environment. 63, 300–306.

Gao Y., Ma M., Yang T., Chen W., Yang T. 2018. Global atmospheric sulfur deposition and associated impaction on nitrogen cycling in ecosystems. Journal of Cleaner Production. 195, 1 – 9.

Garsed S.G. 1985. SO_2 Uptake and Transport. In: Winner W.E., Mooney H.A., Goldstein R.A. (Eds.). Sulphur Dioxide and Vegetation. Stanford University Press, California, United States 75 – 95 pp.

Ghani A., Dexter M., Perrott K.W. 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biology and Biochemistry. 35, 1231 – 1243.

Ghani A., McLaren R.G., Swift, R.S. 1992. Sulphur mineralisation and transformations in soils as influenced by additions of carbon, nitrogen and sulphur. Soil Biology and Biochemistry. 24, 331 - 341.

Gillespie A.W., Farrell R.E., Walley F.L., Ross A.R.S., Leinweber P., Eckhardt K.U., Regier T.Z., Blyth R.I.R. 2011. Glomalin-related soil protein contains non-mycorrhizal-related heat-stable proteins, lipids and humic materials. Soil Biology and Biochemistry. 43, 766 – 777.

Gispert M., Emran M., Pardini G., Doni S., Ceccanti B. 2013. The impact of land management and abandonment on soil enzymatic activity, glomalin content and aggregate stability. Geoderma. 202 – 203, 51 – 61.

Godlewska A. 2018. Sulphur content in test plants and arylsulfatase activity in soil after application of waste materials. Applied Ecology and Environmental Research. 16, 145 – 152.

Goloran J., Chen C., Phillips I., Xu, Z. 2014. Plant phosphorus availability in rehabilitated bauxite-processing residue sand. Plant and Soil. 374 (1 – 2), 565 – 578.

Gosling P., Hodge A., Goodlass G., Bending G.D. 2006. Arbuscular mycorrhizal fungi and organic farming. Agriculture, Ecosystems and Environment. 113, 17 - 35.

Gourav N.K.S., Shrama R.P., Sharma G.D. 2018. Vertical distribution of sulfur fractions in a continuously fertilized acid alfisol under maize-wheat cropping system. Communications in Soil Science and Plant Analysis. 49, 923 – 933.

Grant C.A., Mahli S.S., Karamanos R.E. 2012. Sulfur management for rapeseed. Field Crops Research. 128, 119 – 128.

Gregorich E.G., Beare M.H., Stoklas U., St-Georges P. 2003. Biodegradability of soluble organic matter in maize-cropped soils. Geoderma. 113, 237 – 252.

Grobler L., Bloem A.A., Claassens A.S. 1999. A critical soil sulphur level for maize (*Zea mays* L.) grown in a glasshouse. South African Journal of Plant and Soil. 16, 204 – 206.

Guan X.K., Wei L., Turner C.N., Ma S.C., Yang M.D., Wang, T.C. 2020. Improved straw management practices promote in situ straw decomposition and nutrient release, and increase crop production. Journal of Cleaner Production. 20, 119514.

Günal S., Hardman R., Kopriva S., Mueller J.W. 2019. Sulfation pathways from red to green. Journal of Biological Chemistry. 294, 12293 – 12312.

Gunina A., Kuzyakov Y. 2015. Sugars in soil and sweets for microorganisms: Review of origin, content, composition and fate. Soil Biology and Biochemistry. 90, 87 – 100.

Haas C., Gerker H.H., Ellerbrock R.H., Hallet P.D., Horn R. 2018. Relating soil organic matter composition to soil water repellency for soil biopore surfaces different in history from two Bt horizons of a Haplic Luvisol. Ecohydrology. 11, e1949.

Hamza M.A., Anderson W.K. 2005. Soil compaction in cropping systems - a review of the nature, causes and possible solutions. Soil and Tillage Research. 82, 121 – 145.

Harner M.J., Ramsey P.W., Rillig M.C. 2004. Protein accumulation and distribution in floodplain soils and river foam. Ecology Letters. 7, 829 – 836.

Harrison R.B., Johnson D.W., Todd. D.E. 2009. Adsorption and desorption reversibility in a variety of forest soils. Journal of Environmental Quality. 18, 419-426.

Hawkesford M.J., De Kok L.J. 2006. Managing sulphur metabolism in plants. Plant Cell and Environment. 29, 382 – 395.

Hawkesford M.J., Kopriva S., De Kok L.J. 2016. Nutrient Use Efficiency in Plants. Springer International Publishing Switzerland, Switzerland. 279 pp.

Hawkesford, M.J., Davidian J.C. Grignon C. 1993. Sulphate/H+ co-transport in plasma membrane vesicles isolated from *Brassica napus*: increased transport in membranes isolated from sulphur-starved plants. Planta. 190, 297 – 304.

He Y.T., He X.H., Xu M.G., Zhang W.J., Yang X.Y., Huang S.M. 2018. Long-term fertilization increases soil organic carbon and alters its chemical composition in three wheat-maize cropping sites across central and south China. Soil and Tillage Research. 177, 79 – 87.

Hendrix S., Jozefczak M., Wójcik M., Deckers J., Vangronsveld J., Cuypers A. 2020. Glutathione: A key player in metal chelation, nutrient homeostasis, cell cycle regulation and the DNA damage response in cadmium-exposed *Arabidopsis thaliana*. Plant Physiology and Biochemistry. 154, 498 – 507.

Hoffland E., Kuyper T.W. 2020. Eco-functionality of organic matter in soils. Plant and Soil. 455, 1 – 22.

Holátko J., Brtnický M., Kučerík J., Kotianová M., Elbl J., Kintl A., Kynický J., Benada O., Datta R., Jansa J. 2021. Glomalin - Truths, myths, and the future of this elusive soil glycoprotein. Soil Biology and Biochemistry. 153, 108116.

Houba V.J.G, Temminghoff E.J.M., Gaikhorst G.A., van Vark W. 2000. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent, Communications in Soil Science and Plant Analysis. 3, 1299 - 1396.

Hřivna L., Ryant P., Burešová L. 2011. Vliv síry a dusíku na technologické parametry jarního ječmene. In: Kompendium 2011: sborník konference „Řízení a diagnostika klíčových momentů v technologii jarního ječmene“. 7. – 10. 2. Sdružení pro ječmen a slad, Kralupy nad Vltavou. Česká republika. 15 – 17 pp

Hsieh L.C., Lin S.I., Shih A.C.C., Chen J.W., Lin W.Y., Tseng C.Y., Li W.H., Chiou T.J. 2009. Uncovering small RNA-mediated responses to phosphate deficiency in *Arabidopsis* by deep sequencing. Plant Physiology. 151, 2120 – 2132.

Hu Z., Heneklaus S., Wang S., Xu C.H. Cao Z., Schnug E. 2003. Comparison of mineralization and distribution of soil sulfur fractions in the rhizosphere of oilseed rape and rice. Communications in Soil Science and Plant Analysis. 34, 2243-2257.

Hu Z.Y., Zhao F.J., McGrath S.P. 2005. Sulphur fractionation in calcareous soils and bioavailability to plants. Plant and Soil. 68, 103–109.

Chan K.X., Phua S.Y., Van Breusegem F. 2019. Secondary sulfur metabolism in cellular signalling and oxidative stress responses. Journal of Experimental Botanny. 70, 4237 – 4250.

Chapman S.J. 1997. Barley straw decomposition and S immobilization. Soil Biology and Biochemistry. 29, 109 - 114.

Chen B., Shan X.Q., Shen D.Q., Mou S.F. 1997. Nature of the HCl soluble sulfate in the sequential extraction for sulfur speciation in soils. Fresenius’ Journal of Analytical Chemistry. 357, 941–945

Chievenge P.P., Murwira H.K., Giller K.E., Mapfumo P., Six J. 2007. Long-term impact of tillage and residue management on soil carbon stabilization: Implications for conservation agriculture on contrasting soils. Soil andTillage Research. 94, 328 – 337

CHMI. 2019. Český hydrometeorologický ústav. Znečištění ovzduší na území České republiky v roce 2019. Dostupné online na: https://www.chmi.cz/files/portal/docs/uoco/isko/grafroc/19groc/gr19cz/09_depozice_v1.pdf (otevřeno 10. 08. 2020).

CHMI. 2020. Znečištění ovzduší na území České republiky v roce 2020. Český hydrometeorologický ústav. Dostupné online na: https://www.chmi.cz/files/portal/docs/uoco/isko/grafroc/20groc/gr20cz/20_04_7_SO2_v2.pdf (otevřeno 1. 3. 2022).

Chowdhury M.A.H., Kuono K., Ando T. 1999. Correlation among microbial biomass S, soil properties, and other biomass nutrients. Soil Science and Plant Nutrition. 45, 175-186.

Christensen B.T. 1985. Wheat and barely straw decomposition under field conditions: Effect of soil type and plant cover on weight loss, nitrogen and potassium content. Soil Biology and Biochemistry. 17, 691 – 697.

Irving T.B., Alptekin B., Kleven B., Ané J.M. 2021. A critical review of 25 years of glomalin research: A better mechanical understanding and robust quantification techniques required. New Phytologist. 232, 1572 – 1581.

Islam M.R. (Ed). 2021. Mineralization of farm manures and slurries under aerobic and anaerobic conditions for subsequent release of phosphorus and sulphur in soil. Sustainability. 13, 8605.

Jaćimović G., Aćin V., Mirosavljević M., Brbaklić L., Vujić S., Dunderski D., Šeremešić S. 2023. Effects of combined long-term straw return and nitrogen fertilization on wheat productivity and soil properties in the wheat-maize-soybean rotation system in the Pannonian plain. Agronomy. 13, 1529.

Jaggi A.C., Aulakh M.S., Sharma R. 1999. Temperature effects on soil organic sulphur mineralization and elemental sulfur oxidation in subtropical soils of varying pH. Nutrient Cycling in Agroecosystems. 54, 175-182.

Jandák J., Pokorný E., Prax A. 2007. Půdoznalství 3, Mendelova zemědělská a lesnická Univerzita v Brně. Brno. Česká republika. 142 pp.

Jarecki M.K., Lal R. 2003. Crop management for soil carbon sequestration. Critical Reviews in Plant Sciences. 22, 471 – 502.

Jastrow J.D. 1996. Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. Soil Biology and Biochemistry. 28, 665 – 676.

Jeschke V., Weber K., Moore S.S., Burow M. 2019. Coordination of glucosinolate biosynthesis and turnover under different nutrient conditions. Frontiers in Plant Science. 10, 1560.

Jez J.M. 2019. Structural biology of plant sulfur metabolism: From sulfate to glutathione. Journal of Experimental Botanny. 70, 4089 – 4103.

Jha P., Hati K.M., Dalal R.C., Dang Y.P., Kopittke P.M., McKenna B.A., Menzies N.W. 2022. Effects of 50 years of no-tillage, stubble retention, and nitrogen fertilization on soil respiration, easily extractable glomalin, and nitrogen mineralization. Agronomy. 12, 151.

Jiang J.P., Xiong Y.C., Jiang H.M., Ye D.Y., Song Y.J., Li G.M. 2009. Soil microbial activity during secondary vegetation succession in semiarid abandoned lands of Loess plateau. Pedosphere. 19, 735 – 747.

Jien S.H., Chen, T.H., Chiu C.Y. 2011. Effects of afforestation on soil organic matter characteristics under subtropical forests with low elevation. Journal of Forestry Research. 16, 275 – 283.

Jimenez J., Vedrenne F., Denis C., Mottet A., Déléris S., Steyer J.P., Rivero J.A.C. 2013. A statistical comparison of protein and carbohydrate characterisation methodology applied on sewage sludge samples. Water Research. 47, 1751 – 1762.

Johnson C.M., Nishita H. 1952. Microestimation of sulfur (in plant materials, soils, and irrigation waters). Analytical Chemistry. 24, 736-742.

Jorge-Araújo P., Quiquampoix H., Matumoto-Pintro P.T., Staunton S. 2014. Glomalin related soil protein in French temperate forest soils: Interference in the Bradford assay caused by co-extracted humic substances. European Journal of Soil Science. 66, 311 – 319.

Justes E., Mary B., Meynard J.M., Machet J.M., Thelierhuche L. 1994. Determination of a critical nitrogen dilution curve for winter-wheat crops. Annals of Botany. 74, 397 – 407.

Abbsi K.M., Tahir M.M., Sabir N., Khurshid M. 2015. Impact of the addition of different plant residues on nitrogen mineralization–immobilization turnover and carbon content of a soil incubated under laboratory conditions. Solid Earth. 6, 197 – 205.

Kallenbach C.M., Frey S.D., Grandy A.S. 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature Communications. 7, 13630.

Kataoka T., Watanabe-Takahashi A., Hayashi N., Ohnishi M., Mimura T., Buchner P., Hawkesford MJ., Yamaya T., Takahashi H. 2004. Vacuolar sulfate transporters are essential determinants controlling internal distribution of sulfate in *Arabidopsis*. The Plant Cell. 16, 2693 – 2704

Kätterer T., Bolinder MA., Andren O., Kirchmann H., Menichetti L. 2011. Roots contribute more to refractory soil organic matter than above-ground crop residues, as revealed by a long-term field experiment. Agriculture, Ecosystems & Environment. 141, 184–192.

Ketterings Q., Miyamoto C., Mathur R.R., Dietzel K., Gami S. 2011. A comparison of soil sulfur extraction methods. Soil Science Society of America Journal. 75, 1578 – 1583.

Kawasaki S., Ikeya K., Sugiura Y., Watanabe A. 2015. Changes in the composition of humic acids in various upland field soils with a continuous application of an organic amendment as revealed by fractional precipitation analysis. Soil Science and Plant Nutrition. 61, 450 – 460.

Kertesz M.A. 1999. Riding the sulfur cycle/metabolism of sulfonates and sulfate esters in gram negative bacteria. FEMS Microbiology Reviews. 24, 135 – 175.

Khan M.J., Khan M.H., Khattak R.A., Jan M.T. 2006. Response of maize to different levels of sulphur. Communications in Soil Science and Plant Analysis. 37, 41 – 51.

Kielkopf C.O., Bauer W., Urbatch I.L. 2020. Bradford assay for determining protein concentration. Cold Spring Harbor Protocols. 2020, 102269.

Kinsela A.S., Denmead T.O., Macdonald B.C.T., Melville M.D., Reynolds J.K., White I. 2011. Field-based measurements of sulfur gas emissions from an agricultural coastal acid sulfate soil, eastern Australia. Soil Research. 49, 471 – 480.

Klose S., Moore J.M., Tabatabai M.A. 1999. Arylsulfatase activity of microbial biomass in soils as affected by cropping systems. Biology and Fertility of Soils. 29,46-54.

Klose S., Tabatabai MA. 1999. Arylsulfatase activity of microbial biomass. Soil Science Society of America Journal. 63, 569 - 574.

Knights J.S., Zhao F.J., McGrath S.P., Magan N. 2001. Long-term of land use and fertiliser treatments on sulphur transformations in soils from the Broadbalk experiment. Soil Biology and Biochemistry. 33, 1797– 1804.

Knights J.S., Zhao F.J., Spiro B., McGrath S.P. 2000. Long-term effects of land use and fertilizer treatments on sulfur cycling. Journal of Environmental Quality. 29, 1867 – 1874.

Koide R.T., Peoples M.S. 2013. Behavior of Bradford-reactive substances is consistent with predictions for glomalin. Applied Soil Ecology, 63, 8 – 14.

Kononova M.M. 1966. Soil Organic Matter: Its nature, its role in soil formation and in soil fertility. 2nd ed. Pergamon Press. Oxford. Velká Británie. 544 pp.

Kopáček J., Hejzlar J., Porcal P., Posch M. 2014. Sulphate leaching from diffuse agricultural and forest sources in a large central European catchment during 1900–2010. Science of the Total Environment. 470 - 471, 543 – 550.

Korentajer L., Brynes B.H., Hellums D.T. 1983. The effect of liming and leaching on the sulfur-supplying capacity of soils. Soil Science Society of America Journal. 41, 525 - 530.

Körschens M. (Ed). 2013. Effect of mineral and organic fertilization on crop yield, nitrogen uptake, carbon and nitrogen balances, as well as soil organic carbon content and dynamics: results from 20 European long-term field experiments of the twenty-first century. Archives of Agronomy and Soil Science. 59, 1017 – 1040.

Kotyk A. 2003. Klasifikace transportních proteinů. Chemické Listy. 97. 37 – 40.

Koven C.D., Hugelius G., Lawrence D.M., Wieder W.R. 2017. Higher climatological temperature sensitivity of soil carbon in cold than warm climates. Nature Climate Change. 7, 817 – 822.

Kowalenko C.G., Grimmett M. 2007. Chemical characterization of soil sulfur. In: Carter MR., Gregorich EG. (Eds.). Soil Sampling and Methods of Analysis. Taylor and Francis. Florida. 251 - 261 pp.

Kowalenko G.G., Bittman S., Neilsen G.H., Kennyes E. Hunt D.E., Neilsen D. 2014. Potential for improving sulfur tests on agricultural soils in contrasting ecoregions of British Columbia, Canada. Geoderma Regional. 1, 10 – 20.

Krupová D., Pavlendová H., Pavlenda P., Šrámek V. 2018. Atmospheric deposition of sulphur and nitrogen in forests of the Czech and Slovak Republic. Forestry Journal. 64, 249 – 258.

Kukuis I., Klavinš M., Nikodemus O., Kasparinskis R., Brūmelis G. 2019. Changes in soil organic matter and soil humic substances following the afforestation of former agricultural lands in the boreal-nemoral ecotone (Latvia). Geoderma Regional. 15, e00213.

Kulhánek M., Balík J., Černý J., Sedlář O., Vašák F. 2016. Evaluating of soil sulfur forms changes under different fertilizing systems during long-term field experiments. Plant, Soil and Environment. 62, 408 – 415.

Kulhánek M., Černý J., Balík J., Peklová L. 2014. Winter wheat fertilizing using nitrogen-sulphur fertilizer. Archives of Agronomy and Soil Science. 60, 771260.

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

Kuśmierz S., Skowrońska M., Tkaczyk P., Lipiński W., Mielniczuk J. 2023. Soil Organic Carbon and Mineral Nitrogen Contents in Soils as Affected by Their pH, Texture and Fertilization. Agronomy. 13, 267.

Kwiatkowska-Malina J. 2018. Qualitative and quantitative soil organic matter estimation for sustainable soil management. Journal of Soil & Sediments. 18, 2801 – 2812.

Ladoni M., Basir A., Kravchenko A. 2015. Which soil carbon fraction is the best for assessing management differences? A statistical power perspective. Soil Science Society of America Journal. 79, 848.

Lapa V.V., Seraya T.M., Bogatyreva E.N., Biryukova O.M. 2011. The effect of long term fertilizer application on the group and fractional composition of humus in a soddy-podzolic light loamy soil. Eurasian Soil Science. 44, 100 – 104.

Laskosky J.D., Mante A.A., Zvomuya F., Amarakoon I., Leskiw L. 2020. A bioassay of long-term stockpiled salvaged soil amended with biochar, peat, and humalite. Agrosystems, Geosciences and Environement. 3, e20068.

Lavanya K.R., Kadalli G.G., Patil S., Jayanthi T., Naveen D.V., Channabasavegowda R. 2019. Sulphur fractionation studies in soils of long term fertilizer experiment under finger millet – maize cropping sequence. International Journal of Current Microbiology and Applied Sciences. 8, 1334 – 1345.

Lehmann J., Hansel C.M., Kaiser, C., Markus K., Kate M., Stefano M. 2020. Persistence of soil organic carbon caused by functional complexity. Nature Geoscience. 13, 529 – 534.

Lehmann J., Kleber M. 2015. The contentious nature of soil organic matter. Nature. 528, 60 – 68.

Lehmann J., Solomon D., Zhao F., McGrath S.P. 2008. Atmoshperic SO2 emissions since the late 1800s change organic sulfur forms in humic substance extracts of soils. Environmental Science and Technology. 42, 3550 – 3555.

Leinemann T., Preusser S., Mikutta R., Kalbitz K., Cerli C., Höschen C., Mueller C.W., Kandeler E., Guggenberger G. 2018. Multiple exchange processes on mineral surfaces control the transport of dissolved organic matter through soil profiles. Soil Biology and Biochemistry. 118, 79 – 90.

Leinweber P., Jandl G., Baum C., Eckhardt K.U., Kandeler E. 2008. Stability and composition of soil organic matter control respiration and soil enzyme activities. Soil Biology and Biochemistry. 40, 1496 – 1505.

Lerner S.E., Seghezzo M.L., Molfese E.R., Ponzio N.R., Cogliatti M., Rogers W.J. 2006. N- and S-fertiliser effects on grain composition, industrial quality and end-use in durum wheat. Journal of Cereal Science. 44, 2 – 11.

Leue M., Gerke H.H., Ellerbrock R.H. 2013. Millimetre-scale distribution of organic matter composition at intact biopore and crack surfaces. European Journal of Soil Science. 64, 757 – 769.

Leue M., Hoffman C., Hierold W., Sommer M. 2019. In-situ multi-sensor characterization of soil cores along an erosion-deposition gradient. Catena. 182, 104140.

Leusteck T., Saito K. 1999. Sulfate transport and assimilation in plants. Plant Physiology. 120, 637 – 644.

Li D., Li Z., Zhao B., Zhang J. 2020a. Relationship between the chemical structure of straw and composition of main microbial groups during the decomposition of wheat and maize straws as affected by soil texture. Biology and Fertility of Soils. 56, 11 – 24.

Li P., Jia L., Chen Q., Zhang H., Deng J., Lu J., Xu L., Li X., Hu F., Jiao J. 2024. Adaptive evaluation for agricultural sustainability of different fertilizer management options for a green manure-maize rotation system: Impacts on crop yield, soil biochemical properties and organic carbon fractions. Science of the Total Environment. 908, 168170.

Li Q., Gao Y., Yang A. 2020b. Sulfur homeostasis in plants. International Journal of Molecular Sciences. 21, 8926.

Li X., Han S., Luo X., Chen W., Huang Q. 2019. Arbuscular mycorrhizal-like fungi and glomalin-related soil protein drive the distributions of carbon and nitrogen in a large scale. Journal of Soils and Sediments. 20, 963 – 972.

Li X., Han S., Luo X.S., Chen W.L., Huang Q.Y. 2020c. Arbuscular mycorrhizal-like fungi and glomalin-related soil protein drive the distributions of carbon and nitrogen in a large scale. Journal of Soils and Sediments. 20, 963–972.

Liang B.C., MacKenzie A.F. 1992. Changes in soil organic carbon and nitrogen after six years of corn production. Soil Science. 153, 307 – 313.

Liang G., Wu H., Houssou A.A, Cai D., Wu X., Gao L., Wang B., Li S. 2017. Soil respiration, glomalin content, and enzymatic activity response to straw application in a wheat-maize rotation system. Journal of Soils and Sediments. 18, 697 – 707.

Liang Q., Chen H., Gong Y., Fan M., Yang H., Lal R., Kuzyakov Y. 2012. Effects of 15 years of manure and inorganic fertilizers on soil organic carbon fractions in a wheat-maize system in the North China Plain. Nutrient Cyling in Agroecosystems. 92, 21 – 33.

Lipson D.A., Schmidt S.K., Monson R.K. 1999. Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. Ecology. 80, 1623 – 1631.

Lipson D.A., Schmidt S.K., Monson R.K. 2000. Carbon availability and temperature control the post-snowmelt decline in alpine soil microbial biomass. Soil Biology and Biochemistry. 32,441 – 448.

Liu R.C., Gao W.Q., Srivastava A.K., Zou Y.N., Kuča K., Hashem A., Abd-Allah E.F., Wu Q.S. 2021. Differential effects of exogenous glomalin-related soil proteins on plant growth of trifoliate orange through regulating auxin changes. Frontiers in Plant Science. 12, 745402.

Loges R., Bunne I., Reinsch T., Malisch C., Kluß‌ C., Herrmann A., Taube F. 2018. Forage production in rotational systems generates similar yields compared to maize monocultures but improves soil carbon stocks. European Jounral of Agronomy. 97, 11 – 19.

Lorenz K., Lal R. 2014. Soil organic carbon sequestration in agroforestry systems. A review. Agronomy for Sustainable Development. 34, 443 – 454.

Lou G., Warman P.R. 2004. Labile ester sulphate in organic matter extracted from podzolic soils. Biology and Fertility of Soils. 14, 267 - 271.

Lovelock C., Wright S.F., Clark D.A., Ruess R.W. 2004. Soil stocks of glomalin produced by arbuscular mycorrhizal fungi across a tropical rain forest landscape. Journal of Ecology, 92, 278 – 287.

Ludwig B., John B., Ellerbrock R., Kaiser M., Flessa H. 2003. Stabilization of maize in sandy soil in a long-term experiment. European Journal of Soil Science. 54, 117 – 126.

Luna L., Miralles I., Andrenelli M.C., Gispert M., Pellergini S., Vignozzi N., Solé-Benet A. 2016. Restoration techniques affect soil organic carbon, glomalin and aggregate stability in degraded soils of a semiarid Mediterranean region. Catena. 143, 256 – 264.

Luo Z., Wang E., Sun O.J, 2010. Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. Agriculture, Ecosystems & Environment. 139, 224 – 231.

Ma Y.Y., Zhang H.C., Xiang X.J., Wang D.Z. 2018. Effects of long-term fertilization on arbuscular mycorrhizal fungal community in lime concretion black soil. The Journal of Applied Ecology. 29, 3398 – 3406.

MacCarthy P. 2001. The principles of humic substances, Soil Science. 166, 738 – 751.

Magdoff F., Van es H. 2021. Building Soils for Better Cropts, Ecological management for healthy soils. Sare Outreach, Maryland, United States. 410 pp.

Magdoff F., Weil R.R. 2004. Soil organic matter management strategies. In: Magdoff F., Weil R.R. (Ed.). Soil organic matter in sustainable agriculture. Boca Raton: CRC Press, Florida, United States. 45 – 65 pp.

Margenot A.J., Pulleman M.M., Sommer R., Paul B.K., Parikh S.J., Jackson L.E., Fonte S.J., 2017. Biochemical proxies indicate differences in soil C cycling induced by longterm tillage and residue management in a tropical agroecosystem. Plant and Soil. 420, 315 – 329.

Marchetto A., Simpson D, Aas W., Fagerli H., Hansen K., Pihl-Karlsson G., Karlsson P.E., Rogora M., Sanders T.G.M., Schmitz A., Seidling W., Thimonier A., Tsyro S., de Vries W., Waldner P. 2021. Good agreement between modeled and measured sulfur and nitrogen deposition in Europe, in spite of marked differences in some sites. Frontiers in Environmental Science. 9:734556.

Marschner P. 2012. Marschner´s Mineral Nutrition of Higher Plants. 3rd ed. Academic Press. London. 651 pp.

Martin M.P., Wattenbach M., Smith P., Meersmans J., Jolivet C., Boulonne L., Arrouays D. 2011. Spatial distribution of soil organic carbon stocks in France. Biogeosciences. 8, 1053–1065.

Martinez C.E., Kleinschmidt A.W., Tabatabai M.A. 1998. Sulfate adsorption by variable charge soils: Effect of low-molecular-weight organic acids. Biology and Fertility of Soils. 26, 157 - 163.

Maruyama-Nakashita A., Ohkama-Ohtsu N. 2017. Sulfur assimilation and glutathione metabolism in Plants. In: Hossain M.A., Mostofa M.G., Diaz-Vivancos P., Burritt D.J., Fujita M., Tran L.S.P. (Eds.). 2017. Glutathione in Plant Growth, Development, And Stress Tolerance. Springer International Publishing, Switzerland, 287 – 308 pp.

Maruyama-Nakashita A., Watanabe-Takahashi A., Inoue E., Yamaya T., Saito K., Takahashi H. 2015. Sulfur-responsive elements in the 3′-nontranscribed intergenic region are essential for the induction of sulfate transporter 2;1 gene expression in *Arabidopsis* roots under sulfur deficiency. The Plant Cell. 27, 1279 – 1296.

Mattila T.J., Rajala J. 2022. Do different agronomic soil tests identify similar nutrient deficiencies? Soil Use and Management. 38, 635 – 648.

Matula J. 1999. Use of multinutrient soil tests for sulphur determination. Communications in Soil Science and Plant Analysis. 30, 1733 – 1746.

Matula J. 2007. Výživa a hnojení sírou - metodika pro praxi. Výzkumný ústav rostlinné výroby, v.v.i., Praha, 39 pp.

McGill W.B., Cole C.V. 1981. Comparative aspects of cycling of C, N, S and P through soil organic matter. Geoderma 26, 267-286.

McGrath S.P., Zhao F.J. 1996. Sulphur uptake, yield responses and interaction between nitrogen and sulphur in winter oilseed rape (*Brassica napus*). Journal of Agriculture Science. 126, 53 - 62.

McGrath S.P., Zhao F.J., Blake-Kalff M.M. 2002. History and outlook for sulphur fertilisers in Europe. Proceedings No. 497. International Fertiliser Society, York, United Kingdom.

McLaren R.G., Cameron K.C. 1996. Soil, Plant and Fertilizer Sulphur. In: McLaren R.G., Cameron K.C. (Eds). Soil Science. Oxford University Press. Auckland. 221 – 228 pp.

McLaren R.G., Keer J.I., Swift R.S. 1985. Sulphur transformations in soils using sulphur-35 labelling. Soil Biology and Biochemistry. 17, 73 – 79.

Mehlich A. 1953. Determination of P, Ca, Mg, K, Na and NH_4 by North Carolina Soil Testing Laboratories. Raleigh, University of North Carolina, United States.

Mehlich A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Communications in Soil Science and Plant Analysis. 15, 1409 – 1416.

Mehta Y.K., Shaktawat M.S., Singhi S.M. 2005. Influence of sulphur, phosphorus and farmyard manure on yield attributes and yield of maize (*Zea mays*) in southern Rajasthan conditions. Indian Journal of Agronomy. 50, 203 – 205.

Meier I.C. Leuschner C. 2010. Variation of soil and biomass carbon pools in beech forests across a precipitation gradient. Global Change Biology. 16, 1035 – 1045.

Meng X., Zhang X., Li Y., Jiao Y., Fan L., Jiang Y., Qu C., Filimonenko E., Jiang Y., Tian X., Shi J., Kuzyakov Y. 2024. Nitrogen fertilizer builds soil organic carbon under straw return mainly via microbial necromass formation. Soil Biology and Biochemistry. 188, 109223.

Mengel K., Kirkby E.A., Kosegarten H., Appel, T. 2001. Principles of Plant Nutrition. 5th ed. Springer Netherlands, Dordrecht, Netherlands. 880 pp.

Menšík L., Hlisnikovský L., Pospíšilová L., Kunzová E. 2018. The effect of application of organic manures and mineral fertilizers on the state of soil organic matter and nutrients in the long-term field experiment. Jounral of Soils and Sediments. 18, 2813 – 2822.

Mikha M.M., Marake M.V. 2023. Soil organic matter fractions and carbon distribution under different management in Lesotho, southern Africa. Soil Science Society of America Journal. 87, 140 – 155.

Mockeviciene I., Repsiene R., Amaleviciute-Volunge K., Karcauskiene D., Slepetiene A., Lepane, V. 2022. Effect of long-term application of organic fertilizers on improving organic matter quality in acid soil. Archives of Agronomy and Soil Science. 68, 1192 – 1204.

Morche L. 2008. S-fluxes and spatial alterations of inorganic and organic sulfur fractions in soil as well as their accumulation and depletion in the rhizosphere of agricultural crops by partial use of the radioisotope ^{35}S [dissertation thesis]. Rheinische Friedrich-Wilhelms-Universität. Bonn. Germany. 321 pp.

Mouloubou O.R., Prudent P., Mounier S., Boudenne J.L., Abaker M.G., Théraulaz F. 2016. An adapted sequential chemical fractionation coupled with UV and fluorescence spectroscopy for calcareous soil organic matter study after compost amendment. Microchemical Journal. 124, 139–148.

Mu D., Mu L., Geng X., Mohamed T.A., Wei Z. 2024. Evolution from basic to advanced structure of fulvic acid and humic acid prepared by food waste. International Journal of Biological Macromolecules. 256, 128413.

Mustafa A., Grouz J., Naveed M., Ping Z., Nan S., Minggang X., Núnez-Delgado A. 2022. Stability of soil organic carbon under long-term fertilization: Results from ^{13}C NMR analysis and laboratory incubation. Environmental Research. 205, 112476.

MŽP. 2007. Národní program snižování emisí České republiky. Ministerstvo životního prostředí. Dostupné online na: https://www.mzp.cz/C1257458002F0DC7/cz/strategicke_dokumenty/$FILE/OOO-NPSE_2007-20200217.pdf (otevřeno 28. 2. 2022).

Nannipieri P., Grego S., Ceccanti B. 1990. Ecological Significance of Biological Activity in Soil. In: Bollag JM., Stotzky G. (Eds.). Soil Biochemistry. Marcel Dekker Inc., New York, United States 293 - 355 pp.

Narayan O.P., Kumar P., Yadav B., Dua M., Johri A.K. 2022. Sulfur nutrition and its role in plant growth and development. Plant Signaling and Behavior. 18, 2030082.

Nardi S., Schiavon M., Francioso O. 2021. Chemical structure and biological activity of humic substances define their role as plant growth promoters. Molecules. 26, 2256.

Nelson L.E. 1973. Effect of crop residues on growth of turnips and their recovery of sulfur from soils. Soil Science. 115, 447 – 454.

Neptune A.M.L., Tabatabai M.A., Hanway J.J. 1975. Sulfur fractions and carbon-nitrogen-phosphorus-sulfur relationships in some Brazilian and Iowa soils. Soil Science Society of America Journal. 39, 51-55.

Nie J., Zhou J.M., Wang H.Y., Chen X.Q., Du C.W. 2007. Effect of long-term rice straw return on soil glomalin, carbon and nitrogen. Pedosphere. 17, 295 – 302.

Nichols K.A. 2008. Characterization of Glomalin, a Glycoprotein Produced by Arbuscular Mycorrhizal Fungi. [dissertation thesis]. University of Maryland, Maryland, United States. 285 pp.

Obeng AS., Dunne J., Giltrap M., Tian F. 2023. Soil organic matter carbon chemistry signatures, hydrophobicity and humification index following land use change in temperate peat soils. Heliyon. 9, e19347.

Oktaba L., Odrobinska D., Uzarowicz L. 2018. The impact of different land uses in urban area on humus quality. Journal of Soils and Sediments. 18, 2823 – 2832.

Olk D.C., Bloom P.R., Perdue E.M., McKnight D.M., Chen Y.A., Farenhorst N., Senesi YP., Chin P., Schmitt-Kopplin N., Hertkorn N., Harir M. 2019. Environmental and agricultural relevance of humic fractions extracted by alkali from soils and natural waters. Journal of Environmental Quality. 48, 217 – 232.

Ostatek-Boczynski Z.A., Lee-Steere P. 2012. Evaluation of Mehlich 3 as a universal nutrient extractant for Australian sugarcane soils. Communications in Soil Science and Plant Analysis, 43, 623 – 630.

Ovsepyan L., Kurganova I., de Gerenyu V.L., Kuzyakov Y. 2020. Conversion of cropland to natural vegetation boosts microbial and enzyme activities in soil. Science of Total Environment. 15, 140829.

Pasley H.R., Cairns J.E., Camberate J.J., Vyn T.J. 2012. Nitrogen fertilizer rate increases rate increases plant uptake and soil availability of essential nutrients in continuous maize production in Kenya and Zimbabwe. Nutrient Cycling in Agroecosystems. 115, 373 – 389.

Pavlů L., Zádorová T., Pavlů J., Tejnecký V., Drábek O., Rojas J.R., Thai S., Penížek V. 2023. Prediction of the distribution of soil properties in deep Colluvisols in different pedogeographic regions (Czech Republic) using diffuse reflectance infrared spectroscopy. Soil and Tillage Research. 234, 105844.

Périé C., Ouimet R. 2008. Organic carbon, organic matter and bulk density relationships in boreal forest soils. Canadian Journal of Soil Science 88, 315 – 325.

Piccolo A. 2002. The supramolecular structure of humic substances: a novel understanding of humus chemistry and implications in soil sciences. Advances in Agronomy. 75, 57 – 134.

Piccolo A. 2012. The Nature of Soil Organic Matter and Innovative Soil Managements to Fight Global Changes and Maintain Agricultural Productivity. In: Piccolo A. (Ed.). Carbon Sequestration in Agricultural Soils. Heidelberg: Springer, Heidelberg, Germany. 1 – 19 pp.

Pirela H.J., Tabatabai M.A. 1988. Reduction of organic sulfur in soils with tin and phosphoric acid. Soil Science Society of America Journal. 52, 959 – 964.

Poeplau C., Don A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops - a meta-analysis. Agriculture, Ecosystems & Environment. 200, 33 – 41.

Poeplau C., Don A., Vesterdal L., Leifield J., van Wesemael B., Schumacher J., Censior A. 2011. Temporal dynamics of soil organic carbon after land-use change in the temperate zone - carbon response functions as a model approach. Global Change Biology. 17, 2415 – 2427.

Powlson D.S., Stirling C.M., Jat M.L., Gerard B.G., Palm C.A., Sanchez P.A., Cassman K.G. 2014. Limited potential of no-till agriculture for climate change mitigation. Nature Climate Change. 4, 678 – 683.

Pribyl D.W. 2010. A critical review of the conventional SOC to SOM conversion factor. Geoderma. 186, 75 – 83.

Prietzel J., Thieme J., Eusterhues K., Eichert D. 2007. Iron speciation in soils and soil aggregates by synchrotron-based X-ray microspectroscopy (XANES,μ-XANES). European Journal of Soil Science. 58 (5), 1027 – 1041.

Prietzel J., Weick C., Korintenberg J., Seybold G., Thumerer T., Treml B. 2001. Effects of repeated $(NH_4)_2SO_4$ application on sufur pool in soil, soil microbial biomass, and ground vegetation of two watersheds in the Black Forest/Germany. Plant and Soil. 230, 287 – 305.

Prosser I..M., Purves J.V., Saker L.R., Clarkson D.T. 2001. Rapid disruption of nitrogen metabolism and nitrate transport in spinach plants deprived of sulphate. Journal of Experimental Botany. 52, 113 – 121.

Qaswar M., Jing H., Ahmed W., Dongchu L., Shujun L., Lu Z., Cai A., Lisheng L., Yongmei X., Jusheng G., Huimin Z. 2020. Yield sustainability, soil organic carbon sequestration and nutrients balance under long-term combined application of manure and inorganic fertilizers in acidic paddy soil. Soil andTillage Research. 198, 104569.

Qian P., Schoenau J.J. 1995. Assessing nitrogen mineralization from organic matter using anion exchange membranes. Fertilizer Research 40: 143–148.

Qian P., Schoenau J.J. 2007. Using an anion exchange membrane to predict soil available N and S supplies and the impact of N and S fertilization on canola and wheat growth. Pedosphere. 17, 77 – 83.

Rafael M.Q., Ricardo V.C., Saenz O., Emilio S.O., Oscar P.V., Jose G.H., Jose L.M. 2006. Compositional nutrient diagnosis in maize grown in a calcareous soil. Journal of Plant Nutrition. 29, 2019 - 2033.

Rao T.N., Sharma P.K. 1997. Evaluation of Mehlich III as an extractant for availible soil sulfur. Communications in Soil Science and Plant Analysis. 28, 1033 - 1046.

Rasse D.P., Mulder J., Moni C., Chenu C. 2006. Carbon Turnover Kinetics with Depth in a French Loamy soil. Soil Science Society of America Journal. 70, 2097 – 2105.

Reussi N., Echeverria H.E., Rozas H.S. 2012. Stability of foliar nitrogen:sulfur ratio in spring red wheat and sulfur dilution curve. Journal of Plant Nutrition, 35, 990-1003.

Rheinheimer D.S., Campos B.C., Giacomini S.J., Conceição P.C., Bortoluzzi C. 2008. Comparação de métodos de determinação de carbono orgânico total no solo. Revista Brasileira de Ciência do Solo. 32, 435 – 440.

Rice J.A., MacCarthy P. 1991. Statistical evaluation of the elemental composition of humic substances. Organic Geochemistry. 17, 635 – 648.

Riley N.G., McGrath S.P. 2002. Leaching losses of sulphur from different forms of sulphur fertilizers: a field lysimeter study. Soil Use and Management. 18, 120 – 126.

Rillig M.C. 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. Canadian Journal of Soil Science.84, 355–363.

Rillig M.C., Wright S.F., Eviner V.T. 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: Comparing effects of five plant species. Plant and Soil. 238, 325 – 333.

Rillig M.C., Wright S.F., Nichols K.A., Schmidt W.F., Torn M.S. 2001. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. Plant and Soil. 233, 167 – 177.

Rogers C.W., Dari B., Schroeder K.L. 2019. Comparison of soil-test extractants for potassium, calcium, magnesium, sulfur, and micronutrients in Idaho soils. Agrosystems, Geosciences and Environment. 2, 190067.

Roldán A., Salinas-García J.R., Alguacil M.M., Caravaca F. 2007. Soil sustainability indicators following conservation tillage practices under subtropical maize and bean crops. Soil and Tillage Research. 93, 273 – 282.

Rong Y., Su Y., Wang T., Yang Q. 2016. Effect of chemical and organic fertilization on soil carbon and nitrogen accumulation in a newly cultivated farmland. Journal of Integrative Agriculture, 15, 658 – 666.

Řezáčová V., Czakó A., Stehlík M., Mayerová M., Šimon T., Smatanová M., Madaras M. 2021. Organic fertilization improves soil aggregation through increases in abundance of eubacteria and products of arbuscular mycorrhizal fungi. Scientific Reports. 11, 12548.

Saalbach E., Aigner H. 1987. Zum Diagnosewert der NaCl + $CaC1_2$ extrahierbaren Sulfatmengen von Böden. Landwirtschaftliche Forschung. 40, 8-12.

Sadej W., Źolnowsi AC. 2019. Comparison of the effect of various long-term fertilization systems on the content and fractional composition of humic compounds in Lessive soil. Plant, Soil and Environment. 65, 172 – 180.

Sahrawat L.K., Murthy K.V.S, Wani SP. 2009. Comparative evaluation of Ca chloride and Ca phosphate for extractable sulfur in soils with a wide range of pH. Jounral of Plant Nutrition and Soil Science. 172, 404 – 407.

Sakal R., Sinha R.B., Singh A.O., Bhogal N.S., Ismail M.D. 2000. Influence of sulphur on yield and mineral nutrition of crops in maize-wheat sequence. Journal of the Indian Society of Soil Science. 48, 325 – 329.

Sakal R., Sinha, R.B., Singh A.O., Bhogal N.S., Ismail M.D. 2000. Influence of sulphur on yield and mineral nutrition of crops in maize-wheat sequence. Journal of the Indian Society of Soil Science. 48, 325 – 329.

Sandeep S., Manjaiah K.M., Pal S., Singh A.K. 2016. Soil carbon fractions under maize-wheat system: Effect of tillage and nutrient management. Environmental Monitoring and Assessment. 188, 14.

Santoso D., Lefroy R.D.B., Blair G.J. 1995. Sulfur and phosphorus dynamics in an acid soil/crop system. Australian Journal of Soil Research. 33, 113 – 124

Savarese C., Drosos M., Spaccini R., Cozzolino V., Piccolo A. 2021. Molecular characterization of soil organic matter and its extractable humic fraction from long-term field experiments under different cropping systems. Geoderma. 383, 114700.

Sedlář O., Balík J., Kulhánek M., Černý J., Matěchová M., Suran P. 2021. Crop sulfur status in relation to soil sulfur determined using anion exchange membranes and Mehlich 3. Journal of Plant Nutrition. 44, 1563 – 1570.

Sedlář O., Balík J., Kulhánek M., Černý J., Suran P. 2019. Sulphur nutrition index in relation to nitrogen uptake and quality of winter wheat grain. Chilean Journal of Agricultural Research, 79, 486 - 492.

Sedlář O., Balík J., Kulhánek M., Suran P., Matěchová M. 2020. Inovace diagnostiky výživného stavu plodin fosforem a sírou. Certifikovaná metodika. ČZU v Praze. Praha. 38 pp.

Sekgota W.M. 2018. Arbuscular mycorrhizal fungi as a bio-indicator of soil health under agricultural management practices in South Africa. [diploma thesis]. Rhodes University Makhanda, Grahamstown, Republic of South Africa. 107 pp.

Shah S.A.A, Xu M., Abrar M.M., Mustafa A., Fahad S., Shah T., Shah A, Syed A, Yang X., Zhou W., Zhang S., Nan S., Shi W. 2020. Long-term fertilization affects functional soil organic carbon protection mechanisms in a profile of Chinese loess plateau soil. Chemosphere. 267, 128897.

Shah Z.H., Rehman HM., Akhtar T., Alsamadany H., Hamooh BT., Mujtaba, T., Daur I., Zahrani YA., Alzahrani HAS., Ali S., Yang SH., Chung G. 2018. Humic substances: determining potential molecular regulatory processes in plants. Frontiers in Plant Science. 9, 263.

Shan X.Q., Bin C., Zhang T.H., Li F.L., Bei W., Jin Q. 1997. Relationship between sulfur speciation in soils and plant availability. Science of the Total Environment. 199, 237 – 246.

Shan X.Q., Chen B. 1995. Determination of carbon-bonded sulfur in soils by hydriodic acid reduction and hydrogen-peroxide oxidation. Fresenius´ Journal of Analytical Chemistry. 351, 762 – 767.

Sharma M.K., Kumar M. 2020. Sulphate contamination in groundwater and its remediation: an overview. Environmental Monitoring and Assessment. 192, 74.

Schaumann G.E. 2006. Soil organic matter beyond molecular structure part I: macromolecular and supramolecular characteristics. Journal of Soil Science and Plant Nutrition. 169,145 – 156.

Scherer H.W. 2001. Sulphur in crop production – invited paper. European Journal of Agronomy. 14, 81-111.

Scherer H.W. 2009. Sulfur in soils. Journal of Plant Nutrient and Soil Science. 172, 326 – 335.

Scherer H.W., Welp G., Förster S. 2012. Sulfur fractions in particle-size separates as influenced by long-term application of mineral and organic fertilizers. Plant Soil and Environment. 58, 242-248.

Schiedung H., Bornemann L., Welp G. 2017. Seasonal variability of soil organic carbon fractions under arable land. Pedosphere. 27, 380 – 386.

Schindler F.V., Mercer E.J., Rice J.A. 2007. Chemical characteristics of glomalin-related soil protein (GRSP) extracted from soils of varying organic matter content. Soil Biology and Biochemistry. 39, 320–329.

Schmidt L., Warnstor K., Dörfel H., Leinweber P., Lange H., Merbach W. 2000. The influence of fertilization and rotation on soil organic matter and plant yields in the long-term Eternal Rye trial in Halle (Saale), Germany. Journal of Plant Nutrition and Soil Science. 163, 639 – 648.

Schnitzer M., Khan S.U. 1972. Humic Substances in the Environment. Marcel Dekker. New York. Spojené státy. 327 pp.

Schnug E., Haneklaus S. 1998. Diagnosis of Sulphur Nutrition. In: Schnug E., Heneklaus S. (Eds.). Sulphur in Agroecosystems. Springer: Dordrecht, Dordrecht, Netherlands p. 1 - 38.

Schoenau J.J., Qian P., Huang W.Z. 1993. Ion Exchange resin strips as plant root simulators. Soils and Crops Workshop. University of Saskatchewan, Saskatoon. 191 - 204 pp.

Schofield J.D. 1994. Wheat Proteins: Structure and Functionality in Milling and Bread-making. In: Bushuk W., Rasper V. (Eds.), Wheat: Production, Properties and Quality. Blackie Academic and Professional, Glasgow, Scotland. 73 – 106 pp.

Schöpp W., Posch M., Mylona S., Johansson M. 2003. Long-term development of acid deposition (1880-2030) in sensitive freshwater regions in Europe. Hydrology and Earth Systém Sciences. 7 (4), 436 – 446.

Schulp C.J.E., Veldkamp A. 2008. Long-term landscape - land use interactions as explaining factor for soil organic matter variability in Dutch agricultural landscapes. Geoderma. 146, 457 – 465.

Sible C.N., Seebauer J.R., Below F.E. 2021. Plant biostimulants: a categorical review, their implications for row crop production, and relation to soil health indicators. Agronomy 11, 1297.

Singh A.K., Rai A., Singh N. 2016. Effect of long-term land use systems on fractions of glomalin and soil organic carbon in the Indo-Gangetic plain. Geoderma. 277, 41 – 50.

Singh A.K., Zhu X., Chen C., Wu J., Yang B., Zakari S., Jiang XJ., Singh N., Lie W. 2020. The role of glomalin in mitigation of multiple soil degradation problems. Critical Reviews in Environmental Science and Technology. 52, 1604 – 1638.

Singh B., Hedley M.J., Saggar S., Francis GS. 2004. Chemical fractionation to characterize changes in sulphur and carbon in soil caused by management. European Journal of Soil Science. 55, 79 – 90.

Singh J., Kumar S. 2021. Seasonal changes of soil carbon fractions and enzyme activities in response to winter cover crops under long-term rotation and tillage systems. European Journal of Soil Science. 72, 886 – 899.

Six J., Elliot E.T., Paustian K. 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry. 32, 2099 – 2103.

Smatanová M., Komprsová I. 2019. Determination of organic carbon in soil, interpretation of results. 73 – 91 pp. In: Sborník z 25. mezinárodní konference Racionální použití hnojiv zaměřené na nové trendy ve vývoji a aplikaci hnojiv. Česká zemědělská univerzita v Praze. Praha. Česká republika.

Smith F.W., Rae A.L., Hawkesford M.J. 2000. Molecular mechanisms of phosphate and sulphate transport in plants. Biochemica et Biophysica Acta. 1465, 236 – 245.

Sokol N.W., Sanderman J., Bradford M.A. 2019. Pathways of mineral-associated soil organic matter formation: Integrating the role of plant carbon source, chemistry, and point of entry. Global Change Biology. 25, 12 – 24.

Solomon D., Legmann J., Lobe I., Martinez, CE., Tveitnes S., Du Preet CC., Amelung W. 2005. Sulphur speciation and biogeochemical cycling in long-term arable cropping of subtropical soils: evidence fromwet-chemical reduction and S K-edge XANES spectroscopy. European Journal of Soil Science. 56, 621 – 634.

Sommers L.E., Tabatabai M. A., Nelson DW. 1977. Forms of sulphur in sewage sludge. Journal of Environmental Quality. 6, 42.

Song F., Liu K., Lou Y., Kuzyakov Y., Wang Y. 2022. Divergent responses of aggregate stability to long-term mineral and organic amendments between upland and paddy soils. Journal of Soils and Sediments. 22, 2969 – 2981.

Sootahar M.K., Zeng X., Wang Y., Su S., Soothar P., Bai L., Kumar M., Zhang Y., Mustafa A., Y. 2020. The short-term effects of mineral and plant-derived fulvic acids on some selected soil properties: improvement in the growth, yield, and mineral nutritional status of wheat (*Triticum aestivum* L.) under soils of contrasting textures. Plants. 9, 205.

Sprengel C., 1826. Ueber Pflanzenhumus, Humussaüre und Humussaure Salze. Archiv für die Gesammte Naturlehre. 8, 145 – 220.

Steinfurth D., Zorb C., Braukmann F. Muhling K.H. 2012. Time-dependent distribution of sulphur, sulphate and glutathione in wheat tissues and grain as affected by three sulphur fertilization levels and late S fertilization. Journal of Plant Physiology 169, 72 - 77.

Steinke K., Rutan J., Thurgood L. 2015. Corn response to nitrogen at multiple sulfur rates. Agronomy Journal. 107 (4), 1347 – 1354.

Stevenson F.J. 1994. Humus Chemistry: Genesis, Composition, Reactions. 2nd ed. Wiley. New York. Spojené státy. 512 pp.

Stevenson F.J., Cole M.A. 1999. Cycles of Soil (Carbon, Nitrogen Phosphorus Sulfur, Micronutrients). John Wiley and Sons Publishers, Hoboken, 427 pp.

Stimler K., Montzka S.A., Berry, J.A., Rudich Y., Yakir D. 2010. Relationships between carbonyl sulfie (COS) and CO_2 during leaf gas exchange. New Phytologist. 184, 869 – 878.

Stockmann U. Adams M.A. Crawford J.W., Field D.J., Henakaarchchi N., Jenkins M., Zimmermann M. 2013. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. Agriculture, Ecosystems & Environment. 164, 80 – 99.

Sugimoto K., Sato N., Tsuzuki M. 2007. Utilization of a chloroplast membrane sulfolipid as a major internal sulfur source for protein synthesis in the early phase of sulfur starvation in *Chlamydomonas reinhardtii*. FEBS Letters. 581, 4519 – 452.

Sugiyama R., Hirai, M.Y. 2019. Atypical myrosinase as a mediator of glucosinolate functions in plant. Frontiers in Plant Science. 10:1008.

Suran P., Balík J., Kulhánek M., Sedlář O., Černý J. 2023. Influence of Long-Term Organic fertilization on changes in the content of barious forms of sulfur in the soil under maize monoculture. Agronomy. 13, 957.

Suran P., Balík J., Kulhánek M., Sedlář O., Černý J. 2024. The relationship of soil sulfur with glomalin-related soil protein and humic substances under different mineral and organic fertilisation. Plant, Soil and Environment. 70, 93 – 100.

Suran P., Kulhánek M., Balík J., Černý J., Sedlář O. 2021. Evaluation of Soil S pools under 23 years of maize monoculture. Agronomy. 11, 2376.

Sýkora K. 2006. Utilizace dusíku a síry rostlinami ozimé řepky a kvalita produkce po aplikaci N-S hnojiv. [Disertační práce]. Česká zemědělská univerzita v Praze. Praha. 225 pp.

Šarapatka B., Alvarado-Solano D.P., Čižmár D. 2019. Can glomalin content be used as an indicator for erosion damage to soil and related changes in organic matter characteristics and nutrients? Catena. 181, 104078.

Šimon T, Mikanová O., Cerhanová D. 2008. Long-term effect of straw and farmyard manure on soil organic matter in field experiment in the Czech Republic. Archives of Agronomy and Soil Science. 59, 1 - 13.

Tabatabai M.A. 1982. Sulfur. In: Freney, J.R., Miller R.H. (Eds). Methods of Soil Analysis, Part 2.301 Chemical and Microbilogical Properties, ASA and SSSA. Madison,

Tabatabai M.A., Al-Khafaji, A.A. 1980. Comparison of nitrogen and sulfur mineralization in soils. Soil Science Society of America Journal. 44, 1000 – 1006.

Tabatabai M.A., Bremner J.M. 1970a. Arylsulfatase activity of soils. Soil Science Society of America. 34, 225 - 229.

Tabatabai M.A., Bremner J.M. 1970b. An alkaline oxidation method for determination of total sulphur in soils. Soil Science Society of America. 34, 62 - 65.

Tabatabai M.A., Fu M.H. 1992. Extraction of Enzymes From Soil. In: Bollag J.M., Stotzky G. (Eds.) Soil Biochemistry, Marcel Dekker Inc, New York, United States 197 - 227 pp.

Tan K.H. 2014. Humic Matter in Soil and the Environment: Principles and Controversies. 2nd ed. CRC Press, Boca Raton, Florida. United States. 495 pp.

Tang H., Xiao X., Li C., Wang K., Guo L., Cheng K., Sun G., Pan X. 2018. Impact of long-term fertilization practices on the soil aggregation and humic substances under double-cropped rice fields. Environmental Science and Pollution Research. 25, 11034 – 11044.

Tea I., Genter T., Naulet N., Marie L.M., Kleiber D. 2007. Interaction between nitrogen and sulfur by foliar application and its effects on flour bread-making quality. Journal of the Science of Food and Agriculture 87, 2853 - 2859.

Thai S., Pavlů L., Tejnecký V., Chovancová S., Hin L., Thet B., Němeček K., Drábek O. 2023. Temporal changes in soil chemical compositions in acidified mountain forest soils of Czech Republic. European Journal of Forest Research. 142, 883 – 897.

Tiemann L.K., Grandy A.S., Atkinson E.E., Marin-Spiotta E., McDaniel M.D. 2015. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. Ecology Letters. 18, 761 – 771.

Tisdale S.L., Nelson W.L. Beaton J.D., Havlin J.L. 1993. Soil Fertility and Fertilizers. Macmillan Publishing Company, New York, United States. 634 pp.

Tivet F., Sá J.C.M., Borszowskei P.R., Letourmy P., Briedis C., Ferreira A.O., Santos J.B., Inagaki T.M. 2012. Soil carbon inventory by wet oxidation and dry combustion methods: Effects of land use, soil texture gradients and sampling depth on the linear model of C-equivalent correction factor. Soil Science Society of America Journal. 76, 1048 – 1059.

Tomatsu H., Takano J., Takahashi H., Watanabe-Takahashi A., Shibagaki N., Fujiwara T. 2007. An *Arabidopsis thaliana* high-affinity molybdate transporter required for efficient uptake of molybdate from soil. Proceedings of National Academy of Sciences USA. 104, 18807 – 18812.

Treseder K.K., Turner K.M. 2007. Glomalin in ecosystems. Soil Science Society of America Journal. 71, 1257 – 1266.

Tseng H.M., Lu T.M., Ng, L.T. 2022. Responses of *Cynanchum taiwanianum* and its bioactive compound biosynthesis to levels of nitrogen and potassium fertilization. Agronomy. 12, 180.

Turgay O.C., Buchan D., Moeskops B., De Gusseme B., Ortas I., De Neve S. 2015. Changes in soil ergosterol content, glomalin-related soil protein, and phospholipid fatty acid profile as affected by long-term organic and chemical fertilization practices in Mediterranean Turkey. Arid Land Research and Management. 29, 180 – 198.

Turrión M.B., Gallardo J.F., González M.I. 1999. Extraction of soil-availible phosphate, nitrate and sulphate ions using ion exchange membranes and determination by ion exchange chromatography. Communications in Soil Science and Plant Analysis. 30, 1137 – 1152.

Uchida Y., Nishimura S., Akiyama H. 2012. The relationship of water-soluble carbon and hot-water-soluble carbon with soil respiration in agricultural fields. Agriculture, Ecosystems & Environment. 156, 116 – 122.

Uchoa S.C.P., Alvarez V.H., Freire F.M., Schaefer C.E.G.R., Hartmann L.K. 2003. Method of labile sulphur determination using anion-exchange membranes. Revista Brasileira de Ciencia do Solo. 27, 89 – 99.

Valarini P.J., Curaqueo G., Seguel A., Manzano K., Rubio R., Cornejo P., Borie F. 2009. Effect of compost application on some properties of a volcanic soil from central south Chile. Chilean Journal of Agricultural Research. 69, 416 – 425.

Van der Sloot M., Kleijn D., De Deyn G.B., Limpens J. 2022. Carbon to nitrogen ratio and quantity of organic amendment interactively affect crop growth and soil mineral N retention. Crop and Environment. 1, 161 – 167.

Van Gaelen N., Verschoren V., Clymans W., Poesen J., Govers G., Vanderborght J., Diels J. 2014. Controls on dissolved organic carbon export through surface runoff from loamy agricultural soils. Geoderma. 226–227, 387 – 396.

van Tol de Castro T.A., Berbara R.L.L., Tavares O.C.H., Mello D.F., Pereira, E.G. 2021. Humic acids induce a eustress state via photosynthesis and nitrogen metabolism leading to a root growth improvement in rice plants. Plant Physiology and Biochemistry. 162, 171–184.

Varényiová M., Ducsay L., Ryant, P. 2017. Sulphur nutrition and its effect on yield and oil content of oilseed rape (*Brassica Napus* L.) Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis. 65, 555 – 562.

Vermien C., Smolder R., McLaughlin M.J., Degryse F. 2018. Model-based rationalization of sulphur mineralization in soils using ^{35}S isotope dilution. Soil Biology and Biochemistry. 120, 1 – 11.

Viscarra-Rossel R.A., Webster R., Bui E.N., Baldock J.A. 2014. Baseline map of organic carbon in Australian soil to support national carbon accounting and monitoring under climate change. Global Change Biology. 20, 2953 – 2970.

Vlček V., Pohanka M. 2020. Glomalin-An interesting protein part of the soil organic matter. Soil and Water Research. 15, 67 – 74.

Vona V., Centeri C., Biró Z., Vona M. 2022. Comparing different phosphorus extraction methods: effects of influencing parameters. Sustainability. 14 (4). 2158.

Walkley A., Black I.A. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science. 37, 29 – 38.

Wang H., Wang L., Zhang Y., Hu Y., Wu J., Fu X., Le Y. 2017. The variability and causes of organic carbon retention ability of dierent agricultural straw types returned to soil. Environmental Technology. 38, 538 – 548.

Wang Q., Wang W.J., He X.Y., Zhang W.T., Song K.S., Han S.J. 2015. Role and variation of the amount and composition of glomalin in soil properties in farmland and adjacent plantations with reference to a primary forest in North-Eastern China. PLoS One. 10, e0139623.

Wang Q., Xin L., J.L., Yang X., Guo Z. 2021. Straw application and soil organic carbon change: A meta-analysis. And and Water Research. 13, 112 – 120.

Wang Y., Hu N., Xu M., Li Z., Lou Y., Chen Y., Wu C., Wang Z.L. 2015. 23-year manure and fertilizer application increases soil organic carbon sequestration of a rice–barley cropping system. Biology and Fertility of Soils. 51, 583 – 591.

Wanniarachchi S.D., Voroney D.P., Vyn T.J., Beyaert R.P., MacKenzie A.F. 1999. Tillage effects on the dynamics of total and corn-residue derived soil organic matter in two Ontario soils. Canadian Journal of Soil Science. 79, 473 – 480.

Waqas M.A., Li Y., Smith P., Wang X., Ashraf M.N., Noor M.A., Amou M., Shi S., Zhu Y., Li J., Wan Y., Qin X., Gao Q., Liu S. 2020. The influence of nutrient management on soil organic carbon storage, crop production, and yield stability varies under different climates. Journal of Cleaner Production. 268, 121922.

Wawrzynska A., Moniuszko G., Sirko A. 2015. Links between ethylene and sulfur nutrition- A regulatory interplay or just metabolite association? Frontiers in Plant Science. 6, 1053.

Wei X., Shao M., Gale W., Li L., 2014. Global pattern of soil carbon losses due to the conversion of forests to agricultural land. Scientific Reports. 4, 4062.

Weil R.R., Mughogho S.K. 2000. Sulfur nutrition of maize in four regions of Malawi. Agronomy Journal. 92, 649 – 656.

Wiesmeier M., Urbanski L., Hobley E., Lang B., von Lützow M., Marin-Spiotta E., van Wesemael B., Rabot E., Ließ M., Garcia- Franco N., Wollschläger U., Vogel H.J., Kögel-Knabner I. 2019. Soil organic carbon storage as a key function of soils - a review of drivers and indicators at various scales. Geoderma. 333, 149 – 162.

Wojewódzki P., Cieścińska B. 2012. Effect of crop rotation and long term fertilization on the carbon and glomalin content in the soil. Journal of Central European Agriculture. 13, 814 – 821.

Wright S.F., Anderson R.L. 2000. Aggregate stability and glomalin in alternative crop rotations for the central Great Plains. Biology and Fertility of Soils. 31, 249 – 253.

Wright S.F., Upadhyaya A. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Science. 161, 575 – 586.

Wright S.F., Upadhyaya A. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant and Soil. 198, 97 – 107.

Wu Q.S., Cao M.Q., Zou Y.N., He X.H. 2014. Direct and indirect effects of glomalin, mycorrhizal hyphae, and roots on aggregate stability in rhizosphere of trifoliate orange. Scientific Reports. 4, 5823.

Wu S., Li R., Peng S., Liu Q., Zhu X. 2017. Effect of humic acid on transformation of soil heavy metals. IOP Conference Series: Materials Science and Engineering. 207, 012089.

Xiao H., Li N., Liu C., 2015. Source identification of sulfur in uncultivated surface soils from four Chinese provinces. Pedosphere, 25, 140 - 149.

Xie H.T., Li J.W., Zhang B., Wang L.F., He H.B. Yhang X.D. 2015. Long-term manure amendments reduced soil aggregate stability via redistribution of the glomalin-related soil protein in macroaggregates. Scientific Reports. 5, 14687.

Xu C., Wang S., Chen Z., Lv J., Luo L., Li J., Ma Y. 2016. Sulphur speciation and availability in long-term fertilized soil: evidence from chemici fractionation and S K-edge Xanes spectroscopy. European Journal of Soil Science. 67, 666 – 675.

Xu N., Amgain N.R, Rabbany A., Capasso J., Korus K., Swanson S., Bhada J.H. 2022. Interaction of soil health indicators to different regenerative farming practices on mineral soils, Agrosystems, Geosciences and Environment. 5, e20243.

Xu P., Liang L.Z., Dong X.Y., Shen R.F. 2014. Effect of arbuscular mycorrhizal fungi on aggregate stability of a clay soil inoculating with two different host plants. Acta Agriculturae Scandinavica B – Soil and Plant Science. 65, 23 – 29.

Xue Z., An S. 2018. Changes in soil organic carbon and total nitrogen at a small watershed scale as the result of land use conversion on the loess plateau. Sustainability. 10, 4757.

Yang F., Tang C., Antonietti M. 2021. Natural and artificial humic substances to manage minerals, ions, water, and soil microorganisms. Chemical Society Reviews. 50, 6221 – 6239.

Yang Z., Singh B.R, Hansen .S, Hu Z., Riley H. 2007. Aggregate associated sulphur fractions in longterm (>80 years) fertilized soils. Soil Science Society of America Journal. 70, 163 – 170.

Yoshimoto N., Inoue E., Saito K., Yamaya T., Takahashi H. 2003. Phloem-localizing sulfate transporter, Sultr1;3, mediates re-distribution of sulfur from source to sink organs in *Arabidopsis.* Plant Physiology. 131, 1511 – 1517.

Yoshimoto N., Takahashi H., Smith F.W., Yamaya T., Saito K. 2002. Two distinct high-affinity sulfate transporters with different inducibilities mediate uptake of sulfate in Arabidopsis roots. The Plant Journal. 29, 465 – 473.

Yu Z., She M., Zheng T., Diepeveen D., Islam S., Zhao Y., Zhang Y., Tang G., Zhang Y., Zhang J. 2021. Impact and mechanism of sulphur-deficiency on modern wheat farming nitrogen-related sustainability and gliadin content. Communications Biology. 4, 1 – 6.

Zavarzina A.G., Danchenko N.N., Demin V.V., Artemyeva Z.S., Kogut B.M. 2021. Humic substances: hypotheses and reality (a Review). Eurasian Soil Science. 54, 1826 – 1854.

Zbíral J. (Ed). 2016. Jednotné pracovní postupy – Analýza půd I, postup 30074.1 ÚKZÚZ BRNO.

Zbíral J., Čiižmar D., Malý S., Obdržálková E. 2017. Determination of glomalin in agriculture and forest soils by near-infrared spectroscopy. Plant, Soil and Environment. 63, 226 – 230.

Zbíral J., Smatanová M., Němec P. 2018. Sulphur status in agricultural soils determined using the Mehlich 3 method. Plant Soil and Environment. 64, 255 – 259.

Zhang H., Kariman K., Zhu L., Liu L., Chen J.J., Zhu L., Li D. 2021. Spatial distribution of carbon, nitrogen and sulfur in surface soil across the Pearl River Delta area, South China. Geoderma Regional. 25, e00390.

Zhang L., Kawaguchi R., Morikawa-Ichinose T., Allahham A., Kim S.J., Maruyama-Nakashita A. 2020. Sulfur deficiency-induced glucosinolate catabolism attributed to two β-glucosidases, BGLU28 and BGLU30, is required for plant growth maintenance under sulfur deficiency. Plant and Cell Physiology. 61 (4), 803 – 813.

Zhang X., Wu X., Zhang S., Xing Y., Wang R., Liang, W. 2014. Organic amendment effects on aggregate-associated organic C, microbial biomass C and glomalin in agricultural soils. Catena. 123, 188 – 194.

Zhang Y., Zhang X., Wen J., Wang Y., Zhang N., Jia Y., Su S., Wu C., Zeng X. 2022. Exogenous fulvic acid enhances stability of mineral-associated soil organic matter better than manure. Environmental Science and Pollution Research. 29, 9805 – 9816.

Zhang Z.H., Wang Q., Wang H., Nie S.M. Liang Z.W. 2017. Effects of soil salinity on the content, composition, and ion binding capacity of glomalin-related soil protein. Science of Total Environment. 581, 657 – 665.

Zhao F.J., Evans E.J., Bilsborrow PE., Syers J.K. 1993. Sulphur uptake and distribution in double and single low varieties of oilseed rape (*Brassica napus* L.). Plant and Soil. 150, 69 – 76.

Zhao F.J., Fortune S., Barbosa V.L., McGrath S.P., Stobart R., Bilsborrow P.E. Booth E.J., Brown A., Robson P. 2006. Effects of sulphur on yield and malting quality of barley. Journal of Cereal Science. 43, 369 – 377.

Zhou L., Yuan L., Zhao B., Li Y., Lin Z. 2019. Structural characteristics of humic acids derived from Chinese weathered coal under different oxidizing conditions. PLoS ONE. 14, e0217469.

Zhou W.J., Sha L.Q., Schaefer D.A., Zhang Y.P., Song Q.H., Tan Z.H., Deng Y., Deng X.B., Guan H.L. 2015. Direct effects of litter decomposition on soil dissolved organic carbon and nitrogen in a tropical rainforest. Soil Biology and Biochemistry. 81, 255 – 258.