Crop influence on mobile sulphur content and arylsulphatase activity in the plant rhizosphere

B. Kotková, J. Balík, J. Černý, M. Kulhánek, M. Bazalová

Czech University of Life Sciences in Prague, Prague, Czech Republic

ABSTRACT

The changes of mobile sulphur (S) contents were investigated in the plant rhizosphere using precise model experiments with rhizoboxes. The tested plants were winter wheat (*Triticum aestivum* L.), winter rape (*Brassica napus* L.) and white lupine (*Lupinus albus* L.). In this experiment, a Cambisol from a precise field experiment treated with sewage sludge or manure was used. Total extractable S concentration and mineral S ($S\text{-}SO_4^{2-}$) concentration in the water extract were higher in the plant rhizosphere (< 6 mm from the root) compared to the so-called bulk soil (> 6 mm). The contents of total extractable S^0 decreased in order: lupine (5–35 mg/kg) > rape (4–18 mg/kg) > wheat (1.5–3 mg/kg). The same order was observed for mineral $S\text{-}SO_4^{2-}$ where the contents in soil extracts were 1–10 mg/kg, 2–7 mg/kg, and 0.5–3 mg/kg, respectively. The highest total extractable S and mineral S ($S\text{-}SO_4^{2-}$) contents were found in the treatments fertilized with organic fertilizers. In the case of rape and wheat the arylsulphatase (ARS) activity was higher in the rhizosphere compared to the bulk soil; the opposite was recorded for lupine. It was acknowledged that the ARS activity was higher in the treatments fertilized with organic fertilizers (manure or sewage sludge) with all three tested plants. The highest determined ARS activity was found after wheat cultivation, the lowest after the lupine cultivation. The organic sulphur content followed an opposite tendency (lupine > rape > wheat).

Keywords: sewage sludge; manure; rape; wheat; lupine; rhizosphere; sulphur; arylsulphatase

Sulphur (S) concentration in majority of agricultural soils ranges from 50 to 500 mg S/kg. It is mostly organically bound (up to 98% of the total soil S). The main sources of organic S in soil are basically (dead) plant roots, crop residues, and also organic fertilizers (Tabatabai 1982, Freney 1986, Hu et al. 2003, Knauff et al. 2003, Vaněk et al. 2007).

Rhizosphere is the key zone as for the plant nutrients uptake. Physico-chemical processes between soil and roots generally differ from the processes outside the rhizosphere (Darrah 1993). Changes in nutrient dynamics in the plant rhizosphere were intensively studied for phosphorus (Gahoonia and Nielsen 1992, Zoyza et al. 1997, Balík et al. 2007), nitrogen, potassium, calcium and magnesium (Moritsuka et al. 2000). There are however limited data on S fractionation in the rhizosphere (Han and Yoshida 1982, Hu et al. 2002, 2003). Due to the plant-microorganism relationship, rhizosphere is metabolically a very active zone where many important reactions take place. Nutrient availability and nutrient plant uptake is determined especially by soil conditions in the rhizosphere. Root exudates play an important role here; they are a mixture of organic acids, chelants, sugars, vitamins, amino acids, purines, nucleosides, inorganic ions (HCO_3^-, OH^-, H^+), gas molecules (CO_2, H_2) and enzymes (Dakora and Phillips 2002).

The presence of microorganisms which oxidize S is supported mainly by the rape root exudates (Grayston and Germida 1990). Although it is obvious that plant rhizosphere is concerned in the N and S dynamics in the soil and influences their uptake by plants, this aspect was not examined properly. Grayston and Germida (1990) indicate that higher S oxidation takes place in the rhizosphere and there is also a much bigger and diverse group of heterotrophic organisms compared to the bulk soil.

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Hu et al. (2003) studied S fractionation in the plant rhizosphere of rape and rice. They found that it differs depending on crop type. More inorganic S-SO_4^{2-} was present in the rhizosphere of both tested plants compared to bulk soil. Higher concentrations of organic S were found in the rape rhizosphere but, on the other hand, in the rice bulk soil. Sulphur in the sulphate ester bond was found rather outside the rape rhizosphere. Amino acids like cysteine and methionine are the main S compounds directly bound to carbon (Tabatabai 1982, Freney 1986). Paul and Schmidt (1961) observed slightly higher concentrations of cysteine and methionine in the rhizosphere compared to the bulk soil. Sulphur-containing amino acids do not accumulate as free forms in the soil because they are readily degraded in aerobic conditions (Fitzgerald 1978).

Soil enzymes are potential indicators of soil quality because they correlate with soil biology, their activity is easy to determine and they react on changes in the soil management (Bandick and Dick 1999, Trasar-Cepada et al. 2000). Such indicators include physical, chemical and biological characteristics and can be used for monitoring long-term impacts of management on soil fertility.

Enzymes catalyze biochemical reactions and are an integral part of nutrient cycles in soil. They also play a major role within organic compounds decomposition in soil releasing mineral nutrients (Chròst 1991, Sinsabauch 1994). Some enzymes are tightly connected to microorganisms, other are fully extracellular. Sulphatases participate in biochemical mineralization of organic sulphur and especially arylsulphatase was found in most of the soil samples (Tabatabai and Bremner 1970b, 1971, Gupta et al. 1993, Farrell et al. 1994). Arylsulphatase activity can be used as an indicator of biochemical mineralization intensity of organic sulphate esters in the soil. However, detailed information on factors influencing the activity and kinetics of (aryl)sulphatase in soils is still missing. Sulphatases can be found in the soil also as exoenzymes and are closely related to organic matter. Several studies have shown that organic fertilizers, such as compost and manure improve soil organic sulphur availability (Saranthchandra and Perrott 1981, Knauff and Scherer 1998).

The aim of this experiment was to investigate the influence of different systems of organic fertilization on sulphur distribution and arylsulphatase activity in the rhizospheres of winter wheat, winter rape and white lupine.

MATERIAL AND METHODS

Three tested plants (winter wheat – *Triticum aestivum* L., winter rape – *Brassica napus* L. and white lupine – *Lupinus albus* L.) were planted on the soil taken from a precise field experiment with sewage sludge or manure application. The soil is classified as a Cambisol.

The length of each experimental period using rhizoboxes (Wenzel et al. 2001) was 8 weeks (from sowing till harvest). The soil was sieved (2 mm) and dried. First, the root compartment was sowed with tested plants (10 wheat plants, 8 rape plants and 4 lupine plants per rhizobox). After the striking (approximately 7 days) the rhizosphere section was set and the plants were grown for 7 weeks in stable conditions (day: 16 h, 24°C, humidity 95%; night: 8 h, 16°C, 60% humidity). Plants were watered with demineralized water. At the end of the experiment the plants were harvested and the soil was separated to one-millimeter layers.

The pH value was determined using the extraction with demineralized water with the w/v ratio of 1:10. The suspension was shaken for 16 h and left to settle for 1 h. Then the sample was shaken mechanically and the pH_{H_2O} was measured with the glass electrode.

Modified methodology of Tabatabai and Bremner (1970a) was used for determination of arylsulphatase activity. Methodology: after the addition of a *p*-nitrophenylsulphate [4-nitrophenyl sulfate potassium salt (Sigma)] solution, was added to the soil (1 g) and it was incubated at 37°C for 1 h. *p*-nitrophenol (pNP) released by arylsulphatase activity was extracted and colored with NaOH and determined photometrically.

The total extractable and mineral S concentration in the soil was determined in the water extract (ratio 1:10 w/v). The concentration of total extractable S was determined using ICP-OES, Varian VistaPro, Australia. The concentration of mineral S-SO_4^{2-} was measured on Skalar SAN^{plus} SYSTEM. Organic S was assessed from the difference of total extractable sulphur concentration and mineral S-SO_4^{2-}. The total S amount was determined using CNS device LECO.

RESULTS AND DISCUSSION

Main agrochemical characteristics of the Cambisol used in the experiment are summarized in Table 1 (soil prior to the experiment). The soil was taken from the long-term precise field experiment. On

Table 1. Nutrient concentrations (mg/kg) and pH_{CaCl_2} of the tested Cambisol at the beginning of the experiment

Treatment	$pH/CaCl_2$	Available concentration*				S_{tot}
		P	Ca	Mg	K	
Control	5.0	116	1603	78	217	762
Sewage sludge	5.1	180	1664	83	201	807
Manure	5.1	152	1166	87	253	793

*Mehlich III

Table 2. Plants yield (g of dry matter per rhizobox)

Treatment	Lupine		Wheat		Rape	
	aboveground biomass	roots	aboveground biomass	roots	aboveground biomass	roots
Control	4.5	0.28	1.6	0.3	3.7	1.08
Sewage sludge	3.7	0.20	2.2	0.5	4.0	1.05
Manure	4.7	0.27	1.8	0.2	3.8	0.98
F-test	5.94	1.11	11.89	10.03	0.89	1.15
d_{min} (α = 0.05)	0.7	n.s.	0.3	0.2	n.s.	n.s.

n.s. – not significant

average, 88.4 kg S/ha was added every three years with the sewage sludge application and 55.8 kg S/ha with manure application. It means there was on average 0.9% S in the dry matter of sewage sludge and 0.3% in the dry matter of manure.

At the end of the rhizobox experiment, the harvested biomass was divided into the aboveground part and roots and the dry matter yield was determined (Table 2). In the case of wheat and rape the lowest yield was found on unfertilized control and the highest yield after the sewage sludge treatment. In the case of lupine, the lowest yield was found after the sewage sludge treatment and the highest yield after the manure treatment. Wheat had the lowest yield from the tested plants, rape and lupine had similar but higher yields.

Table 3 shows total S content in the aboveground biomass of tested plants. The lowest S concentrations were found in all tested plants after the manure treatment and the highest after the sewage sludge treatment. While rape and lupine had the highest S concentration in the aboveground biomass (0.11–0.32%, 0.12–0.24%, respectively), wheat had the lowest (0.07–0.10%). Sulphur uptake by the plants' aboveground biomass is shown in Table 4.

In the rhizosphere of wheat, pH_{H_2O} was the highest close to the roots compared to the bulk soil. Only minor differences were found in the pH_{H_2O} values after different treatments (Figure 1a). Youssef and Chino (1989) measured the pH changes in the barley rhizosphere. Barley was planted in

Table 3. Sulphur concentrations in the aboveground biomass (%)

Treatment	Lupine	Wheat	Rape
Control	0.206	0.070	0.255
Sewage sludge	0.235	0.088	0.324
Manure	0.127	0.100	0.106
F-test	35.84	7.07	80.03
d_{min} (α = 0.05)	0.027	0.012	0.037

Table 4. Sulphur uptake by the aboveground biomass (mg per rizobox)

Treatment	Lupine	Wheat	Rape
Control	9.236	1.099	9.395
Sewage sludge	8.645	1.906	13.079
Manure	5.980	1.772	3.972
F-test	18.11	44.82	23.73
d_{min} (α = 0.05)	1.304	0.207	3.008

the sand culture with a pH value 5. The increase of pH appeared up to 2 units. In our experiments the pH value increased on average by 0.46 in the wheat rhizosphere compared to bulk soil. The pH increase could be caused by the cation accumulation (Ca^{2+}, Mg^{2+}) in the rhizosphere (data not presented here).

In the rhizosphere of rape, pH_{H_2O} was the lowest close to the roots and the highest in the bulk soil. The decrease in pH_{H_2O} value was 0.47. Comparing the treatments, the lowest pH_{H_2O} was in the soil fertilized with manure and the highest after the sewage sludge treatment compared to the unfertilized control (Figure 1b). Chaignon et al. (2002) found that pH increased in the rape and tomato rhizosphere grown on acidic soil and on the contrary decreased in the alkaline soil. Hinsinger et al. (2003) observed that when the pH value was 4.8 in the bulk soil, it was significantly increased in the rape rhizosphere and, on the other hand, rhizosphere acidification occurred when the bulk soil pH value exceeded 7.1.

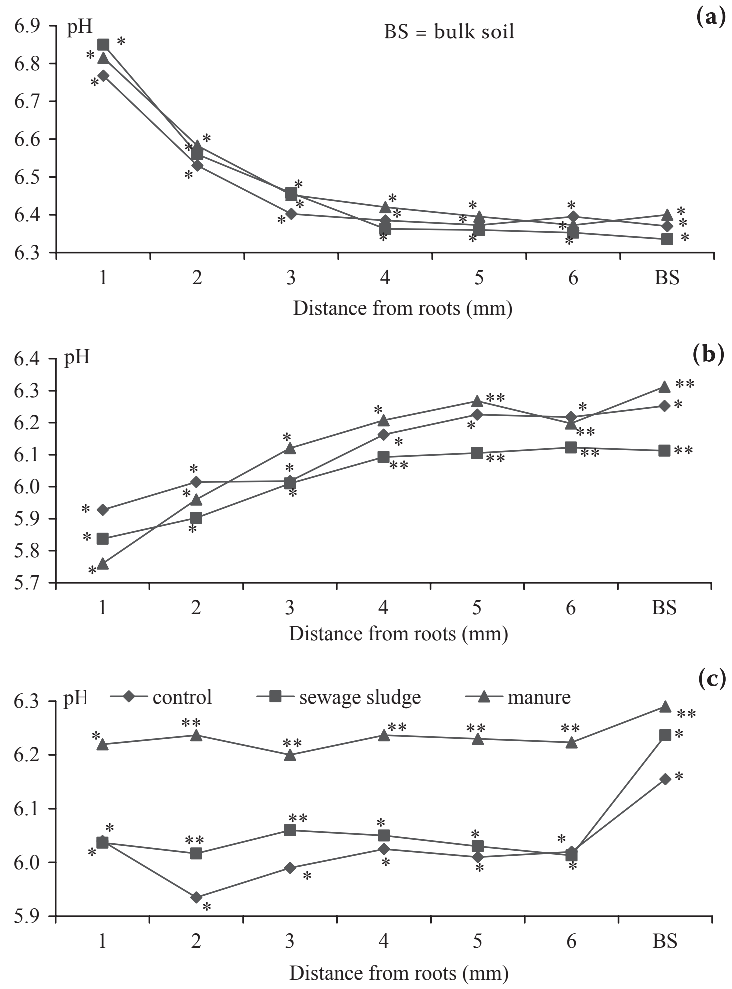

Figure 1. Changes of pH_{H_2O} in plant rhizosphere – (a) winter wheat, (b) winter rape, (c)white lupine

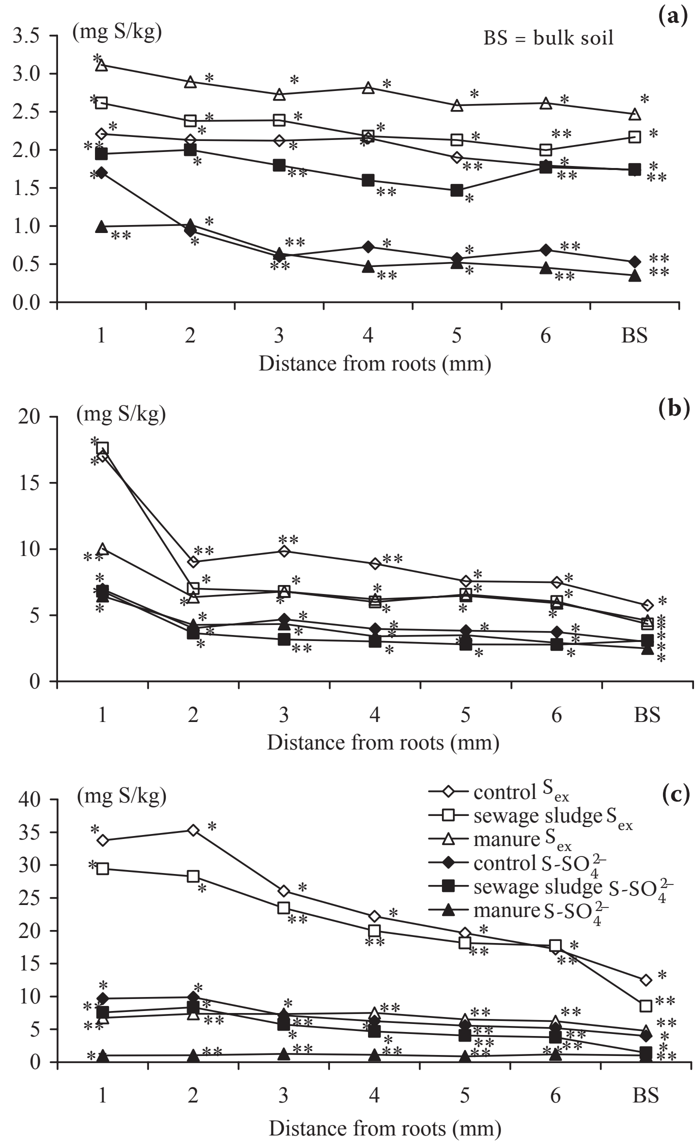

Figure 2. Total extractable sulphur concentrations (S_{ex}) and mineral sulphur concentrations ($S\text{-}SO_4^{2-}$) (water extract) in plant rhizosphere (mg S/kg soil) – (a) winter wheat, (b) winter rape, (c)white lupine

In the lupine rhizosphere acidification also occurred (pH decrease of 0.16) but it was not as pronounced as in the case of rape (Figure 1c). The highest pH_{H_2O} values were found after the manure treatment.

Root-induced pH increase and decrease in the rhizosphere depend mainly on the initial soil pH value, plant species and nitrogen nutrition (Youssef and Chino 1989, Hu et al. 2002, Hinsinger et al. 2003).

Total extractable S content (S_{ex}) in the water extract was the highest in the distance of 1 mm from the roots in the wheat rhizosphere. The S_{ex} value slightly decreased with the distance from the root. The highest S_{ex} was found on the soil fertilized with manure and the lowest one was on the unfertilized control (Figure 2a). The S_{ex} value reached from 1.5 to 3.0 mg/kg. Figure 2a also shows mineral S ($S\text{-}SO_4^{2-}$) concentrations, which follow a similar pattern. These results correspond well with Lasserre et al. (2000) who determined a significantly higher content of S_{ex} in the barley rhizosphere in comparison with the bulk soil.

The S_{ex} values in the water extract were higher in the rape rhizosphere compared to the wheat rhizosphere. S_{ex} reached values from 4 to 18 mg/kg and it was the highest in the distance of 1 mm from the root (Figure 2b). Higher S_{ex} values were measured on the unfertilized control compared to the sewage sludge treatment. It is obvious that mineral S content ($S\text{-}SO_4^{2-}$) accounts approximately for 50% of the total extractable sulphur (S_{ex}).

The S_{ex} values in the lupine rhizosphere determined in the water extract were the highest from

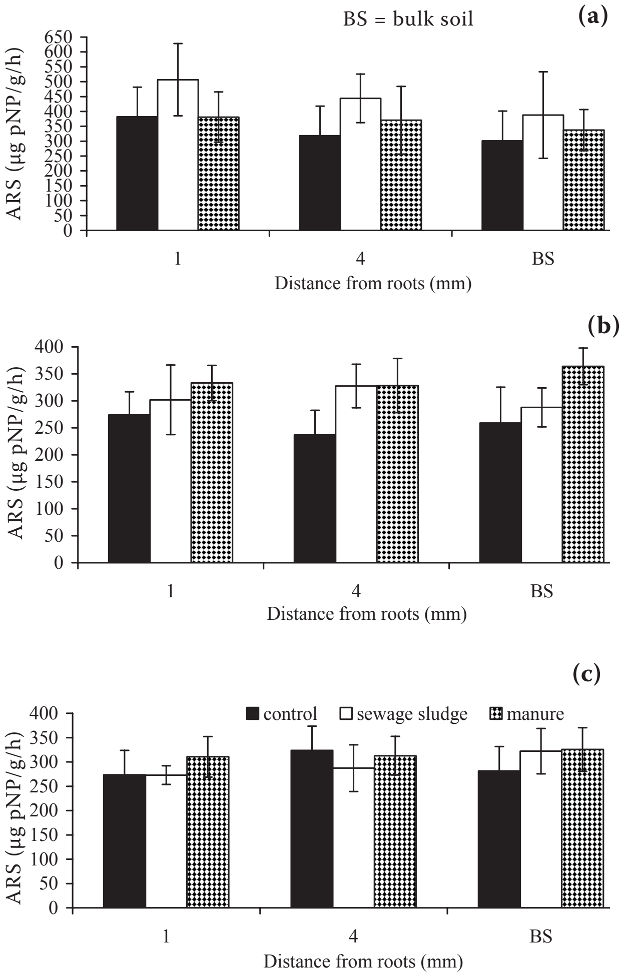

Figure 3. Changes of arylsulphatase (ARS) activity in plant rhizosphere (µg pNP/g/h) – (a) winter wheat, (b) winter rape, (c)white lupine

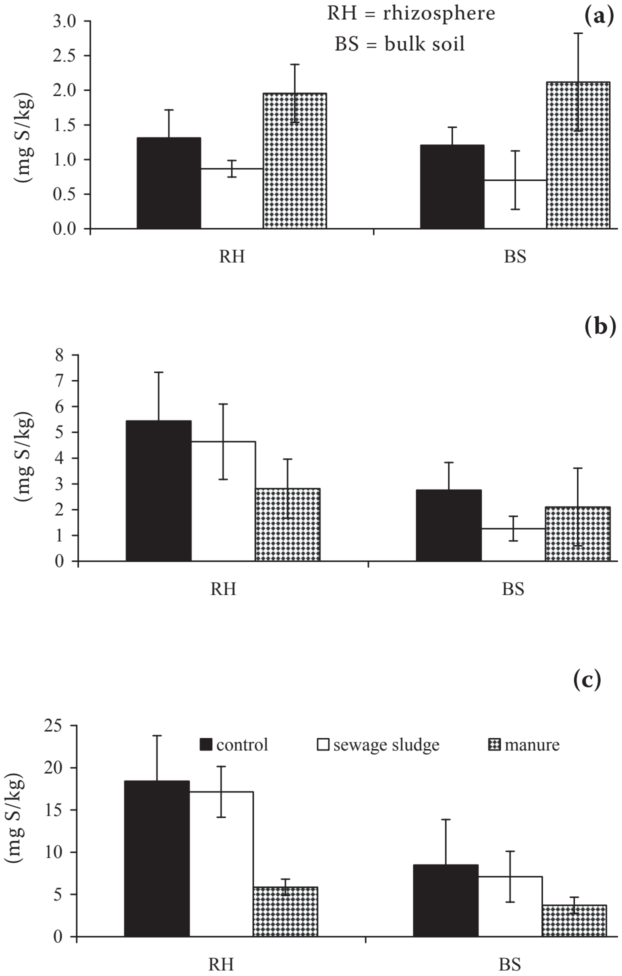

Figure 4. Organic sulphur (S_{org}) concentrations in plant rhizosphere and bulk soil (mg S/kg) – (a) winter wheat, (b) winter rape, (c)white lupine

three tested plants. S_{ex} reached values from 5 to 35 mg/kg and it was the highest in the unfertilized control, slightly lower after the sewage sludge treatment and the lowest in the manure treatment (Figure 2c). The amount of mineral S-SO_4^{2-} ranged from 1 to 10 mg/kg.

The content of total extractable S is on average 6 times higher in the rhizosphere of all tested plants compared to the bulk soil. Similar results were presented by Barber (1995) and Hu et al. (2003) who determined higher concentrations of total extractable S outside the rhizosphere of rape and rice. Results of Morche et al. (2007), who dealt with S distribution in the rape and rye-grass rhizosphere, correspond well with our findings. Authors determined higher S-SO_4^{2-} concentrations in the water extract of the rhizosphere soil with the above-mentioned two plants compared to the bulk soil. They also compared S-SO_4^{2-} in treatments fertilized either with an organic fertilizer (compost) or a mineral fertilizer. They concluded that higher S-SO_4^{2-} concentrations were found in organically fertilized soil (Morche et al. 2007).

When wheat was cultivated on the Cambisol, arylsulphatase (ARS) activity was the highest in the distance of 1 mm from the root and decreased with distance from the root (Figure 3a). This correlates with Knauff et al. (2003) who found higher enzyme activity in the immediate vicinity of roots. Comparing treatments, the highest ARS activity was after the sewage sludge treatments; comparing the tested plants, ARS activity values were the highest in the wheat rhizosphere and reached 300–500 µg *p*-nitrophenol/g/h. However, Knauff et al. (2003) found the highest ARS activity in the rape rhizosphere. Figure 3a shows a significant relationship of ARS activity and the distance from the roots. It decreases with distance from the roots. S_{org} concentrations on the Cambisol ranged from 0.5 to 2 mg/kg and there are no substantial differences among the treatments (Figure 4a). The highest S_{org} content was determined after the manure treatment. Wheat rhizosphere contained the lowest S_{org}.

Figure 3b shows that the highest ARS activity while growing rape was recorded in the treatments with organic fertilizers (sewage sludge and especially manure) compared to the unfertilized control. Similar results were acknowledged by Knauff et al. (2003). The ARS activity value was 240–400 μg *p*-nitrophenol/g/h. As it is further obvious from Figure 3b there was no significant relationship between the ARS activity and the distance from the roots. Knauff et al. (2003) also found out that the distance from the roots has no impact on the ARS activity, except for the significant increase of ARS activity 0.25 mm from the *Sinapis albus* root. We discovered higher S_{org} concentrations in the treatments with organic fertilizers and in the rhizosphere (Figure 4b). Hu et al. (2003) also confirmed more organic S in the rape rhizosphere.

In the case of lupine, the lowest ARS activity was observed in the rhizosphere and it gently increased towards the bulk soil (Figure 3c). It was also acknowledged that the highest ARS activity was in the treatments fertilized with organic fertilizers. Comparing the tested plants, ARS activity values were the lowest and reached 250–350 μg *p*-nitrophenol/g/h. As in the case of rape, there was no significant relationship of the ARS activity and the distance from the roots. In the lupine rhizosphere, the highest S_{org} concentrations were observed (Figure 4c). Again more S_{org} was found in the lupine rhizosphere compared to the bulk soil.

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Corresponding author:

Prof. Ing. Jiří Balík, CSc., Česká zemědělská univerzita v Praze, Fakulta agrobiologie, potravinových a přírodních zdrojů, 165 21 Praha 6-Suchdol, Česká republika
phone: + 420 224 382 732, fax: + 420 224 382 535, e-mail: balik@af.czu.cz