Plant, Soil and Environment, 70, 2024 (2): 93–100 *Original Paper*

https://doi.org/10.17221/493/2023-PSE

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

Pavel Suran, Jiří Balík, Martin Kulhánek, Ondřej Sedlář, Jindřich Černý*

Department of Agro-Environmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic

**Corresponding author: suranp@af.czu.cz*

Citation: 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 Environ., 70: 93–100.

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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Supported by the Ministry of Agriculture of the Czech Republic, Project No. QK21010124.

https://doi.org/10.17221/493/2023-PSE

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

https://doi.org/10.17221/493/2023-PSE

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.

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-

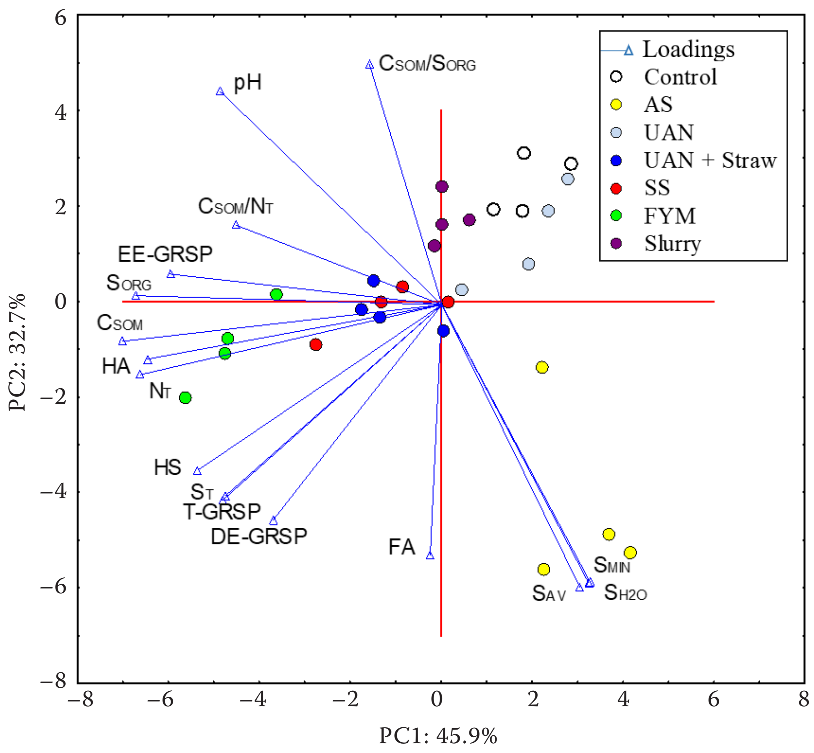

Figure 1. Loading vectors for both principal components and their relation to fertiliser treatment cases show the biplot position of PC1 *vs.* PC2. Points of different colours represent cases of different treatments. There are four cases per treatment. n = 28 Control – unfertilised control; AS – ammonium 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.

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Received: December 19, 2023
Accepted: January 12, 2024