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INFLUENCE OF SULFUR AND IRON FERTILIZATION ON NUTRIENT UTILIZATION BY PLANTS

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Abstract

The aim of the three-year pot experiment was to determine the effect of standard mineral fertilization enriched with sulfur and iron on the content of nitrogen and sulfur in plants and on nutrient utilization by plants. Abundance of sulfates in soil after sulfur fertilization was also assessed. The direct effect of fertilization was assessed during the first and second year of the pot experiment, and the after-effect was analyzed during the third year. Rape (first year) and maize (second and third year) were the test plants. Solid mineral fertilizers (A: a mixture of ammonium nitrate and dolomite; B: a mixture of ammonium nitrate and sulfate) enriched with iron sulfate were used. Nitrogen content in the plants varied depending on applied fertilization as well on plant species and part. Sulfur application increased sulfur content in the aboveground parts of plants by 25-457% and in roots by 95-708%. Iron application ambiguously influenced nitrogen and sulfur content in the plants. Hovewer, simultaneous application of iron and sulfur (as fertilizer B enriched with iron) resulted in the highest coefficient of nitrogen (84%) and sulfur (39%) utilization. Sulfur fertilization caused a 5-20 fold increase in sulfate sulfur content in the soil.

Key words: mineral fertilizer, sulfur, sulfates, nitrogen, iron

INTRODUCTION

One of the most important functions of sulfur is building proteins. Sulfur is a component of aminoacids (methionine, cystyne, cysteine), and disulfide bridges are crucial for the creation of a protein structure. Sulfur takes part in photosynthesis, antioxidative mechanisms or heavy metal detoxification (Kopcewicz and Lewak 2002). It is one of the factors determining yield quantity (Kaczor i Łaszcz-Zakorczemnna 2010, Meena *et al.* 2013). A sufficient sulfur supply is indispensable for high nitrogen utilization, and sulfur deficiency increases the content of non-protein nitrogen in plants (Kaczor i Łaszcz-Zakorczemnna 2010, De Bona *et al.* 2011). Low content of available sulfur in soils is one of the factors limiting plant yield. Sulfate deficiency concerns most soils of agricultural use in Europe (Scherer 2009). The main reason for that situation is the reduction in sulfur deposition from the atmosphere onto the soil surface, stemming from the reduction in sulfur dioxide emission (mainly from combustion processes) (Vega *et al.,* 2018). This tendency has been observed since the end of the twentieth century and will continue at least until 2050 (Engardt *et al.* 2017). Sulfur deficiency is also a result of uptake of this element with plant yield, sulfate leaching into the soil profile, and reduced use of organic fertilizers. Therefore, mineral fertilization with sulfur has become necessary in sustainable fertilization that ensures maximization of nutrient utilization from fertilizers. On the other hand, sulfur fertilization can lead to sulfate accumulation in soil and to acidification of the environment (Filipek-Mazur *et al.* 2018, 2019).

Rational fertilization should also take providing the plant with microelements into account. Iron is one of these crucial microelements and approximately one third of global soils are characterized by its deficiency (Aref 2012 based on Yi *et al.* 1994). Iron plays a role, among other things, in antioxidative mechanisms, photosynthesis, ATP formation, reduction of nitrates, biological nitrogen fixation (Kopcewicz and Lewak 2002). The yield-forming effect of iron fertilization has been confirmed (Rahman *et al.* 2011, Meena *et al.* 2013, Zuchi *et al.* 2015). Iron and sulfur are parts of proteins that are enzymes in, among other things, the respiratory chain (Balk and Pilon 2011).

The aim of the research was to determine the effect of mineral fertilization with sulfur and iron on the content of nitrogen and sulfur in plants and on nutrient utilization by plants. The abundance of sulfates in soil after sulfur fertilization was also assessed.

MATERIALS AND METHODS

Conducting the pot experiment

The pot experiment was conducted in a greenhouse located at the experimental station of the University of Agriculture, in Krakow-Mydlniki, Poland.

The pot experiment was set up on medium soil of a slightly acid reaction, low content of sulfate sulfur and total sulfur, and medium content of available phosphorus and potassium (Table 1). The experiment was set up in 2013 and continued in 2014 and 2015. The experiment comprised eight treatments (conducted in triplicate):

1. no fertilization (control) – C;
2. NPK fertilization with chemically pure salts – NPK;
3. NPK+S fertilization with chemically pure salts – NPK+S;
4. NPK fertilization with chemically pure salts + Fe chelate – NPK+Fe;
5. NPK fertilization with fertilizer A (fertilizer A: a mixture of ammonium nitrate and ground dolomite; 27% N) and chemically pure salts – A;
6. NPK fertilization with fertilizer A with the addition of Fe (27% N; 0.5% Fe) and chemically pure salts – A+Fe;
7. NPK fertilization with fertilizer B (fertilizer B: a mixture of ammonium nitrate and ammonium sulfate; 26% N, 13% S) and chemically pure salts – B;
8. NPK fertilization with fertilizer B with the addition of Fe (26% N, 13% S, 0.1% Fe) and chemically pure salts – B+Fe.

Table 1. Soil properties prior to setting up the experiment

Parameter	Value
Soil texture – fraction 1-0.1 mm, %	39
Soil texture – fraction 0.1-0.02 mm, %	31
Soil texture – fraction < 0.02 mm, %	30
pH_{H2O}	6.36
pH_{KCl}	5.22
Hydrolityc acidity Hh, mmol (+)·kg^{-1} d.m.	16.1
Total N, g·kg^{-1} d.m.	0.701
Organic C, g·kg^{-1} d.m.	7.35
Total S, g·kg^{-1} d.m.	0.124
Sulfate S, mg·kg^{-1} d.m.	12.5
Available P, mg·kg^{-1} d.m.	54.5
Available K, mg·kg^{-1} d.m.	182

Doses of nutrients added to the soil of the fertilized treatments in the first and second year of the experiment were as follows: 0.8 g N; 0.3 g P; 1.2 g K and 0.4 g S·pot^{-1}. The soil mass per pot was 5 kg d.m. Nitrogen was added to the soil from the treatments fertilized with mineral fertilizers in those fertilizers. It was added to the soil of the other fertilized treatments in the form of analytically pure NH_4NO_3 solution. A part of nitrogen was added in the form of analytically pure $(NH_4)_2SO_4$ solution only in the treatment with fertilization with NPK+S in the form of chemically pure salts, because sulfur was added to the soil in that form. Sulfur was also added to the soil of treatments fertilized with fertilizer B. Phosphorus and potassium were added to the soil of all fertilized treatments in the form of solutions of chemically pure salts: $Ca(H_2PO_4)_2 \cdot H_2O$ and KCl. Iron was added to the soil of three treatments, including two fertilized with mineral fertilizers. Due to the different iron content in mineral fertilizers (0.5% Fe in fertilizer A and 0.1% Fe in fertilizer B), iron doses added to the soil fertilized with these fertilizers also differed (0.015 and 0.003 g $Fe \cdot pot^{-1}$, respectively). The form of iron added to both fertilizers (A and B) was the same – iron sulfate. This element was added to the soil of the third of the treatments fertilized with iron in chelated form (product: Fe HBED with 9% Fe, PPC ADOB), at a dose of 0.015 g $Fe \cdot pot^{-1}$. In the third year of the experiment, the after-effect of the applied fertilizers was evaluated. Doses of nutrients added to the soil of fertilized treatments were as follows: 0.8 g N; 0.3 g P and 1.2 g $K \cdot pot^{-1}$; fertilization was carried out using solutions of analytically pure salts.

In 2013, spring rape ‘Feliks’ cv. was cultivated, whereas in 2014 and 2015 – maize ‘Bejm’ cv. (FAO 230-240). The plants were sown on 6 May 2013, 8 May 2014 and 7 May 2015. Twenty rape seeds or seven maize grains were planted in each pot. After plants emergence, density was adjusted to five rape plants and four maize plants per pot. Rape was harvested on 4 July 2013 (60 days of vegetation), at the stage of flowering/pod formation. Maize was harvested on 10 July 2014 (64 days of vegetation) and on 16 July 2015 (72 days of vegetation), at the stage of 8-10 leaves. Moisture content of the soil during plant vegetation was maintained at 60% of its maximum water capacity.

Methods of laboratory analyses

Aboveground parts and roots of the plants were washed and dried (70°C) in order to determine dry matter yield. After that, the material was ground to prepare samples for determination of sulfur and nitrogen content. Determination of sulfur content was carried out using inductively coupled plasma-optical emission spectrometry (ICP-OES), on a Perkin Elmer Optima 7300 DV apparatus. The material was digested with concentrated nitric acid, evaporated and (after binding sulfur by magnesium nitrate) burned in a muffle furnace (2h at 300°C, 3h at 450°C). The remains were dissolved in nitric acid solution (Ostrowska *et al.* 1991). Nitrogen content was determined by the Kjeldahl method on Kjeltec 2300 (FOSS) apparatus. The amount of elements taken up by plants was calculated as a product of the element content in dry matter yield and the yield amount. To calculate the coefficient of utilization of nutrients from fertilizers, the difference between total uptake of the elements from the fertilized soil and the control soil was calculated. The obtained value was divided by the dose of element introduced to the soil, and the results were expressed in percentage (%).

Samples of the soil material were collected after plant harvest. The material was air-dried and sieved through a 1-mm sieve, after which sulfate sulfur content was determined by the ICP-OES method. Sulfates were rinsed with 0.03 mol·dm^{-3} acetic acid (30 min., 40 rpm, 1:10 m/v) (Ostrowska *et al.* 1991). The analyses were conducted after each year of research.

To characterize the soil properties before setting up the experiment, additional analyses were conducted: granulometric composition was determined by the Bouyoucos-Casagrande's areometric method in Prószyński's modification (Warzyński *et al.* 2018); hydrolytic acidity by the Kappen method after extraction with 1 mol·dm^{-3} sodium acetate (1h, 40 rpm, 2:5 m/v); total nitrogen content by the Kjeldahl method; and total sulfur by the ICP-OES method after binding sulfur by magnesium nitrate, dry mineralization (12h at 450^{o}C) and after dissolving the remains in nitric acid solution (Ostrowska *et al.* 1991). Organic carbon was determined after oxidation with potassium dichromate (Skjemstad and Baldock 2007). Available forms of phosphorus and potassium were determined by the Egner-Riehm method (Ivanov *et al.* 2012), after extraction with pH 3.55 calcium lactate (90 min., 40 rpm, 1:50 m/v).

Statistical analysis of the results

A univariate analysis of variation was carried out (factor: type of fertilization). The significance of differences between mean values for individual treatments was estimated by Tukey test, at the significance level of $\alpha = 0.05$. Dell Statistica ver. 13 (Dell Inc.) was used for calculations.

Principal component analysis (PCA) was applied to show relationships between the analyzed parameters and treatments. The amount of plant yield (not discussed in detail in this paper) was also taken into account in the analysis. Iron content in the plants was not taken into account since it was not correlated with the content of nitrogen and sulfur in the plants. Statistical software PQStat was used.

RESULTS

Nitrogen content in the test plants varied depending on applied fertilization as well as on species and part of the plant (Table 2). During the first and the second year, nitrogen content in aboveground parts of the fertilized plants was significantly higher than in the control plants. In the third year, aboveground parts of the control plants contained more nitrogen than those of some of the fertilized plants, which was a result of element concentration in a low yield; a similar situation was observed in relation to nitrogen content in roots of the test plants for all years of research. Nitrogen content in the plants fertilized with sulfur was statistically lower or the same, compared to the plants fertilized with NPK without sulfur, regardless of plant species and part or year. Application of iron chelate increased nitrogen content only in rape aboveground parts. Iron addition to mineral fertilizers increased nitrogen content only in maize aboveground parts and roots during the third year of research, in the case of enrichiment of fertilizer A. In other cases, iron application significantly decreased nitrogen content or the content remained the same.

Table 2 Nitrogen content in plants

Treatment	**Rape (1st year)**		**Maize (2nd year)**		**Maize (3rd year)**	
	Above-ground parts	**Roots**	**Above-ground parts**	**Roots**	**Above-ground parts**	**Roots**
	[g·kg^{-1} d.m.±SD]					
Control	9.65^{a}*±0.26	6.47^{c}±0.05	6.07^{a}±0.19	4.80^{a}±0.27	12.94^{b}±0.18	5.98^{a}±0.86
NPK	14.14^{c}±0.52	6.98^{d}±0.10	17.72^{e}±0.47	14.49^{d}±0.19	16.96^{c}±0.56	14.76^{d}±0.96
NPK+S	11.44^{b}±0.31	4.76^{a}±0.05	7.52^{b}±0.13	5.32^{a}±0.05	12.25^{b}±0.72	6.76^{ab}±1.00
NPK+Fe	16.37^{d}±0.80	6.38^{c}±0.22	12.71^{c}±0.76	12.00^{c}±0.13	15.50^{c}±1.00	15.31^{d}±0.76
Fertilizer A**	16.32^{d}±0.08	6.74^{cd}±0.21	14.80^{d}±0.03	14.12^{d}±0.83	10.27^{a}±0.31	7.92^{b}±0.16
Fertilizer A+Fe	14.35^{c}±0.35	6.43^{c}±0.17	12.99^{c}±0.51	9.97^{b}±0.35	16.47^{c}±0.40	12.21^{c}±0.05
Fertilizer B	13.29^{c}±0.60	6.40^{c}±0.08	7.39^{b}±0.07	5.61^{a}±0.01	10.27^{a}±0.18	7.02^{ab}±0.24
Fertilizer B+Fe	14.01^{c}±0.93	5.67^{b}±0.07	8.45^{b}±0.14	5.31^{a}±0.17	9.86^{a}±0.46	7.42^{ab}±0.07

* mean values in the columns marked with the same letters do not differ statistically significantly at the significance level $\alpha = 0.05$, according to Tukey test

** A – mixture of ammonium nitrate and ground dolomite, fertilization supplemented with chemically pure salts; B – mixture of ammonium nitrate and ammonium sulfate, fertilization supplemented with chemically pure salts

SD – standard deviation

Sulfur fertilization increased sulfur content in the plants by 25-708%, compared to NPK treatment (Table 2). As a result, the N:S ratio in the plants fertilized with sulfur was considerably lower than in the other fertilized plants (Fig. 1). The relatively high sulfur content in the control plants was a result of element concentration in low yield. No clear effect of iron application on sulfur content in the plants was observed.

Table 3. Sulfur content in plants

Treatment	Rape (1st year)		Maize (2nd year)		Maize (3rd year)	
	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots
	[g·kg^{-1} d.m.±SD]					
Control	4.29^{d}*±0.29	1.81^{c}±0.13	0.73^{cd}±0.05	1.32^{c}±0.07	0.67^{ab}±0.02	1.12^{b}±0.08
NPK	0.73^{a}±0.04	0.66^{a}±0.05	0.49^{b}±0.03	0.37^{a}±0.03	0.85^{c}±0.06	0.66^{a}±0.05
NPK+S	4.06^{cd}±0.06	1.83^{c}±0.12	0.75^{d}±0.01	2.82^{e}±0.11	1.09^{d}±0.11	1.28^{b}±0.06
NPK+Fe	0.72^{a}±0.01	0.95^{b}±0.05	0.43^{ab}±0.04	0.42^{a}±0.02	0.58^{a}±0.06	0.63^{a}±0.03
Fertilizer A**	0.79^{a}±0.07	0.68^{ab}±0.07	0.37^{a}±0.04	0.31^{a}±0.01	0.50^{a}±0.01	0.60^{a}±0.06
Fertilizer A+Fe	0.76^{a}±0.01	0.84^{ab}±0.02	0.39^{ab}±0.02	0.75^{b}±0.05	0.77^{bc}±0.06	0.75^{a}±0.01
Fertilizer B	3.18^{b}±0.26	1.73^{c}±0.19	0.75^{d}±0.04	2.96^{e}±0.04	1.07^{d}±0.03	2.14^{c}±0.21
Fertilizer B+Fe	3.49^{bc}±0.43	2.40^{d}±0.04	0.62^{c}±0.06	2.59^{d}±0.06	1.13^{d}±0.06	2.14^{c}±0.12

*, ** as in Table 2
SD – standard deviation

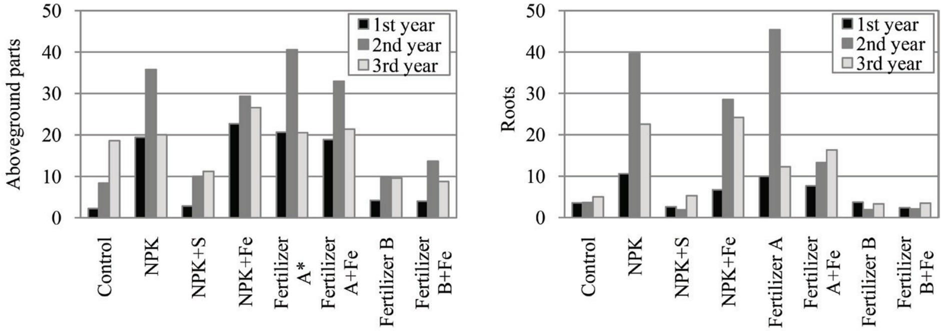

Figure 1. N:S ratio in plants
* A – mixture of ammonium nitrate and ground dolomite, fertilization supplemented with chemically pure salts;
B – mixture of ammonium nitrate and ammonium sulfate, fertilization supplemented with chemically pure salts

The amount of nitrogen and sulfur taken up by plants confirmed the effect of nitrogen and sulfur fertilization (Fig. 2). Total amount of nitrogen taken up by the plants fertilized without any sulfur was 5.6-6.2 times higher than the amount taken up by the control plants. After sulfur application (treatments: NPK+S, fertilizer B, fertilizer B+Fe), nitrogen uptake was 6.6-7.5 times higher compared to the control plants. Total amount of sulfur taken up by the plants fertilized without any sulfur was 1.3-1.4 times higher than the amount taken up by the control plants; sulfur addition considerably increased the uptake (up to a 6.0-6.8 times higher value). The value of coefficient of nutrient utilization was the highest in the case of fertilizer B+Fe (Fig. 3). No clear effect of iron addition to fertilizer A or of chelate application on nitrogen and sulfur uptake by the plants was observed.

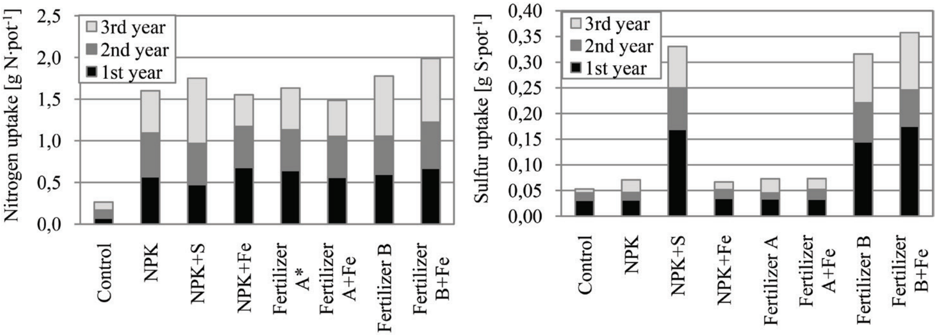

Figure 2. Amount of nitrogen and sulfur taken up by plants
* as in Fig. 1

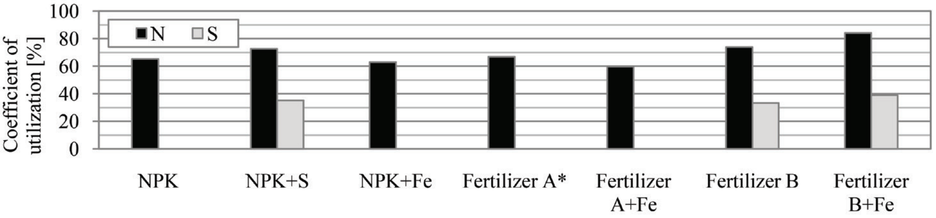

Figure 3. Values of coefficient of nutrient utilization from fertilizers
* as in Fig. 1

Sulfur fertilization (treatments: NPK+S, fertilizer B, fertilizer B+Fe) significantly increased sulfate sulfur content in the soil in all years of research (Table 4). After the first year the content was 5-7 times higher than in the NPK treatment. After the second year (which means after double application of sulfur), the content was 19-20 times higher. During the third year of research, no sulfur fertilization was conducted. However, sulfate sulfur content in the soil fertilized with this element was still 9-13 times higher than in the soil with the NPK treatment.

Table 4. Sulfate sulfur content in soil

Treatment	1st year	2nd year	3rd year
		[mg·kg^{-1} d.m.±SD]	
Control	12.07^{a}*±1.05	7.42^{a}±0.52	9.91^{ab}±0.09
NPK	10.31^{a}±0.01	5.30^{a}±0.62	5.60^{ab}±1.02
NPK+S	50.53^{b}±4.26	103.46^{b}±2.04	70.10^{d}±6.45
NPK+Fe	9.34^{a}±0.82	5.77^{a}±0.86	8.45^{ab}±0.18
Fertilizer A**	9.96^{a}±1.47	5.68^{a}±0.25	2.12^{a}±0.61
Fertilizer A+Fe	7.91^{a}±0.06	3.75^{a}±0.01	12.00^{b}±1.21
Fertilizer B	70.97^{c}±1.70	105.68^{b}±10.98	64.36^{d}±5.40
Fertilizer B+Fe	64.56^{c}±8.32	98.55^{b}±8.25	49.82^{c}±2.52

*, ** as in Table 2
SD – standard deviation

Nitrogen content in plant aboveground parts and roots was positively correlated (Fig. 4). There was a negative correlation between nitrogen content in plants (aboveground parts and roots) and sulfur content in plants roots, as well as with sulfate sulfur content in the soil. There was a positive correlation between sulfate sulfur content in the soil, sulfur content in plant roots, and the plant yield.

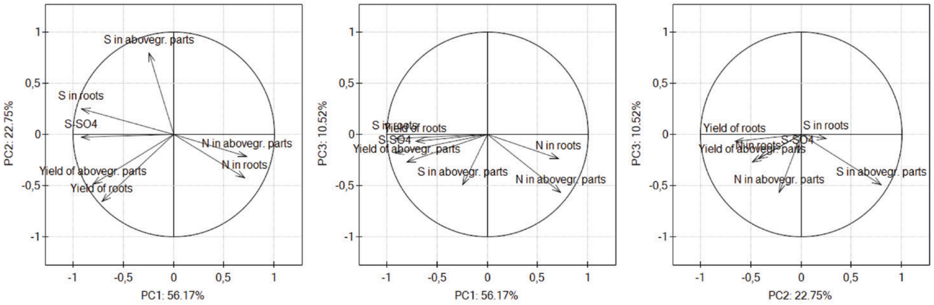

Figure 4. Principal component analysis (PCA) showing relationships between parameters

DISCUSSION

Sulfur application decreased nitrogen content in the plants, compared to fertilization without sulfur addition (Table 2). Moreover, a negative correlation between nitrogen content in the plants and sulfate sulfur content in the soil was observed (Fig. 4). A similar effect was recorded by Kaczor and Łaszcz-

Zakorczemnna (2010), who observed a decrease in nitrogen content in spring rape, regardless of plant part, with increasing sulfur dose. The cited authors explained this dependency by the dilution of the component in the increased (as a result of fertilization) plant yield. Jankowski *et al.* (2015) obtained similar results. Filipek-Mazur *et al.* (2017) did not observe a significant effect of sulfur fertilization on nitrogen content in rape seeds and wheat grains.

Sulfur fertilization significantly increased sulfur content in the plants and soil (it was both direct and after-effect of the fertilization) (Tables 3, 4). The same effect of sulfur fertilization was recorded by Filipek-Mazur *et al.* (2017, 2018), Gondek and Kopeć (2010) and Jankowski *et al.* (2015).

No clear effect of iron fertilization on nitrogen and sulfur content in plants was found. However, the highest total amount of both elements was taken up by the plants fertilized with fertilizer B (a mixture of ammonium nitrate and ammonium sulfate) enriched with iron. This confirms the beneficial effect of simultaneous iron and sulfur application on element uptake and utilization from fertilizers. Podleśna *et al.* (2017) recorded higher nitrogen utilization after simultaneous sulfur and nitrogen application. However, element utilization also depends on weather conditions (precipitation, air temperature) during vegetation period – unfavorable conditions decrease the yield-forming potential of plants, and, as a result, the coefficient of utilization is lower (Trawczyński and Wierzbicka 2014). High values of the coefficient of nutrient utilization from fertilizers indicate low risk of environmental pollution, associated with fertilizer application (and consisting in leaching of elements deep into soil). Mineral fertilization may led to nitrate and sulfate leaching (Fernández-Escobar *et al.* 2004, Gondek and Kopeć 2010, Jakubowski *et al.* 2014). Improved element utilization by plants also translates into economic profit for the farm.

CONCLUSIONS

1. Nitrogen content in the test plants varied depending on applied fertilization as well as on plant species and part. Nitrogen content in the plants fertilized with fertilizer A (a mixture of ammonium nitrate and powdered dolomite) was significantly higher than after application of fertilizer B (a mixture of ammonium nitrate and ammonium sulfate) or the content did not differ statistically.
2. Sulfur application significantly increased sulfur content in the plants and did not increase nitrogen content. The effect of iron addition on the chemical composition of the plants was ambiguous. However, taking both sulfur and iron into account in fertilization (application of fertilizer B+Fe) allowed us to obtain the highest coefficient of nitrogen and sulfur utilization.

3. Sulfur fertilization significantly increased sulfate sulfur content in the soil.

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