Sulfur fractions in particle-size separates as influenced by long-term application of mineral and organic fertilizers

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ABSTRACT

A field experiment established in 1962 was chosen to investigate the effect of long-term application of farmyard manure (FYM), compost (COM) and sewage sludge (SS), respectively, in two increments as compared to mineral fertilizer on inorganic and organic S fractions in particle-size separates (< 0.002 mm, 0.002–0.02 mm, 0.02–2 mm). Independent of the particle-size the application of the high amounts of COM and SS resulted in the highest total S contents. It is evident that the particle-size fractions < 0.002 mm contained the majority of total soil sulfur (S). The content of plant available S (water-soluble and adsorbed SO_4^{2-}) decreased with increasing particle-size, while the influence of the kind and amount of organic fertilizers was negligible. As compared to C-bonded S sulfate esters were the dominant organic S fraction in size separates. The content of both organic S fractions was highest in the particle-size fraction < 0.002 mm and lowest in the particle-size fraction 0.02–2 mm. The influence of the application of organic fertilizers was less pronounced. Only high application rates of COM and SS, respectively, resulted in the highest contents of both organic S fractions in the particle-size fractions 0.002–0.02 mm and 0.02–2 mm.

Keywords: inorganics; organics; farmyard manure; compost; sewage sludge

Total soil sulphur (S), which comprises inorganic and organic binding forms ranges between 250 and 2500 kg/ha in most topsoils of arable land. Inorganic S, composed of water soluble SO_4^{2-} and adsorbed SO_4^{2-}, is generally believed to be the immediate source for plants. Generally it accounts for less than 5% of total soil S (Kulhánek et al. 2011). In soil solution SO_4^{2-} is present only in small concentrations (Balík et al. 2009), which vary continuously and depend at any time on the balance between S plant uptake, fertilizer input, mineralization and immobilization (McLaren and Cameron 2004). Organic S is the main S binding form in soils (Scherer 2009) and contributes to up to 95% of total soil S in cultivated temperate soils. According to Kertesz and Mirleau (2004) organic S is a heterogeneous mixture and very little is known about the chemical identity. However, two main groups can be distinguished: C-bonded S (C-S) and ester sulfate (C-O-S). Although some other organic S compounds like sulfonates and heterocyclic sulfur occur they are thought to be of minor importance. C-bonded S, which mainly comprises the S-containing amino acids methionine and cystein, is correlated with microbial biomass S (Castellano and Dick 1990). In German topsoils this S fraction reaches up to 57% of soil organic S (Förster et al. unpublished). Ester sulfates forming between 27 and 45% of soil organic S include sulfated polysaccharides, cholin sulfate and phenolic sulfates (Edwards 1998).

While C-bonded S is directly derived from plant residues and root inputs as well as from microbial protein synthesis, sulfate ester is mainly generated through biochemical processes by the soil microorganisms, metabolizing organic residues (Solomon et al. 2001).

In soils S is continuously cycled between inorganic and organic binding forms. According to

Tabatabai and Al-Khafaji (1980) in incubation experiments S mineralization rates range between 16 and 86 mg S/kg soil during a period of 26 weeks, while Knights et al. (2001) reported that between 14 and 35 mg S/kg soil were mineralized under optimum conditions over 28 weeks. However, because mineralization of organic S compounds occurs via biochemical as well as biological processes, factors like soil moisture, pH, temperature and substrate availability may have an impact on SO_4^{2-} delivery under field conditions. Therefore any interpretation of S mineralization under laboratory conditions must be made with caution.

While Zhao et al. (2006) found that the bulk of the mineralized is S derived from the C-bonded S pool, studies using ^{35}S-labeled SO_4^{2-} showed that ester sulfate is of a more transitory nature than C-bonded S (Shan et al. 1997), and therefore appears to make a higher contribution to S mineralization. However, according to Lou and Warman (1992) only a portion of the ester sulfate is biochemically reactive, because it can be protected from degradative enzymes by inclusion in soil microaggregates (Eriksen et al. 1998). This means that not all of the ester sulfate groups are equally labile (Lou and Warman 1992). For this reason it may be assumed that beside the concentration also the stability of ester sulfates affects the amount of S mineralized.

The association of C and N in various size fractions was studied from the aspect of the relative distribution (Wright and Hons 2004), but only little information exists on the long-term effects of different organic fertilizers on the form and distribution of S in size fractions. However, the knowledge of the association of S with particle-size separates may provide a better understanding of S transformations in soils. Therefore a long-term field experiment initiated in 1962, which includes mineral fertilizer application and organic amendments, provided a unique opportunity to study long-term effects of mineral fertilizer and compost, sewage sludge and farmyard manure application, respectively, on total S content and inorganic S and organic S fractions in soil particle-size separates.

MATERIAL AND METHODS

Field experiment. In 1962 a long-term field experiment (randomized complete block design with four replicates) was established at the experimental farm of INRES – Plant Nutrition (50°32'42''N, 6°59'14''E), University of Bonn, Germany, on a luvisol derived from loess (World Reference Base for Soil Resources 2006).

The treatments selected for the present investigation are: mineral fertilizer (NH_4NO_3 plus $CaCO_3$, KCl, CaH_2PO_4) [MIN], 9 t and 18 t/ha farmyard manure [FYM1, FYM2], 14.5 t and 58 t/ha compost from organic household waste [COM1, COM2] and 3.7 and 14.8 t/ha sewage sludge [SS1, SS2]. Until 1997 these amounts were applied every second year to a root crop-cereal crop rotation. In 1999 the crop rotation was changed to root crop-cereal-cereal and organic fertilizers were applied every third year. The amounts now are 9 and 18 t farmyard manure/ha, 30 and 120 t compost/ha and 5 and 20 t sewage sludge/ha. The amounts of organic fertilizers are based on dry weight. Mineral fertilizers were applied according to the demand of the crop in the rotation.

The mean total S content of the organic fertilizers (based on dry matter) is 0.22% (FYM), 0.12% (COM) and 0.67% (SS).

The most important soil parameters are shown in Table 1.

Soil samples. Soil samples (cores with dimension of 20 × 20 × 30 cm) were randomly taken with a spade from the four plots (24.5 m^2 each) of the corresponding treatments early in spring 2006 one year after the last application of organic fertilizers. For the determination of soil characteristics subsamples were air dried, ground and passed through a 2 mm sieve.

Particle-size fractionation. Soil samples were separated into three particle size fractions (< 0.002 mm, 0.002–0.02 mm, 0.02–2 mm according to Séquaris and Lewandowski (2003). The frac-

Table 1. Particle distribution (% of total) as affected by different organic manures

	< 0.002 mm	0.002–0.02 mm	0.02–2 mm
MIN	2.50	9.14	88.36
FYM1	5.95	18.34	75.71
FYM2	1.64	8.78	89.58
COM1	8.20	14.06	77.72
COM2	1.77	10.92	87.31
SS1	8.51	16.35	76.11
SS2	2.30	8.30	89.40

MIN – mineral fertilizer; FYM1 – 9 t/ha farmyard manure; FYM2 – 18 t/ha farmyard manure; COM1 – 14.5 t/ha compost from organic household waste; COM2 – 58 t/ha compost from organic household waste; SS1 – 3.7 t/ha sewage sludge; SS2 – 14.8 t/ha sewage sludge

tionation was achieved by soil phase cold water extraction in the following three steps:

Step 1: Weigh 100 g of soil into a 1 000 mL glass bottle containing 200 mL of distilled water and shake on a horizontal shaker for 6 h (150 RPM); add 600 mL of distilled water, agitate by hand for one minute and leave undisturbed for sedimentation; after 6 min remove 700 mL of the supernatant and transfer it into another glass bottle; dry the sediment (0.02–2 mm) in the rest of the liquid in an oven at 40°C.

Step 2: Shake 700 mL of supernatant from step 1 on a horizontal shaker for 2 min and afterwards leave undisturbed for 12 h for sedimentation; remove 600 mL and dry the sediment (0.002–0.02 mm) at 40°C.

Step 3: Transfer the rest into a 250 mL centrifuge tube and centrifuge for 90 min at 12 000 g and 20°C; discharge the supernatant and dry the sediment (< 0.002 mm) at 40°C.

Sequential extraction/Procedure for sulfur speciation analysis. Inorganic sulfur (water-soluble SO_4^{2-} (a), and adsorbed SO_4^{2-} (b) were extracted using the sequential extraction method according to Freney et al. (1970) and Shan et al. (1997): (a) Transfer 1 g of soil into a centrifuge tube; add 10 mL of distilled water; shake the soil suspension on a reciprocal shaker for 30 min; centrifuge at 12 000 g for 10 min; decant the supernatant into test tube without disturbing the soil at the bottom of the centrifuge tube. (b) Add 10 mL of 0.032 mol/L NaH_2PO_4 to the residual soil in the centrifuge tube; continue as described above.

Ester sulfate and C-bonded sulfur. Organic S fractions were determined according to Shan and Chen (1995), modified by Morche (2008).

HI extraction. Transfer 0.5 g of soil into a boiling flask; connect the boiling flask to a Johnson-Nishita digestion apparatus and add 15 mL reducing solution (a mixture of hydriodic, hypophosphoric and formic acid); reflux the suspension gently for 1 h under nitrogen atmosphere. The H_2S gas was trapped and converted to sulfate.

Ester sulfate was calculated from the difference between HI reducible S and inorganic S and C-bonded S from the difference between total S and HI reducible S.

Sulfur of all extracts from the sequential extraction was determined by anion chromatography and from HI extraction by ICP-OES. All samples were filtered through a 0.45 μm cellulose acetate filter (Macherey & Nagel, Düren, Germany) before measuring.

Total S was measured with a CNS analyzer (Eurovector EUROEA, Redavalle, Italy).

Statistical analysis. Statistical analysis of the data was done by SPSS 17 (Chicago, USA). Means were compared using a one way ANOVA including descriptives and test of homogeneity of variance. The differences between treatments were compared using the Tukey's test at a significance level of 0.05.

RESULTS AND DISCUSSION

Independent of the kind and the amount of organic manure applied, we found that particles of 0.02–2 mm comprised the greatest proportion, followed by particles in the 0.002–0.02 mm and < 0.002 mm size class (Table 1). Within the organic fertilizer treatments the formation of larger particles was favoured by the higher application rate. Confirming results of Singh and Singh (1996), who emphasize the contribution of organic matter in the formation of soil aggregates, we found a significant relationship (r = 0.618; Figure 1) between the percentage of the largest fraction and soil carbon levels (Table 2). Because microbial derived polysaccharides act as cementing agents, we assume that the formation of larger particles was favoured by the enhanced microbial activity (Zaller and Köpke 2004). Therefore the higher share of larger particles could indeed be contributed to the input of organic residues to the soils with organic fertilizers as compared to mineral fertilizer (Yang et al. 2007a).

The distribution of total S in different particle-size classes is shown in Figure 2. Examination of particle-size separates show that significantly higher amounts of total S were present in the particle-size class < 0.002 mm, followed by particle-size class 0.002–0.02 mm and 0.02–2 mm. The values of the smallest soil separates were up to five times higher and ranged between 455 and 630 mg S/kg than the values of the largest separates, ranging
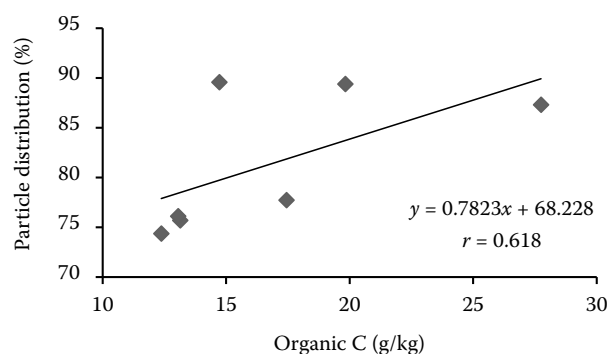

Figure 1. Correlation between soil organic carbon and particle distribution

Table 2. Chemical soil characteristics

	MIN	FYM1	FYM2	COM1	COM2	SS1	SS2
pH	6.06	5.95	6.22	6.73	7.10	6.01	6.52
C_t (g/kg)	12.38	13.15	14.73	17.45	27.75	13.06	19.83
N_t (g/kg)	1.09	1.15	1.31	1.45	2.27	1.13	1.51
C/N ratio	11.36	11.43	11.24	12.03	12.22	11.56	13.13

MIN – mineral fertilizer; FYM1 – 9 t/ha farmyard manure; FYM2 – 18 t/ha farmyard manure; COM1 – 14.5 t/ha compost from organic household waste; COM2 – 58 t/hacompost from organic household waste; SS1 – 3.7 t/ha sewage sludge; SS2 – 14.8 t/ha sewage sludge

between 115–275 mg S/kg. This is in accordance with results of Solomon et al. (2001), who reported that total S increased with decreasing size of the particles. As compared to mineral fertilizer application the influence of farmyard manure on total S in the different particle fractions was negligible, while the impact of compost and sewage sludge was more pronounced, but as compared to the control, only the high application rate of compost and sewage sludge, respectively, resulted in a significant increase of total S. However, it is difficult to explain, why we could not find an impact of farmyard manure.

Examination of particle-size separates of the different treatments show that the amount of water-soluble S decreased in the order: < 0.002 mm > 0.002–0.02 mm > 0.02–2 mm. In total the amounts ranged from 9.86 (SS1) to 23.06 mg/kg (COM2) in the small particles, 6.99 (FYM1) to 13.79 mg/kg

(FYM2) in the medium particles and 3.44 (SS1) to 7.06 mg/kg (COM2) in the large particles (Figure 3). However, independent of the particle-size the influence of organic fertilizers on the content of water-soluble S was less pronounced. In the particle-size separate < 0.002 mm only the high compost application rate resulted in a significantly higher content, while in the particle-size separate 0.02–2 mm it was increased after application of high amounts of farmyard manure and compost, respectively. Confirming results of Förster et al. (unpublished) with bulk soil we suppose that these small differences between the different treatments must be linked to the date of soil sampling, which was early in spring. Differences are assumed to be higher under optimum conditions for S mineralization. Further it may be suspected that a part of the water-soluble S was lost during the wet-sieving procedure.
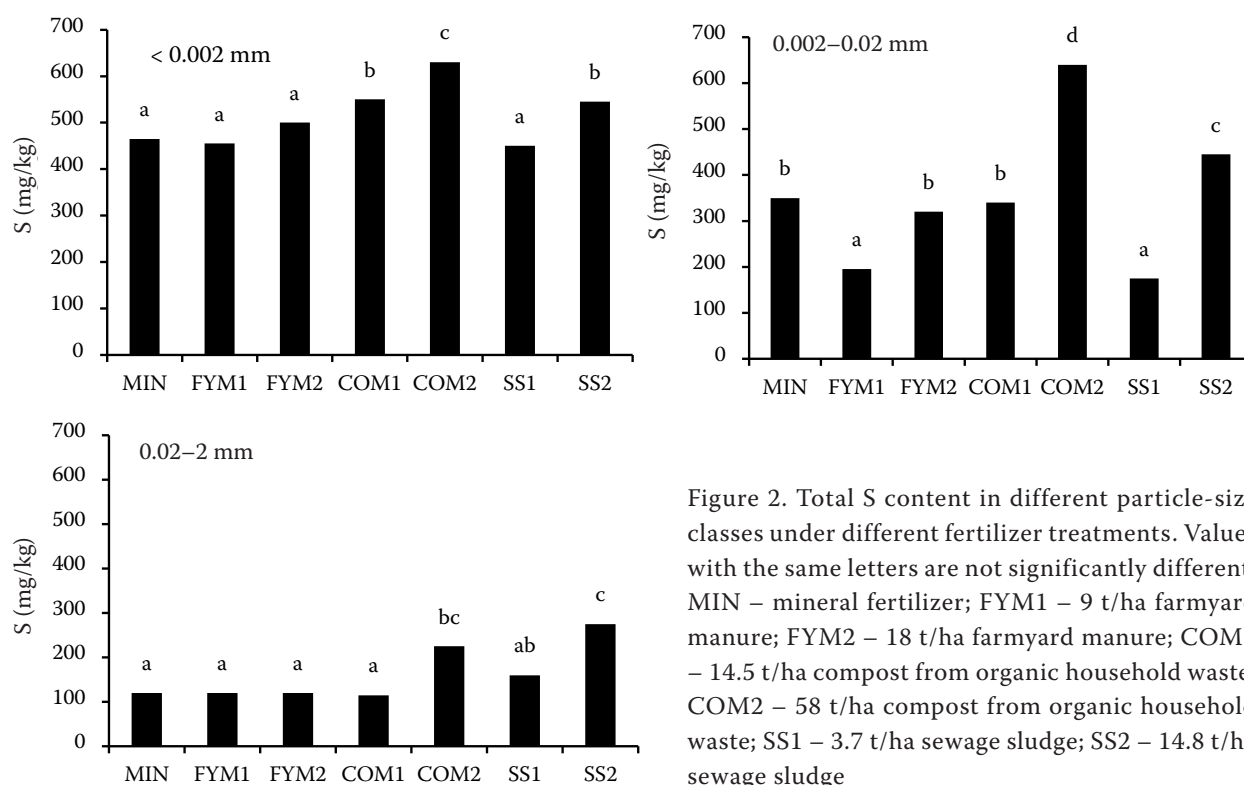

Figure 2. Total S content in different particle-size classes under different fertilizer treatments. Values with the same letters are not significantly different. MIN – mineral fertilizer; FYM1 – 9 t/ha farmyard manure; FYM2 – 18 t/ha farmyard manure; COM1 – 14.5 t/ha compost from organic household waste; COM2 – 58 t/ha compost from organic household waste; SS1 – 3.7 t/ha sewage sludge; SS2 – 14.8 t/ha sewage sludge
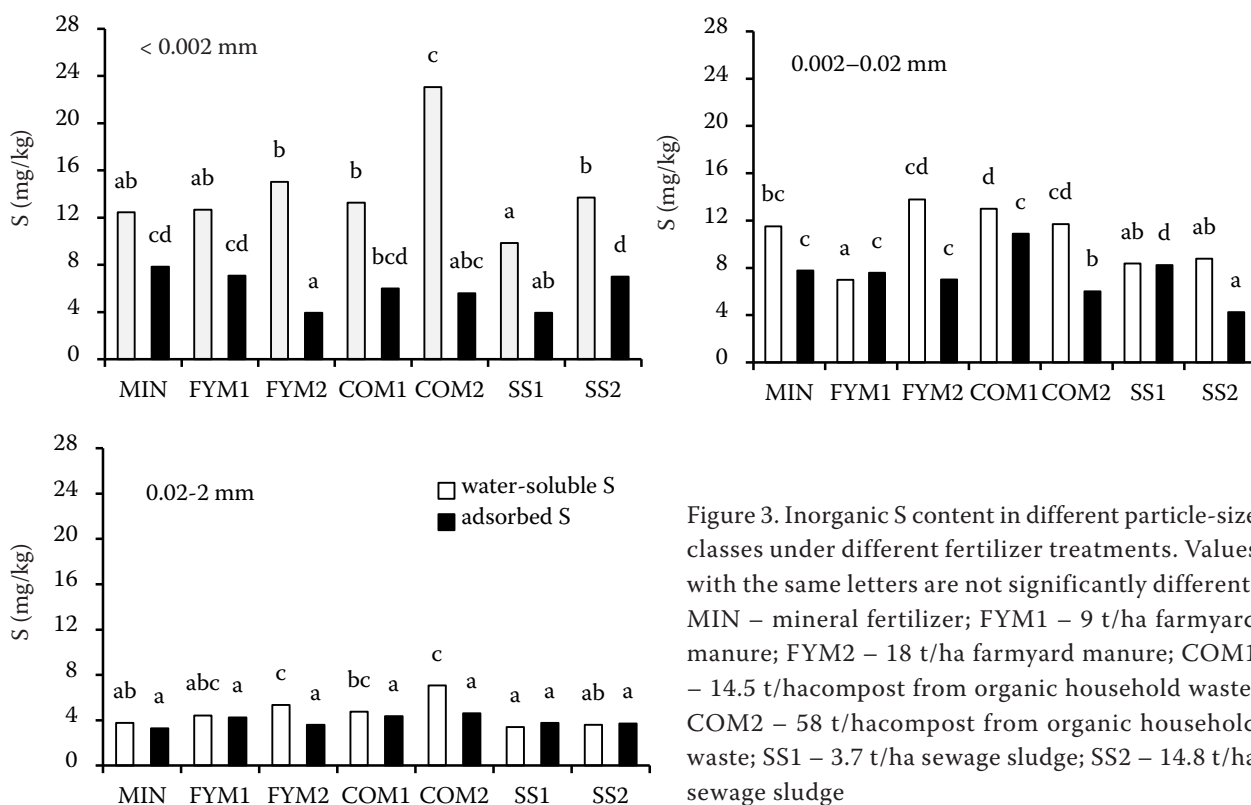
Figure 3. Inorganic S content in different particle-size classes under different fertilizer treatments. Values with the same letters are not significantly different. MIN – mineral fertilizer; FYM1 – 9 t/ha farmyard manure; FYM2 – 18 t/ha farmyard manure; COM1 – 14.5 t/hacompost from organic household waste; COM2 – 58 t/hacompost from organic household waste; SS1 – 3.7 t/ha sewage sludge; SS2 – 14.8 t/ha sewage sludge

The amounts of adsorbed S were in the same order of magnitude in the smallest and the medium size particles, and were lower in the size class 0.02–2 mm (Figure 3). However, regardless of the type of organic manure the adsorbed amounts of SO_4^{2-} mostly decreased with the higher application rate. This is most likely the result of competition between anionic groups supplied with organic matter and SO_4^{2-} for adsorption sites on Fe and Al hydroxides (Johnson and Todd 1983). It should be further pointed out that adsorption of SO_4^{2-} is pH dependent and gets stronger at low soil pH. At pH > 6.5, adsorption is negligible and most SO_4^{2-} is in soil solution (Curtin and Syers 1990). Therefore generally little SO_4^{2-} adsorption is expected in the pH range of our soil samples (Table 2), because maximum is reached at pH 3 and falls rapidly as the pH rises to levels normally prevailing in limed soils.

While water-soluble and adsorbed SO_4^{2-} are directly available to plants mineralization of ester sulfate and C-bonded S also makes a contribution to plant uptake of S (**Eriksen 2009**). According to Hu et al. (2002) ester sulfate is considered the more labile of these two fractions. Therefore it may be assumed that the extent of SO_4^{2-} release in soils depends on the concentration of ester sulfates (Ganesmurthy and Nielsen 1990). However, according to Eriksen et al. (1998) the ester sulfates are a fraction of organic S compounds with widely

differing accessibilities. Their biochemical stability may depend on their location in the structure of humic polymers (Lou and Warman 1992). While ester sulfate groups attached to the inner structure of humic polymers are less available, the sulfate groups located on the external surfaces may be accessible to sulfatases and therefore easily mineralizable (Eriksen et al. 1998). Furthermore, it was suggested that a large part of ester sulfates is in close association with clay. Therefore ester sulfates are protected from microbial transformations and the turnover is reduced (Eriksen 1997). As compared to C and N the interaction with clay to protect easily decomposable organic compounds from microbial decomposition seems even more important for S (Eriksen et al. 1998).

Therefore to provide a better understanding of S transformations in soils, knowledge of the association of ester sulfate with different sizes of soil aggregates is obligatory.

Even though the content of ester sulfate was highest in the size class < 0.002 mm, ranging between 280.1 (FYM1) and 366.4 mg/kg S (SS2) we observed that, compared with mineral fertilizer application, this S fraction was barely influenced by organic fertilizers (Figure 4). The medium size class (0.002–0.02 mm) contained between 128.2 (SS1) and 238.5 mg/kg S (COM2) as ester sulfates. They increased with each increment of the different organic fertilizers. However, as compared to the
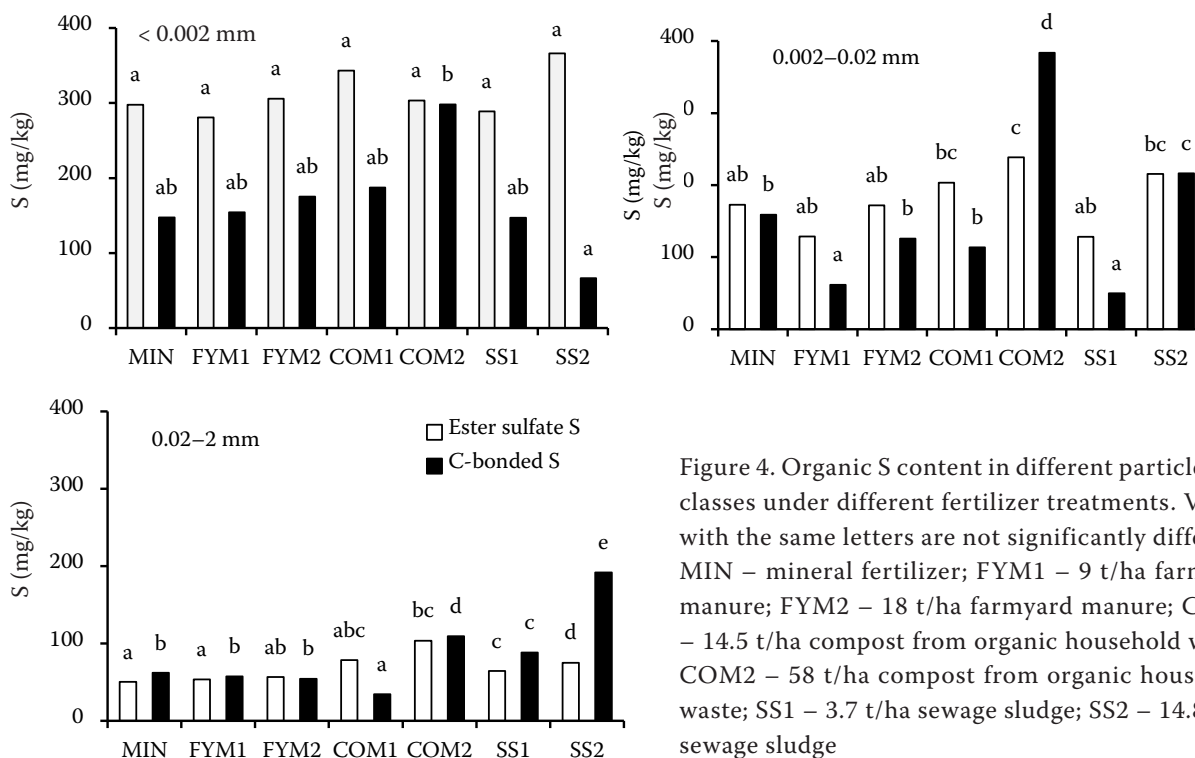
Figure 4. Organic S content in different particle-size classes under different fertilizer treatments. Values with the same letters are not significantly different. MIN – mineral fertilizer; FYM1 – 9 t/ha farmyard manure; FYM2 – 18 t/ha farmyard manure; COM1 – 14.5 t/ha compost from organic household waste; COM2 – 58 t/ha compost from organic household waste; SS1 – 3.7 t/ha sewage sludge; SS2 – 14.8 t/ha sewage sludge

control the increase was only significant with the high compost application rate. For the large particles ester sulfate content varied between 50.3 (MIN) and 103.6 mg/kg S (COM2) and was significantly higher with the high compost application rate as compared to the control. The increase of the ester sulfate content with decreasing particle size confirms that the biochemical release of SO_4^{2-} from ester sulfate is at least partly controlled by the extent to which they are protected against hydrolysis in soil particles, or in micropores to which sulfatase enzymes cannot gain access.

In comparison to ester sulfate, independently of the particle-size, C-bonded S contents were mostly lower. Confirming results of Yang et al. (2007b) we found the highest contents in the smallest particles, while the lowest value was detected in size class 0.02–2 mm. The content varied between 147.7 (MIN) and 298.2 mg/kg S (COM2), 49.9 (SS1) and 383.8 mg/kg S (COM2) and 62.1 (MIN) and 191.8 mg/kg (SS2) in the small, medium and large particles, respectively. This could be ascribed to stabilization of C-bonded S produced during decomposition of organic fertilizers by small soil particles. Again the influence of the different organic fertilizers was less pronounced.

Our results indicate that ester sulfate and C-bonded S tend to concentrate in the finest soil matter. Nevertheless, due to their abundance, smaller particles account for only a very small proportion of the total soil mass and their influ-

ence on S delivery is therefore more limited than that of larger particles. Therefore we assume that organic S associated with larger particles is the predominantly available S pool for plants.

Acknowledgement

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