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The efficiency of nitrogen and sulphur fertilization on yields and value of N:S ratio for *Lolium* × *boucheanum*

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ABSTRACT

This paper deals with the efficiency of nitrogen (N) and sulphur (S) fertilization on yields and value of N:S ratio for hybrid ryegrass cultivated in monoculture and in a mix with white clover. The research was carried out from 2011–2013 near Krakow in southern Poland. Plants were evaluated in the context of diversified fertilization with nitrogen at rates of 50 and 100 kg N/ha as well as two sulphur-based fertilizers: Arista siarka and Pro-siarka, used at rates of 5, 10, 15 kg S/ha. Independent research findings have shown a clear synergism between nitrogen and sulphur, namely fertilization with sulphur in the form of Arysta siarka and Pro-siarka fertilizers that increased the yields of total aboveground biomass of the plants, from all cuts and decreased the values of the N:S ratio. The highest average dry matter yield was determined after the use of 50 kg N/ha and 15 kg S/ha in the form of Pro-siarka fertilizer, compared to fertilization with 50 kg N/ha, amounting to 0.52–2.08 t/ha for hybrid ryegrass and 0.15–2.40 t/ha for hybrid ryegrass with white clover, respectively. The highest average narrowing of the N:S ratio was calculated in plants fertilized with 100 kg N/ha and 15 kg S/ha in the form of Pro-siarka fertilizer.

Keywords: crop production; nutrient; *Trifolium repens*; sulphur deficiency; grassland management

In recent years, due to pro-ecological activities as well as the changing range of fertilizers, many countries have experienced sulphur (S) shortages in crop production (Stern 2005, Morris 2007). In Poland, sulphur shortages in agroecosystems tend to occur particularly in crop production with intensive nitrogen (N) fertilization. Therefore, there is a concern that the traditionally used NPK fertilization is not balanced whilst the sulphur deficit may limit the utilization of other nutrients by plants, particularly nitrogen (Lošák et al. 2000). Nitrogen assimilation in plants is closely related to their sulphur supply because with high doses of this nutrient its utilization from fertilizers and its agricultural and physiological efficiency are significantly higher in the sulphur fertilization environment (Podleśna 2005). According to Blake-Kalffa et al. (2003), mineral sulphur fertilization cannot become a routine agrotechnical treatment but must be preceded by diagnostic tests. At the moment, the research concerning mitigation and prevention of sulphur deficiency is mainly focused on species exhibiting a high demand for this element. Most studies were carried out on rapeseed (Lošák and Richter 2003). Fewer studies were completed on agricultural crops (Zhao et al. 2006), and there are only a few papers on Fabaceae (Sator et al. 2002) and Poaceae plants (Richards 1990).

The research hypothesis asks which level of fertilization with sulphur in two forms and with nitrogen had the most impact on yields and N:S ratio for hybrid ryegrass grown in monoculture and mixed with *Trifolium repens*, as well as whether and how the agents interact with each other.

MATERIAL AND METHODS

The study was carried out in Gnatowice, near Krakow in southern Poland (50°11'51,3600''N,

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20°10'36,4440''E, 220 m a.s.l.). The experiment was conducted from 2011–2013, in a split-plot layout, in four replications, on 12 m^2 (3 m × 4 m) fields. The study covered hybrid ryegrass (cv. Gala tetraploidal) grown in monoculture and in a 50% mix with white clover (cv. Romena), 50% crops each. Hybrid ryegrass was evaluated in the context of diverse fertilization with nitrogen at 50 and 100 kg N/ha, which were used twice: in early spring and after the first swath, each dose was applied in equal parts, in the form of 34% ammonium nitrate. Phosphorus (P) was also used in fertilization, at 35 kg P/ha, in the form of triple superphosphate (20% P), once in spring. Potassium (K) was used at 83 kg K/ha in the form of high concentration potassium salt (50% K); in two equal doses in early spring and after the first swath.

The field experiment also involved foliar fertilization with elementary sulphur, with the following agents included:

- agent I – fertilizer type: Arysta siarka (80% S in the total form) and Pro-siarka S 800 SC (80% S in the total form),
- agent II – sulphur doses: sulphur in the form of Pro-siarka S 800 SC liquid fertilizer was used once, before the start of vegetation, in three doses (5, 10 and 15 kg S/ha). Sulphur in the form of Arysta siarka fertilizer was used in three doses as well. Plants were sprayed with Arysta siarka fertilizer solutions during the following plant vegetation phases identified with EU BBCH scale codes:
- 5 kg S/ha – one-time dose during late tillering phase (26–29 BBCH),
- 10 and 15 kg S/ha – doses divided into two equal parts, used at the following times: tillering phase (22–23 BBCH) (5 and 7.5 kg S/ha) and at the start of the stem elongation phase (31–33 BBCH) (5 and 7.5 kg S/ha). Trial establishments were set up on degraded chernozem produced of loess. The chemical properties of this soil are presented in Table 1.

In gross samples of soil material (created through four replications), chemical determinations were made using the following methods: pH per 1 mol/dm^3 KCl using the potentiometric method; organic carbon content: Oleksynowa modification of the Tiurin's method; total nitrogen: the Kjeldahl method using Kjeltec apparatus; assimilable phosphorus: Egner–Riehm's colorimetric method; assimilable potassium: Egner-Riehm with the use of flame photometry; assimilable magnesium: atomic absorption spectroscopy, after extraction in 0.0125 mol $CaCl_2/dm^3$; total sulphur content: ICP OES – inductively coupled plasma optical emission spectrometry, on thermo elementar – IRIS advantage.

The proportion of white clover was determined before gathering the first swath according to the Stebler-Schröter method.

The plants were mowed three times during the vegetation season. The gathered biomass was weighed, then a 1 kg sample was dried at 105°C to fixed weight, to determine the dry matter content by weight. Dried plants were then cut and milled. Chemical analysis was conducted in the material thus prepared, determining the following contents: total nitrogen (N_{tot}) – the Kjeldahl's method; total sulphur (S_{tot}) – ICP OES on thermo elementar – IRIS advantage device, after the prior mineralization of samples in the mix of concentrated acids: sulphuric (VI) and chloric (VII) in 4:1 proportion.

The results were analyzed statistically through the analysis of variance, with Statistica. The least significant differences (*LSD*) for fertilization parameters were verified using the Tukey's test, at confidence level α = 0.05.

The total yearly precipitation during the study period ranged from 581–700 mm, and during the vegetation period from 358–478 mm. The average yearly temperature ranged from 7.9–8.6°C and in the vegetation period from 15.2–16.0°C. In the long-term perspective (1977–2013), the total average yearly precipitation reached 690 mm

Table 1. Soil characteristics of luvic chernozems from trial location (0–20 cm layer)

Specification	Unit	Value
pH (1 mol/L KCl)		6.5
Organic C	(g/kg)	24.7
Total N		1.8
P available	(mg/kg)	62.5
K available		136.7
Mg available		54.9
Total S		321.5
Sand	(%)	17.4
Silt		58.3
Clay		21.4

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and during the vegetation period it was 466 mm. Average yearly temperatures in the long-term perspective was 8.4°C and during the vegetation period it increased to 15.2°C.

RESULTS AND DISCUSSION

The average proportion of white clover in the control treatment without fertilization was 20%, while in establishments with only N fertilization it was at 23% and 8%, respectively (Table 2). Under the influence of fertilization with 50 kg N/ha and S in the form of Arysta siarka fertilizer, the average proportions of white clover ranged from 24–33%. The highest proportion of the latter plant was discovered after applying S at 10 kg S/ha. On the other hand, with lower N fertilization combined with Pro-siarka fertilizer, the average proportions of white clover ranged from 22–37%. Most clover was identified in the establishments where 15 kg S/ha was used in combination with 50 kg N/ha. When N was used at 100 kg N/ha and S applied in the form of Arysta siarka, the proportions of white clover ranged from 9–16%, and after the use of Pro-siarka fertilizer it ranged from 12–16%.

Yields of the total aboveground biomass of hybrid ryegrass grown in monoculture as well as in mixture with white clover in establishments fertilized with N only, regardless of the applied dose, varied for particular regrowths (Figure 1). Nitrogen application at 100 kg N/ha resulted in a major increase of hybrid ryegrass yields as compared to 50 kg N/ha application, so that the yield increases were recorded at 47.9–56.3%. For the mixture of ryegrass and clover, the increase of dry matter yield caused by 100 kg N/ha, as compared to 50 kg N/ha, was at 46.2–66.0%.

When N and S were used in combination, both in the form of Arysta siarka and Pro-siarka fertilizers, a clear synergism was discovered between N and S, which was represented by plant yields. The positive effect of S on the dry matter content of plants was also demonstrated by Zhao et al. (1999) and Salvagiotti and Miralles (2008).

Variance analysis indicates that with the low level of test probability ($P < 0.001$), significant differences were discovered between mean values of dry matter with the two levels of N fertilization and three levels of S fertilization as applied in the experiment (Table 3).

Furthermore, according to Taube et al. (2000), there are significant differences between mean dry matter values for comparable plants under the influence of synergism of fertilization with N and S with the low level of test probability ($P < 0.01$). Tallec et al. (2008), though, stated similar dependence only for the monoculture of perennial ryegrass. However, for the mixture of perennial ryegrass and white clover the significance of yield under the influence of synergism of fertilization with those two fertilizers was with the level of probability ($P < 0.01$).

The average dry matter gains after the use of S in Arista siarka and 50 kg N/ha, compared to fertilization with 50 kg N/ha, ranged from 0.26–1.80 t/ha. On the other hand, after the application of Pro-siarka, it was between 0.51 and 1.90 t/ha. For comparison, the average yield gain for a mixture of hybrid ryegrass and white clover after the use of Arista siarka and 50 kg N/ha, compared to fertilization with 50 kg N/ha, ranged from 0.23–1.65 t/ha, and after the application of Pro-siarka, it was between 0.16 and 2.09 t/ha.

Only for hybrid ryegrass increased yields were recorded in further regrowths under the influence of higher N and S application with reference to 100 kg N/ha application. An exception in this respect were the first regrowth plants fertilized with 100 kg N/ha and 15 kg S/ha in the form of Arista siarka fertilizers, which gave 360 kg/ha less yields than those fertilized with 100 kg N/ha. The average dry matter gains after the use of Arista siarka and 100 kg N/ha, compared to fertilization

Table 2. Mean share of white clover in the sward mixtures of *Lolium × boucheanum + Trifolium repens* (%)

Treatment				
Without N fertilization (control)	20			
With dose N – without S fertilization	N_{50}		N_{100}	
	23		8	
Fertilization N	N_{50}	N_{100}	N_{50}	N_{100}
With dose S (kg/ha)	Arysta siarka		Pro-siarka	
5	26	11	22	15
10	33	16	35	12
15	24	9	37	16

N_{50} – 50 kg N/ha; N_{100} – 100 kg N/ha

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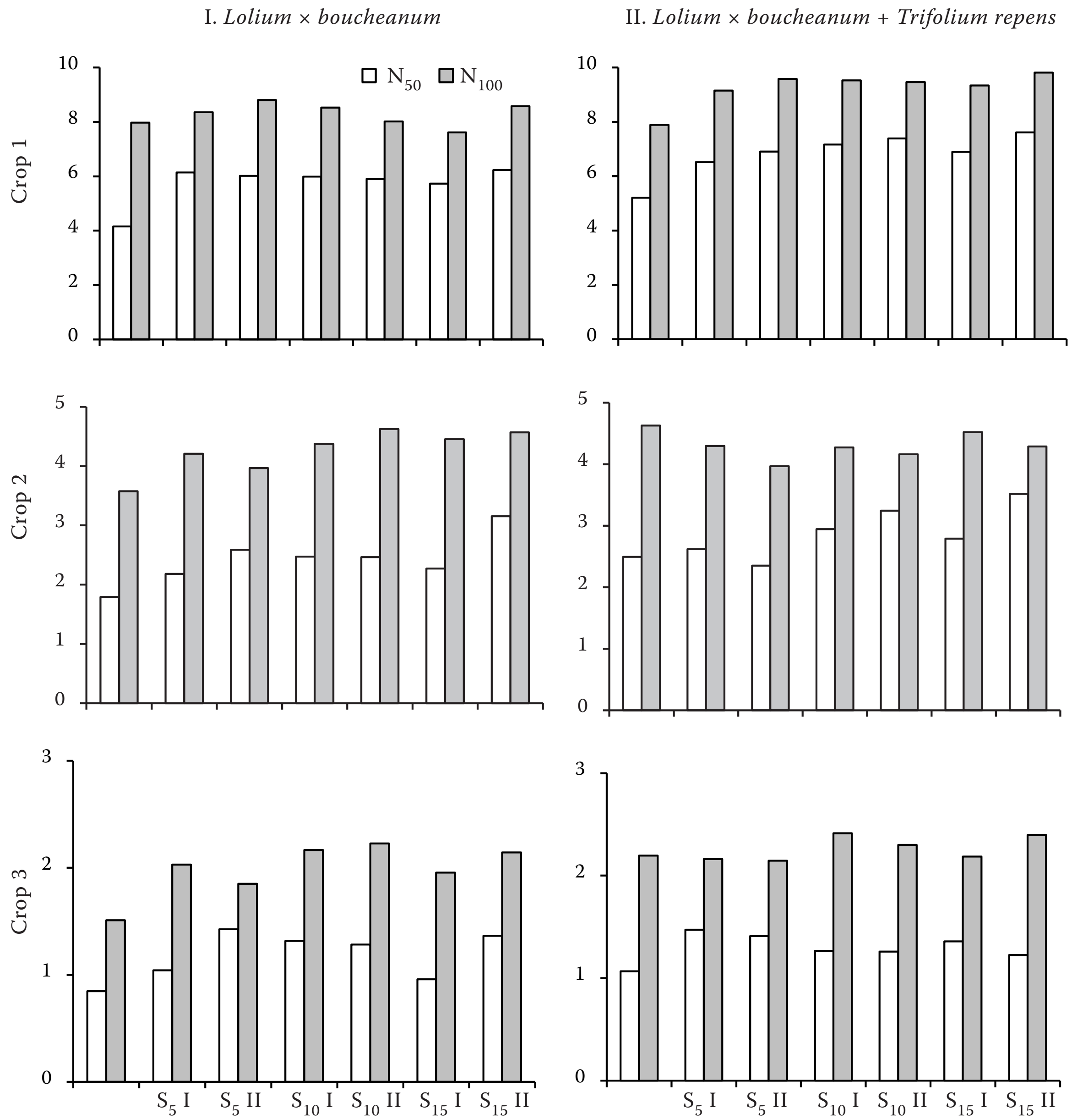

	Lolium × *boucheanum*			*Lolium* × *boucheanum* + *Trifolium repens*		
Crop	1	2	3	1	2	3
$LSD_{0.05}$	2.13	0.70	0.45	2.11	0.62	0.53
Control	1.65	0.85	0.52	2.02	1.10	0.77

Figure 1. Yield of dry matter (t/ha). N_{50} – 50 kg N/ha; N_{100} – 100 kg N/ha; S_5 – 5 kg S/ha; S_{10} – 10 kg S/ha; S_{15} – 15 kg S/ha; crop: 1, 2, 3 – regrowths

with 100 kg N/ha, ranged from 0.19–0.77 t/ha. On the other hand, after the application of Pro-siarka it was between 0.49 and 0.82 t/ha.

In the first regrowth only, the application of N at 100 kg N/ha and S in any dosage of the mixture compared to the application of N at 100 kg N/ha only, caused higher yields in all of the experiments. In the second regrowth, the relation was reversed, i.e. higher yields from N fertilization were obtained than from N + S fertilization, by 380 kg dry matter per hectare on average. In the third regrowth, the average yield gain for hybrid ryegrass and white

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Table 3. ANOVA *F*-test probabilities for the effects of fertilization on plants and their interaction effects

Specification	Crop		
	1	2	3
Dry matter yield		< *P*	
Fertilization	< 0.001	< 0.001	< 0.001
Plants	< 0.001	< 0.001	< 0.001
Fertilization × plants	0.841	< 0.001	0.007
N:S ratio		< *P*	
Fertilization	< 0.001	< 0.001	< 0.001
Plants	0.003	0.073	0.966
Fertilization × plants	0.009	0.006	0.185

Crop: 1, 2, 3 – regrowths

clover after the use of Arista siarka and 100 kg N/ha, compared to fertilization with 100 kg N/ha, was 90 kg of dry matter per hectare; after the application of Pro-siarka it was 70 kg of dry matter. In establishments where 100 kg N/ha and 5 kg S/ha in both forms were applied, lower yields were determined, by 30–40 kg dry matter per 1 hectare, than in establishments where N was applied at 100 kg N/ha.

However, there are no grounds ($P = 0.841$) for rejecting the zero hypothesis concerning the lack of interaction between the agents concerned in the first regrowth only. In this regrowth, the fertilization applied caused identical changes in mean values of dry matter in the plants under consideration. In the second and third regrowth (< 0.001; 0.007), with low test probability ($P < 0.05$), agent concurrence was determined, i.e. fertilization was proved to affect the mean value of dry matter depending on the plants used.

According to Grant et al. (2003) and Mathot et al. (2009), quantity proportions of N:S in indicator parts of plants are among the best, and commonly accepted S fertilization needs testing. The N:S ratio only expresses the quantitative proportion of the elements and not the actual contents. Zhao et al. (1997) indicate a disadvantage of the index at hand, namely that the same N:S ratio may correspond to different S levels and different N levels in the plant. Brown et al. (2000) state that in grassland biomass, depending on the location, fertilization and regrowth, N:S proportions may range from 8.5–18.4:1.

In the author's own research, N fertilization at 100 kg N/ha, compared to fertilization at 50 kg N/ha, increased the N:S value in hybrid ryegrass in the first and second regrowth by 1.0% and 6.3%, respectively (Figure 2). In the third regrowth plant, this proportion was the opposite, namely the N:S ratio decreased by 1.1%.

In hybrid ryegrass and white clover, the N:S value was 5.1–14.2% higher when fertilized with N at 100 kg N/ha. Additional S application for each dosage and N application in two doses resulted in a significant decrease of N:S ratio in plants. In a study by Podleśna (2005), S decreases at 2.4 and 2.2 g/kg dry matter in clover and ryegrass, respectively, led to the N:S ratio being narrowed to 8 and 6. According to Mathot et al. (2008), fertilization with S at 40 kg S/ha resulted in a 14-fold concentration of the N:S ratio in grassy vegetation.

With a low level of test probability ($P < 0.001$), significant differences occurred between the mean values of N:S with the fertilization levels applied in the experiment. Similarly, with a low level of the test probability ($P = 0.003$), a zero hypothesis concerning the lack of impact of the plants was used in the experiment on the mean N:S value in the first regrowth only. Therefore, significant differences occurred between the mean values of N:S for the compared plants in this regrowth. On the other hand, for the first and second regrowth, there are no grounds ($P < 0.05$) for rejecting the zero hypothesis concerning the lack of impact of the plant used on the mean N:S value. Therefore, there were no significant differences between the mean values of N:S for the plants under consideration. In addition, there are no grounds ($P = 0.185$) for rejecting the hypothesis concerning the lack of interaction between the agents concerned in the third regrowth only. For the first and second regrowth, the zero hypothesis concerning interaction, meaning interaction between agents, or the impact of the fertilizer dosage on the mean value of N:S according to the plants used, was rejected.

Fertilization with S in the form of Arista siarka fertilizer (3 doses) and N at 50 kg N/ha, compared to N fertilization at 50 kg N/ha, lowered the N:S ratio in hybrid ryegrass by 5.9–10.3 and in the mixture by 7.2–11.3. A similar effect was obtained for the application of S in the form of Pro-siarka fertilizer, which also reduced the value of N:S by 6.8–11.1 in hybrid ryegrass, and by 6.2–11.1 in the mixture.

On the other hand, fertilization with S in the form of Arista siarka fertilizer (3 doses) and N at

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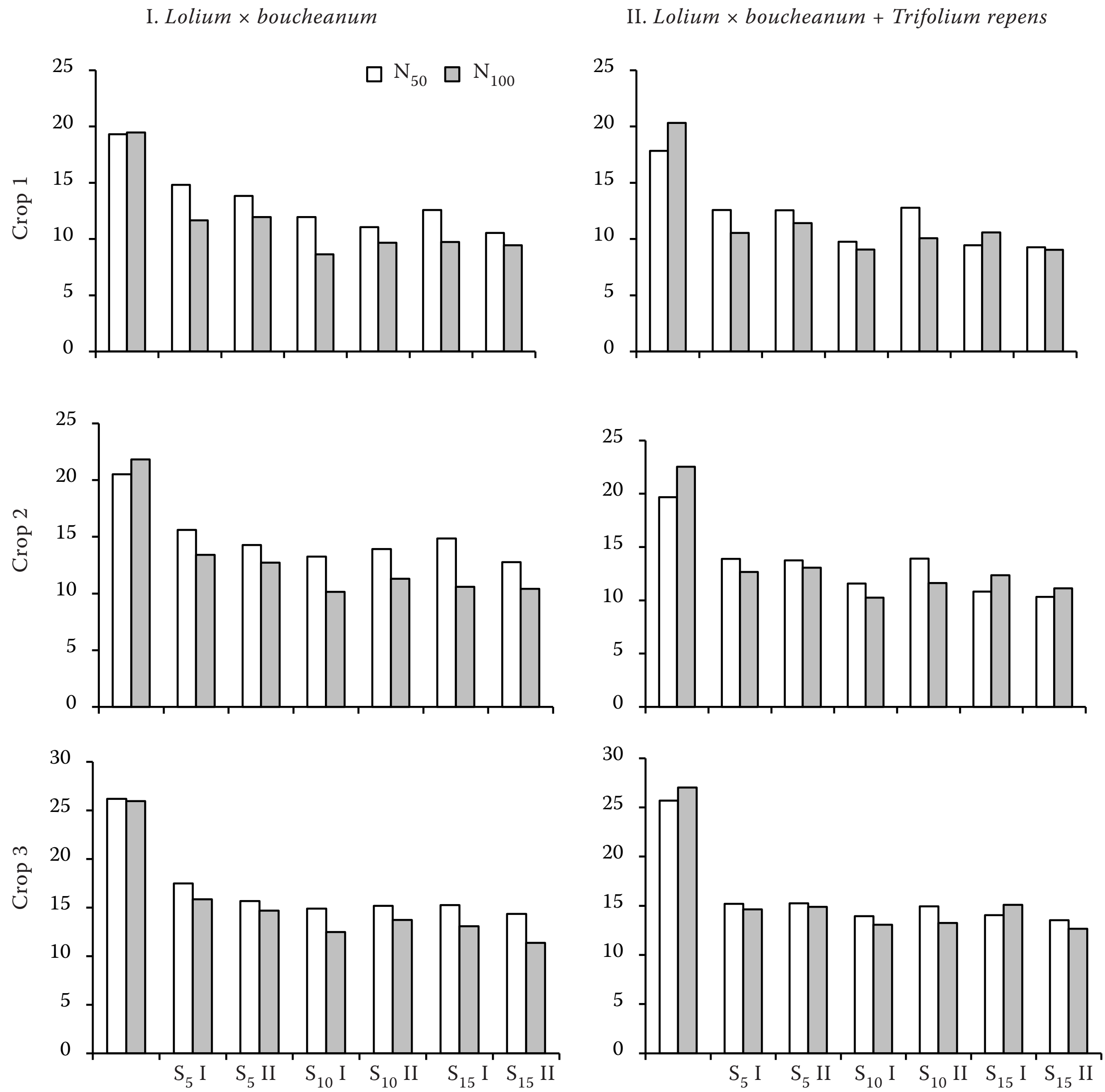

	Lolium × *boucheanum*			*Lolium* × *boucheanum* + *Trifolium repens*		
Crops	1	2	3	1	2	3
$LSD_{0.05}$	3.29	3.49	3.75	2.93	3.21	3.95
Control	17.8	18.6	21.5	16.6	19.7	24.3

Figure 2. Mean ratio of total nitrogen to total sulphur in subsequent regrowths of plant. N_{50} – 50 kg N/ha; N_{100} – 100 kg N/ha; S_5 – 5 kg S/ha; S_{10} – 10 kg S/ha; S_{15} – 15 kg S/ha; crop: 1, 2, 3 – regrowths

100 kg N/ha, compared to N fertilization at 100 kg N/ha, lowered the N:S ratio in hybrid ryegrass by 9.5–12.1 and in the mixture by 10.2–12.7. A similar relation was determined after the application of S in the form of Pro-siarka fertilizer, which also reduced the value of N:S by 9.2–12.6 in hybrid ryegrass, and by 10.1–13.4 in the mixture.

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