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INTERNATIONAL Agrophysics
www.international-agrophysics.org

Int. Agrophys., 2016, 30, 359-367
doi: 10.1515/intag-2015-0098

Arylsulphatase activity and sulphate content in relation to crop rotation and fertilization of soil

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Received November 17, 2015; accepted June 6, 2016

A b s t r a c t. The aim of the study was to investigate the effect of varying rates of FYM (0, 20, 40, 60 Mg ha^{-1}) and nitrogen N_0, N_1, N_2, and N_3 on the content of sulphate sulphur (VI) and the activity of arylsulphatase, which participates in the transformations of this element in Haplic Luvisol. The study report is based on a long-term field experiment with two different crop rotations: A – recognized as exhausting the humus from soil and B – recognized as enriching the soil with humus. During the cultivation of the plants, the soil was sampled four times from corn and a red clover cultivar and grass. The FYM fertilization rate for which the highest arylsulphatase activity and the content of sulphates were identified was 60 Mg ha^{-1}. An inhibitory effect of high rates (90 and 135 kg N ha^{-1}) of ammonium nitrate on the arylsulphatase activity was also observed. A significant correlation between the content of carbon, nitrogen, and sulphates and the arylsulphatase activity was recorded. The investigation on the effect of combined application of farmyard manure and mineral nitrogen fertilization on the activity of arylsulphatase participating in the sulphur cycling was launched to examine the problem in detail.

K e y w o r d s: arylsulphatase, sulphate sulphur (VI), Luvisol, FYM, nitrogen fertilization

INTRODUCTION

Cultivation practices, such as fertilization, application of farmyard manure and crop rotation affect differently carbon, nitrogen, phosphorus and sulphur cycling in soils (Acosta-Martinez *et al.,* 2011; Dodor and Tabatabai, 2003). Applying organic supplements, such as manure, to agrosystems as a nutrient source in management practice can increase soil organic matter (SOM) and improve the nutrient status in the soil (Mikha and Rice, 2004). Nutrient cycling is essential for the transformation of nutrients into plant available forms. The influence of manure application occurs due to the changes in soil microbial communities (Acosta-Martinez and Harmel, 2006), strongly affecting the soil potential for enzyme-mediated substrate catalysis that control soil nutrient availability and SOM quality and quantity (Acosta-Martinez *et al.,* 2011). According to Eivazzi *et al*. (2003), more often enzyme activities increased more often when organic and inorganic fertilizers were added together. Mineral nitrogen can directly affect the microbial production of soil enzymes but the effect varies with the type of soil and enzyme as well as the kind of enzymatic reaction (Iyyemperumal and Shi, 2008). According to the review by Alvarez (2005), nitrogen addition generally results in an increase in the SOM level but only when crop residues are returned to soil. Liu *et al*. (2010) reported a decrease in soil microbial biomass after the addition of mineral nitrogen.

Over the last two decades, sulphur deficiency has been recognized as a constraint on crop production all over the world (Scherer, 2009; Yang *et al.,* 2007; Zhao *et al.,* 2003), mostly due to the reduction of sulphur dioxide emissions from power plants and other industrial sources (Lehmann *et al*., 2008), the increasing use of low-S-containing fertilizers, the decreasing use of S-containing fungicides and pesticides and high-yielding crops (Eriksen *et al*., 2004; Kost *et al*., 2008; Scherer, 2009). The bioavailability of organic and inorganic sulphur in soils can be controlled through enzyme activities (Scherer, 2009; Tabatabai, 1994). Arylsulphatase (EC 3.1.6.1) is an enzyme that hydrolyzes aromatic sulphate esters ($R\text{-}O\text{-}SO_3^{2-}$) into phenols (R-OH) and inorganic sulphate (SO_4^{2-}) (Tabatabai, 1994). These

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enzymes are commonly present in soil and are believed to contribute to the provision of sulphur nutrition to plants (Elsgard and Vinter, 2004). The knowledge of the activity of soil enzymes and the cycle of field-grown plant roots is essential.

Long-term experiments can provide insights into the response of soils to management. However, the impact of long-term simultaneous fertilization with farmyard manure and ammonium nitrate on the arylsulphatase activity in crop rotation species is not well understood. Knauf *et al.* (2003) investigated the arylsulphatase activity within the rhizosphere of *Sinapis album*, *Lolium perenne, Triticum aestivum* and *Brassica napus*, which were fertilized using organic and mineral fertilizers. The inclusion of a leguminous crop in the crop rotation increases soil enzymatic activity since leguminous crops may secrete higher amounts of exudates into the rhizosphere than non-leguminous crops (Sainju *et al*., 2006). Therefore, in the present investigations, arylsulphatase activity was determined in soil with two crop rotations: exhausting the soil from organic matter and crop rotation enriching the soil with humus in a long-term field experiment.

The aim of the present research was to determine the effect of selected plants and fertilization with various rates of FYM and ammonium nitrate on the content of available sulphur and the activity of the enzyme that participates in the transformations of sulphur compounds in soil. The variety of root systems among plant species leads to the variation in nutrient uptake and losses and, hence, possibly to changes in the soil microbial activity. We hypothesized that:

- the arylsulphatase activity and the content of sulphates (VI) will change with increasing rates of ammonium nitrate application;
- the content of sulphates (VI) and arylsulphatase will increase with increasing FYM rates;
- the content of carbon, nitrogen, and sulphur will increase due to the cultivation of corn and the red clover and grass plants, and
- there will be a significant relationship between the parameters studied.

MATERIAL AND METHODS

The research material was sampled from a long term field experiment located in the area of the Agricultural Experiment Station at Grabów, Mazowieckie Province, Zwoleński County, Przyłęki Commune. The location of the experiment station is as follows: altitude (51°21'8"N), longitude (21°40'8"E), with lowland climate of moderate altitudes. The experiment was established in 1980 by the Department of Plant Nutrition and Fertilization, the Institute of Soil Science and Plant Cultivation (IUNG) in Puławy. The soils of the Agricultural Experiment Station at Grabów represent Haplic Luvisols (LVh), class IV a, agricultural land and very good rye soil.

The analysis of the granulometric composition (sand fraction 2.0-05 mm 61-84%, silt fraction 0.05-0.002 15-34% and clay fraction <0.002 2-7%) demonstrated that the soil in the study area shows a loamy sand texture.

The experiment was carried out in a four-year crop rotation design; it was set up using the split-plot method in which the first factor was the type of the crop rotation: A – recognized as exhausting the humus from soil (potato, winter wheat, spring barley, corn grown for silage) and B – recognized as enriching the soil with humus: (potato, winter wheat with white mustard, spring barley with undersown grass, a red clover cultivar with meadow fescue). Four 40 m^2 replications of the cultivated plots were created.

The second factor was farmyard manure (FYM) fertilization. FYM was applied in October 2004 at the following rates: 0, 20, 40, and 60 Mg ha^{-1}. The chemical composition of fresh cattle FYM applied in the experiment nutrient was as follows: dry matter 21 mg kg^{-1}, total organic carbon (TOC) 81 mg kg^{-1}, nitrogen (N) 4.5 mg kg^{-1}, phosphorus (P) 1.22 mg kg^{-1}, potassium (K) 5.56 mg kg^{-1}, calcium (Ca) 3.0 mg kg^{-1}, magnesium (Mg) 0.84 mg kg^{-1}, and sodium (Na) 1.0 mg kg^{-1}. The third factor included nitrogen added in the form of ammonium nitrate (34% N) at the rates of N_0, N_1, N_2, and N_3, where the rate of N_2 was an adequate multiplication of the rate of N_1. The following rates of N_1 in kg ha^{-1} N in crop rotation A were applied: N_1: 45 kg under potato and corn and 40 kg under winter wheat and spring barley. In crop rotation B: N_1: 45 kg under potato, 40 kg under winter wheat with mustard and the red clover cultivar with meadow fescue, 30 kg under spring barley with undersown crops as well as combined for three cuts 120 kg (3 x 40 kg each). The next doses of N were established for N_2: 90 kg N under potato and corn, 80 kg N under winter wheat, spring barley and red clover + grasses, 60 kg N under spring barley with undersown crops and N_3: 135 kg N under potato and corn, 120 kg N under winter wheat, spring barley, and red clover + grasses, 90 kg N under spring barley with undersown crops. During a single rotation, 170 kg N in both rotations with mineral fertilizers was applied into the soil in plots N_1. Phosphorus fertilizers (granulated triplesuperphosphate 20% P) and potassium fertilizers (50% K) were used at the same doses in all the experimental treatments: potato: 24 kg ha^{-1} of P, 100 kg ha^{-1} of K, winter wheat: 21 kg ha^{-1} of P, 60 kg ha^{-1} of K; spring barley: 24 kg ha^{-1} of P, 72 kg ha^{-1} of K; corn: 24 kg ha^{-1} of P , 132 kg ha^{-1} of K; and red clover: 24 kg ha^{-1} of P, 100 kg ha^{-1} of K.

The soil was sampled four times under corn and the mixture of clover and grasses during the vegetation period in April, June, July, and September in 2008. The weather conditions were extremely diverse (Table 1). The vegetation of plants was recorded in the third week of March. The fairly balanced distribution of precipitation during the vegetable period was favourable for the plants. There were relatively low temperatures including frost in mid-May, which resulted in some plant freezing. June was characterized

T a b l e 1. Air temperature (°C) and rainfall (mm) during the vegetation period

Specification	Months						
	IV	V	VI	VII	VIII	IX	IV-IX
Air temperature (°C)	8.2	12.0	15.9	18.0	18.6	13.0	14.3
Rainfall (mm)	67.0	41.3	83.7	112.1	58.7	17.5	380

by low temperatures and only in July, along with higher temperatures and significant rainfall, rapid growth and the development of plants was recorded.

Soil samples were sieved to pass through a 2 mm sieve. Arylsulphatase activity was determined according to the method of Tabatabai (1994). The soil samples (1.0 g) were incubated with 4 ml of acetate buffer (pH 5.8), 125 µl of toluene, and 1 ml of a potassium p-nitrophenyl sulphate solution at 37°C for 1 h. Afterwards, 0.5 M $CaCl_2$ and 4 ml 0.5 M NaOH were added and then the solution was filtered. The intensity of the colour of the p-nitrophenol formed was determined by reading the absorbance at 420 nm using a calibration absorbance curve versus the p-nitrophenol concentrations. The content of sulphate sulphur was determined according to the Bardsley-Lancaster turbidimetric method (1960) as modified by COMN-IUNG using a UV-visible spectrophotometer Evolution 201. The following parameters were also analysed in the soil material: 1. pH in KCl, 2. organic carbon with the TOC Primacs analyser by Scalar, and 3. total nitrogen was determined using the Kjeldahl method (PN-ISO 11261, 2002).

All the assays were made in three replicates. The paper presents mean arithmetic values. The results were subjected to the analysis of variance and the significance of the differences between the mean values was verified using the Tukey test at p = 0.05. All the calculations were performed using FR-ANALWAR software based on Microsoft Excel. The results of the analyses were also exposed to the analysis of simple correlation using Statistica for Windows Pl software (R=8 treatments × 2 replications × 2 crop species ×4 times= 64 objects for one crop rotation).

RESULTS AND DISCUSSION

Optimal balanced crop rotation involving forage legumes can improve the organic carbon status in the soil. Soon *et al*. (2007), who tested the influence of 12 years of four crop sequences on the organic C pools in a Grey Luvisolic sandy loam soil, found increased total organic C content in the top 15 cm of soil in the red clover rotation compared with pea. It was demonstrated that the farming practices led to changes in the SOM content. These changes are difficult to detect over a short or medium time; thus, the content of total organic carbon (TOC) in the soil from Grabów was not related to the plant grown (Table 2). In our study, fertilization exerted a crucial impact on the content of TOC. Irrespective of the rates of FYM and nitrogen fertilizer applied, a higher content of TOC was found in the soil sampled from the plots at the rates of 40 and 60 Mg ha^{-1} of FYM. The effect of the high (N_2 and N_3) rates of ammonium nitrate, on the other hand, decreased the content of organic carbon in the samples, compared with the control objects. The increase in SOC due to organic amendments can be substantial. Results from a Rothamsted long-term agricultural experiment in Great Britain showed that continuous

T a b l e 2. Total organic carbon and total nitrogen content

Specification		TOC g kg^{-1}	TN g kg^{-1}
Crop rotation I factor	A	9.802 a	0.976 a
	B	10.59 a	1.088 b
FYM t ha^{-1} II factor	0	8.599 a	0.872 a
	20	10.015 b	1.062 b
	40	10.826 c	1.071 b
	60	11.348 c	1.123 c
Nitrogen kg kg^{-1} III factor	N_0	10.538 b	0.964 a
	N1	10.556 b	1.038 b
	N_2	9.971 a	1.063 c
	N_3	9.723 a	1.061 c
Mean		10.2	1.03
SD		1.37	0.13

A, B – crop rotation, SD – standard deviation, a, b – values differ significantly at p < 0.05.

application of farmyard manure almost tripled the SOC content over 100 years (McLauchlan, 2006). Melero *et al.* (2006) reported that organic management maintained soil organic matter at higher levels than inorganic fertilization.

The content of total nitrogen (TN) was related to the plant species applied (Table 2). The higher amount of mineral nitrogen was found in the soil from the crop rotation with the mixture of clover and grasses. Similar results were reported by Pecio *et al*. (2005) for a four-field crop rotation design, in which corn for grain, spring barley, oat, and winter wheat were grown. The effect of crop rotation on the nitrogen content in the soil was also confirmed by Melero *et al*. (2011), reporting the lowest soil nitrate content in wheat-sunflower rotation and the highest in the wheat-faba bean rotation. The lowest content of total nitrogen was determined in the soil sampled from the objects without FYM fertilization (Table 2). The difference between the N content in the soils from the control objects and fertilization with 60 Mg ha^{-1} 4 year^{-1} FYM was 22%. In turn, a significant increase in soil N to 68% was reported by Nett *et al*. (2011), comparing the non-fertilized treatment with the fertilization rate of 60 Mg FYM ha^{-1} year^{-1}. The FYM was applied on loamy sand every year since 1989. The application of increasing rates of nitrogen fertilizers increased the content of mineral nitrogen in soil. Melero *et al*. (2011), on the other hand, reported that nitrogen fertilization (N rate: 50 and 150 kg N ha^{-1}) had no great effect on nitrate content in the upper layers (0-10 cm depth).

The ratio of C:N in the soil under corn ranged from 9.1 to 11.8; however, it declined within the range from 8.0 to 14.5 for clover and grasses (Table 3). The crop rotation with the plants that 'enrich' soil with organic matter widened the ratio of C:N crop, which can affect the biological activity in soil, mostly through the increase in the population of microorganisms that can easily use both components. Soil microorganisms that transform organic and mineral compounds enrich the soil with nitrogen, promote plant growth, and deliver antibiotic and biologically active substances; a substantial role in these transformations is played by enzymatic processes (Janvier *et al.*, 2007). One can thus assume that the arylsulphatase activity in crop rotation with a mixture of clover and grasses is of microbiological origin.

Based on the analysis of active and exchangeable acidity, it was found that the soil represents acid and slightly acid soils (Table 3). A lower pH value was observed in the soil sampled from the plots with the mixture of clover and grasses. The soil pH affects the activity of enzymes and the content of sulphates available to plants (Gianfreda and Ruggiero, 2006; Scherer, 2009). The prevailing form of sulphur in soil is organic sulphur and the content of the mineral forms available to plants is related to the intensity of mineralization of plant residues, organic fertilizers, and humus. The transformations of organic sulphur into its mineral forms are enhanced by high pH. An increase in this parameter to pH 7.5 increases the mineralization rate. In soils with pH above 6.0, sulphate sulphur occurs in the soil solution and is easily available to plants (Scherer, 2009).

Arylsulphatase activity depends on the type of crop, FYM, and ammonium nitrate fertilization (Table 4). Irrespective of the plant, the highest activity of the enzyme was found in the soil sampled at the beginning and at the end of the vegetation period (Table 4). The seasonal decrease in the activity of hydrolase may be due to a change in soil moisture and temperature increase (Table 1). Thus,

Table 3. Value of C:N and pH in H_2O and KCl

FYM t ha^{-1}	C:N				pH in H_2O				pH in KCl			
	Rates of nitrogen											
	N_0	N_1	N_2	N_3	N_0	N_1	N_2	N_3	N_0	N_1	N_2	N_3
Crop rotation A												
0	10.9	10.6	9.4	10.1	6.2	6.0	6.0	5.9	5.8	5.7	5.5	5.3
20	11.8	11.7	8.4	9.6	6.0	5.8	5.9	5.8	5.3	5.4	5.4	5.3
40	10.8	11.0	8.6	9.1	5.9	5.9	5.8	5.8	5.4	5.4	5.4	5.2
60	11.2	9.6	9.2	9.5	5.9	5.9	5.9	5.8	5.4	5.5	5.5	5.3
Crop rotation B												
0	10.4	8.6	10.1	9.1	5.6	5.9	5.6	5.4	4.9	4.7	4.8	4.8
20	9.3	8.6	8.7	8.2	5.6	5.4	5.5	5.4	5.0	4.9	4.9	4.9
40	14.5	10.4	9.8	8.0	5.7	5.6	5.7	5.6	5.0	5.0	5.0	5.0
60	9.6	11.1	10.7	10.1	5.7	5.6	5.6	5.7	5.3	5.2	5.1	5.2

Table 4. Dynamics of arylsulphatase activity in soil depending on the crop rotation, rates of FYM and ammonium nitrate

Properties		Arylsulphatase activity mM pNP kg^{-1} h^{-1}				
		April	June	July	September	Mean
Crop rotation	A	0.278 a	0.199 a	0.183 a	0.308 a	0.445
I factor	B	0.380 b	0.294 b	0.259 b	0.366 b	0.535
FYM	0	0.253 a	0.174 a	0.139 a	0.367 ab	0.433
t ha^{-1}	20	0.317 b	0.246 b	0.191 b	0.298 a	0.450
II factor	40	0.337 b	0.253 b	0.243 c	0.290 a	0.471
	60	0.409 c	0.314 c	0.312 d	0.395 b	0.609
Nitrogen	N_0	0.339 ab	0.261 c	0.239 b	0.433 b	0.568
kg kg^{-1}	N_1	0.305 a	0.274 d	0.232 b	0.316 a	0.481
III factor	N_2	0.334 a	0.232 b	0.216 a	0.304 a	0.463
	N_3	0.337 ab	0.219 a	0.196 a	0.296 a	0.448
Mean		0.329	0.247	0.221	0.338	0.491
SD		0.100	0.098	0.101	0.256	

0, 20, 40, 60 – rates of FYM t ha^{-1}, N_0, N_1, N_2, N_3 – rates of ammonium nitrate. Other explanations as in Table 2.

environmental conditions inhibited the activity of soil microorganisms in the soils. The soil moisture content changes over time, and therefore the distribution of soil enzymes and decomposition rates are probably highly dynamic (Baldrian, 2014).

A significant effect of the type of plants on the arylsulphatase activity in the Luvisol under study was recorded. The activity of the enzyme ranged from 0.174 mM pNP kg^{-1} h^{-1} to 0.433 mM pNP kg^{-1} h^{-1} (Table 4). A higher activity of arylsulphatase was recorded in the soil sampled from the crop rotation that included clover (Table 4). The type of crop can affect soil enzyme activities. Generally, a monoculture causes physical soil degradation with possible negative effects on soil microbial properties, whereas crop rotation enhances the microbial activity of soil. The high input and diversity of organic materials stimulate microbial activity, leading to higher concentrations of microbial biomass and enzyme activities than monocropping systems (Dodor and Tabatabai, 2003). Other factors that affect enzyme activity are the crop type and the root system due to the rhizosphere effect (Gianfreda and Ruggiero, 2006). As reported by Knauff *et al*. (2003), the highest activities of arylsulphatase from a long-term field experiment were found within the rhizosphere of *Brassica napus* and *Triticum aestivu*m, and the lowest values in the case of *Sinapus album* and *Lolium perenne*. Plants actively respond to an insufficient sulphur supply by producing and excreting sulphatases, which may help them to exploit the organic soil sulphur compounds. The activities are relatively low, but they may increase with more severe S deficiency. Knauff *et al*. (2003) assumed that arylsulphatase activity determined in roots of sterile-grown plants is derived from endophytic bacteria and not by higher plants.

The application of farmyard manure in the experiment increased the arylsulphatase activity (Table 4). Usually, an increase in the FYM rate increased the hydrolase activity. The long-term effect of organic amendments on enzyme activities is probably a combined effect of a higher degree of stabilization of enzymes to humic substances and an increase in microbial biomass with increased soil carbon concentration (Elfstrand *et al*., 2007). The optimal FYM rate for which the highest arylsulphatase activity in soil was observed for each of the soil sampling dates was 60 Mg ha^{-1} (Table 4). In general, the addition of organic residues can stimulate enzyme activities as a result of microbial proliferation or enzyme induction in response to addition of inhibitors, such as heavy metals, not present in the organic residue (Gianfreda and Ruggiero, 2006). Eivazi *et al*. (2003) reported that the ratios between enzymes such as acid phosphatase and alkaline phosphatase activities as well as urease and arylsulphatase activity were significantly higher after the application of manure, regardless of the crop type. Knauff *et al*. (2003) also reported that arylsulphatase activity was correlated with the amount of organic carbon and can be increased by long-term amendment of organic manure. Similar results were reported by Elfstrand *et al*. (2007), *ie* the protease and arylsulphatase activities in soil with FYM were significantly higher than in green manure and sawdust treatments. In contrast, Liang *et al*. (2014) detected no significant difference in arylsulphatase activity among three fertilization schemes: control without fertilization, mineral fertilization, and FYM treatments in a long-term fertilizer experiment started in 1993.

T a b l e 5. Dynamics of sulphate sulphur ($S\text{-}SO_4^{2-}$) content in soil depending on the crop rotation, rates of FYM and ammonium nitrate

Properties		$S\text{-}SO_4^{2-}$ mg kg^{-1}				
		April	June	July	September	Mean
Crop rotation	A	12.912a	13.792b	16.865b	15.033b	14.650
I factor	B	10.411a	10.221a	9.818a	9.963a	10.103
FYM	0	8.053a	10.788a	12.562b	10.016a	10.355
t ha^{-1}	20	10.374b	12.440c	11.052a	10.474a	11.085
II factor	40	14.473cd	12.838c	13.149c	13.194b	13.413
	60	13.746c	11.960b	16.602d	16.309c	14.654
Nitrogen	N_0	13.393c	11.941a	13.321ab	12.445b	12.775
kg kg^{-1}	N_1	10.276a	12.213b	12.446a	11.789a	11.681
III factor	N_2	11.599ab	11.500a	12.675a	13.096c	12.217
	N_3	11.377a	12.373b	14.922c	12.663b	12.833
Mean		11.662	12.007	13.342	12.498	12.377
SD		4.763	2.202	4.826	3.997	

Explanation as in Tables 2 and 4.

T a b l e 6. Correlation matrix between the chemical properties and arylsulphatase activity (n = 64)

Crop rotation		TOC	TN	pH_{KCl}	pH_{H_2O}	$S\text{-}SO_4^{2-}$
A	$S\text{-}SO_4^{2-}$	0.27	0.39	-0.21	-0.24	–
	Arylsulphatase	0.43	0.44	–	–	n.s.
B	$S\text{-}SO_4^{2-}$	0.62	0.54	-0.30	-0.30	-
	Arylsulphatase	0.58	0.47	n.s.	n.s.	0.62

TOC – total organic carbon, TN – total nitrogen, n.s – differences not significant, significant at $p<0.0$.

Fertilization with ammonium nitrate also affected the activity of arylsulphatase. Usually, the highest activity of this enzyme was recorded in the soil from the plots fertilized with the lowest rate of the nitrogen fertilizer. This was especially true in the soil sampled in the case of the last two dates, in which the arylsulphatase activity from the plots with a fertilizer rate of N_0 was by 18 and 32% higher than in the soil from the plots with the highest rate of ammonium nitrate, respectively (Table 4). Nitrogen fertilization, especially in mineral forms, may have an indirect impact on the activities of soil enzymes *via* changes in soil properties such as pH (Gianfreda and Ruggiero, 2006). Enzyme activities increased more often when organic and mineral nitrogen fertilizers were added together (Eivazi *et al.*, 2003). Zhang *et al.* (2015) reported that long-term N fertilization decreased soil α- and β- glucosidase activities. This can be explained by the fact that salts tend to modify the ionic conformation of the active centre of enzymes; specific ion toxicities can also result in nutritional imbalances for microbial growth and subsequent enzyme synthesis.

The present research has demonstrated that the effect on the content of sulphur (VI) depends on crop rotation, term of sampling, and plant fertilization (Table 5). The content of sulphates in the soil ranged from 12.912 mg kg^{-1} to 16.865 mg kg^{-1} in the soil sampled from crop rotation A and from 9.818 to 10.411 mg kg^{-1} in the samples from experiment B (Table 5). In general, in the soils under agricultural use in Poland, the content of $S\text{-}SO_4^{2-}$ does not exceed 25 mg kg^{-1}. Most soils, *ie* 70% of the agricultural acreage, contain from 5.0 to 20.0 mg kg^{-1} of this component (Lipiński *et al.*, 2003). In our study, the content of available sulphur in the soil classifies it as representing a class of soils with medium (10.1-15.0 mg kg^{-1}) and high (15.1-20.0 mg kg^{-1}) content of the component for crop B and for crop A, respectively. The medium content of sulphates (VI) in soil requires supplementation with sulphur. The lowest content of sulphur in available forms was recorded in the soil sampled during the penultimate and the final soil sampling date in crop rotation B (Table 5). Growing a mixture of clover with grasses in the experimental field at Grabów would require an additional use of 15 kg S ha^{-1} (Lipiński *et al.*, 2003). A concentration of sulphates (VI) in soil below 10 mg kg^{-1} is considered low and does not ensure that plants have an adequate supply of sulphur. Sulphur (VI) deficit in plant production has become a problem of contemporary agriculture (Eriksen *et al.*, 2004; Scherer, 2009). Sulphur plays a dual role in the *Fabaceae*; it is essential for symbiotic nitrogen fixation and for protein synthesis. When exposed to sulphur deficit, plants produce lower amounts of valuable protein which contains lower levels of indispensable amino acids. Biological nitrogen fixation, nodulation, and yield of peanut crops are reduced with S-deficiency. Varin *et al*. (2010) examined whether the effect of SO_4^{2-} addition on nitrogen fixation resulted from a stimulation of host plant growth, a specific effect of sulphur on nodulation, or a specific effect of S on nodule metabolism. The application of sulphate (VI) increased whole plant dry mass, root length, and nodule biomass expressed on a root-length basis. Nitrogen fixation was drastically reduced in S-deficient plants as a consequence of a low nodule development, but also due to low nitrogenase and leghaemoglobin production due to down-regulation by a nitrogen feedback mechanism, as the high concentration of whole plant nitrogen and the accumulation of N-rich amino acids under severe sulphur deficiency indicated that the assimilation of nitrogen exceeded the amount required for plant growth. Compared with subterranean clover supplied with S, nodulation was markedly decreased in the S-deficient clover. This is attributed to the decline in the requirement for N with reduced S supply (Mazid *et al*., 2011).

In the investigation in Grabów, an influence of the FYM doses on the sulphate (VI) content during plant vegetation was reported. The lowest sulphate content was observed in soil without FYM (exceptionally: July). It was found that the optimal rates of a natural fertilizer for which the highest contents of sulphates in soil were determined were the amounts of 40 and 60 Mg ha^{-1} (Table 5). Knauff *et al.* (2003) assumed that the long-term amendments of FYM or compost, respectively, not only increased the organic matter and total sulphur content of soils but also the sulphatase activity; these findings show that agronomic measures such as compost and FYM application improve the availability of soil organic sulphur.

An influence of the mineral nitrogen fertilizer application on the content of available sulphur was observed during the vegetation period of corn and grasses with clover. The interaction between the sulphate (VI) content and the doses of nitrogen from ammonium nitrate was changed. The highest sulphate content in April was noticed in soil without N, but in July with N_3. This may be a result of different nitrogen and sulphur requirements of the plants or the varying uptake of these nutrients. This may be also an effect of application of ammonium nitrate, which acidifies the soil and thus contributes to varied rates of sulphur sorption by soil components. Adsorption of SO_4^{2-} is stronger at low soil pH; at pH > 6.5, adsorption is negligible and most sulphates are found in the soil solution (Scherer, 2009). Tables 3 and 4 show changes in the content of sulphates as well as the arylsulphatase activity during the vegetation period in both species. It can be noticed that there was higher arylsulphatase activity in the plot with the lower content of available sulphur. Similarly, Speir *et al*. (1980) found that low SO_4^{2-} concentrations in the soil solution were associated with poor sulphur availability to plants and microorganisms that stimulate the biotic production of sulphohydrolases. Conversely, the synthesis of sulphohydrolases and their activity in soil decrease in the case of lower amounts of sulphates. According to another author (Dick, 1992), the activities of the soil enzymes involved in the cycling of a given nutrient are often negatively associated with the availability of the nutrient in soil.

The present study has presented the effect of other parameters on the content on arylsulphatase activity in soil. Soil organic carbon and nitrogen are the most important soil components that may influence the activities of enzymes in soil since they reflect the amount of organic matter (Gianfreda and Bollag, 1996). The activity of arylsulphatase was positively correlated with the content of total organic carbon ($r = 0.43$ for crop rotation A and $r = 0.58$ for crop rotation B $p < 0.05$) and total nitrogen ($r = 0.44$ for crop rotation A and $r = 0.47$ for crop rotation B $p < 0.05$) (Table 6). Arylsulphatase activity was found to be positively correlated with the content of sulphate sulphur ($r = 0.62$ for crop rotation B $p < 0.05$) (Table 6). The activity of this enzyme was negatively correlated with pH in 1M KCl. The negative significant correlation confirms the results that have been reported by other authors (Kotkova *et al*., 2008; Vong *et al*., 2004). They also observed that high contents of sulphates in soil decrease the activity of arylsulphatase.

CONCLUSIONS

1. The content of total nitrogen and sulphate sulphur in soil was related to the plant species and the type of fertilization applied. Higher contents of nutrients were recorded in the soil sampled from the crop rotation with mixtures of clover and grasses.

2. The highest activity of arylsulphatase and high content of sulphates in soil were noted in the plot with the farmyard manure rate of 60 Mg ha^{-1}.

3. A varied effect of fertilization with ammonium nitrate on the content of sulphate sulphur and the arylsulphatase activity was recorded. The ambiguous effect of increasing the rates of nitrogen fertilizer may be caused by the acidifying effect of the nitrate fertilizer on soil.

4. The statistical correlation between the organic matter and the content of sulphate sulphur and the arylsulphatase activity indicates a significant relationship between these parameters in soil.

Conflict of interest: The Authors do not declare conflict of interest.

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