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Sowing ratio, NS fertilisation and interactions of *Lolium* sp. and *Festulolium* grown in mixtures with *Trifolium repens*

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Abstract: Current ecological trends for production of forage for livestock indicate a need for small usage of fertilisers while increasing productivity. The study aimed to assess yield and interactions of selected *Lolium* and *Festulolium* species grown in mixtures with *Trifolium repens* and fertilised with NS, regarding mutual interactions based on available indicators. A two-year field study (2016–2017) was carried out in southern Poland and the research objects were designed on degraded chernozems. The highest yield was observed for mixtures: *L. multiflorum* + *T. repens* and *L.* × *boucheanum* + *T. repens* sown in the proportion 50:50, fertilised with $N_{50}S_{15}$. The mean dry matter yield was higher in the 1st year of the study, in all variants of fertilisation and types of mixtures. The land equivalent ratio differed for species in individual objects, for individual cuts and study years, and was most often higher than 1.0, which points to a positive interference between species in mixtures. Also, the competitive ratio index was often higher than 1.0, which means that grass species were more competitive than white clover. However, in the second year of study *T. repens* was dominant in multiple objects.

Keywords: grasses; legume; dry matter yield; nutrients; competition interactions

Short-term grasslands are significant elements of crop rotation in balanced farming systems (Begna et al. 2011). Mixtures of grasses with legumes sown on arable land for 2–3 years simplify arranging fodder for livestock during summer, which reduces costs. The basic advantage of grasses and legumes mixtures is a more stable yield compared to species sown individually, which results from the fact that components of mixture use water and feed ingredients to a larger extent (Esmaeili et al. 2011, Hakl et al. 2018). Both mixtures composed of several plant species and included a few cultivars of the same species to be better suited to the specific habitat conditions (Kramberger et al. 2013). However, there are positive and negative interactions between components of mixtures, which makes structure, chemical composition and yield of mixtures different from the yield observed for pure sowing (Lazzarotto et al. 2009). Thus, an adequate selection of mixture components and their proportions is crucial for obtaining highs yield of good quality (Annicchiarico and Proietti 2010).

Proper crop rotation using legumes results in lower production costs, because the amount of organic nitrogen used in fertilisers and washed away from the soil is limited (Kramberger et al. 2013). Absorption of nitrogen (N) by plants is closely related to the amount of sulfur (S) they receive; in Poland S shortages in agroecosystems tend to occur particularly in crop production with intensive N fertilisation. Therefore, there is a concern that the traditionally used NPK fertilisation is not balanced and the S deficit may limit the utilisation of other nutrients by plants (Grygierzec et al. 2015). One of the key fodders among the legumes grown in moderate climate

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is *Trifolium repens* L. The main reason why it is used in mixtures is the ability to assimilate N from the atmosphere. Growing of this species in pure sowing and in mixtures with *Lolium perenne* L. was studied (Guckert and Hay 2001). However, mixtures with *L. multiflorum*, *L.* × *boucheanum* or with *Festulolium* still require more in-depth research. It seems crucial especially as regards their mutual interactions (Mead and Willey 1980, Willey and Rao 1980).

According to Oljaca et al. (2000), interspecific competition is weaker than intraspecific. Competition between species is also impacted by agrotechnical factors, such as mineral fertilisation or sowing amount (Morgado and Willey 2003). The land equivalent ratio (LER) and competitive ratio (CR) are some of the competition indexes frequently used to compare mixtures and pure stands (Dhima et al. 2007, Atis et al. 2012). The most common index adopted to measure land productivity and to determine the efficacy of intercropping in arable lands is LER (Brintha and Seran 2009). The practice of growing more than one crop simultaneously, in alternating rows of the same field is called intercropping. This has been of increasing interest in sustainable arable systems in temperate regions (Neugschwandtner and Kaul 2014). The main advantage of intercropping is more efficient utilisation of available resources such as light, nutrients and water, and at the same time, increased productivity compared with each sole crop of the mixture (Amanullah et al. 2016). Nonetheless, little has been published on how to achieve similar results on short-term grasslands. This seems important in the context of current ecological trends aimed at increasing productivity based on using small amounts of fertilisers. Moreover, there are no studies available on competitive interactions between grasses and legumes mixtures of *Lolium* and *Festulolium* species with *T. repens*, under the influence of NS fertilising.

The research hypothesis asks which proportion of mixture and level of fertilisation with NS had the most significant impact on the dry matter yields and competitive interactions in mixtures as well.

MATERIAL AND METHODS

Environmental conditions. The field study was carried out in Gnatowice (50°11'51.3600''N, 20°10'36.4440''E, 220 m a.s.l.), in southern Poland, in 2016–2017. The research objects were designed on degraded chernozem made of loess. The chemical properties of this kind of soil were as follows: pH (1 mol/L KCl) – 6.2, organic C – 23.1 g, total N – 1.6 g/kg, available: P – 60.4 mg, K – 128.3 mg, Mg – 51.7 mg/kg, sand – 171 g, silt – 586 g, clay – 210 g/kg.

The average annual temperature in 2016–2017 ranged from 9.1–9.4 °C, whereas in the vegetation period it was 15.4–15.8 °C. It increased by 0.7 °C in 2016 and by 0.4°C in 2017, compared to the multiannual average temperatures over the years 1990–2015 (Table 1). The annual precipitation in 2016–2017 was from 702.3 to 745.3 mm, in the vegetation period it was 436.3 to 467.9 mm. In the latter, there was a higher annual amount of precipitation in both years, compared to the average multiannual period, by 53.0 and 10.0 mm, respectively. All meteorological data come from the meteorological station in Prusy, the experimental station of the Institute of Plant Production, University of Agriculture in Krakow, located 8 km from Gnatowice.

Experimental setup. The experiment was arranged according to the split-plot method, in four replica-

Table 1. Long-term average monthly temperature and amount of precipitation (1990–2015) and deviations during the 2016 and 2017 growing seasons

	Temperature (°C)			Precipitation (mm)		
	mean	deviations		mean	deviations	
	1990–2015	2016	2017	1990–2015	2016	2017
April	9.1	0.1	–1.6	47.4	4.0	44.6
May	14.1	0.1	–0.4	78.7	–33.6	–25.8
June	17.3	1.4	1.5	82.3	–25.0	–52.3
July	19.3	0.1	–0.2	94.5	121.4	–44.1
August	18.6	–0.7	1.3	72.8	–25.1	–2.3
September	13.6	1.9	–0.1	65.0	–46.1	107.1
Multiannual average temperature (1990–2015)	8.7	0.7	0.4	–	–	–
Average amount of annual rainfall (1990–2015)	–	–	–	692.3	53.0	10.0

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tions, on fields with the surface of 12 m^2 (3 m × 4 m). It covered: *Lolium* × *boucheanum* (LB) (Gala type), *L. multiflorum* (LM) (Temida), *Festulolium* (F) (Agula) and *Trifolium repens* (TR) (Romena). The grasses were grown in monoculture or 2-species mixtures with TR in the following proportions of weight ratio of seeds: 50:50 and 75:25, respectively. The plants were assessed in treatment with variable dose of N fertilisation of 30 and 50 kg N/ha, applied in the form of ammonium nitrate three times: in early spring, after the 1^{st} and 2^{nd} cut. The first dose (30 kg N/ha) was applied in even portions of 10 kg N/ha, whereas the second dose (50 kg N/ha) was divided into three parts: 17, 17 and 16 kg N/ha. In the fertilisation, phosphorus (P) was also used in the amount of 38 kg P/ha, in a single dose applied in early spring in the form of superphosphate. Potassium was used in the amount of 82 kg K/ha, applied in 2 even doses in early spring and after 1^{st} cut. Also, S fertilisation was used (Prosiarka S 800), applied one-off before the beginning of vegetation in 3 doses: 5, 10 and 15 kg S/ha. The plants were cut 3 times in the vegetation season and dry matter (DM) content was determined using the gravimetric method.

Indicators of mutual interactions. The land equivalent ratio and competitive ratio were used to determine the efficacy of mixtures in short-term grasslands and the possibility of better use of available environmental resources, compared to pure sowing. The LER was determined by the formula: LER = $LER_a + LER_b$, so that:

$$LER_a = \frac{Y_{ab}}{Y_{aa}}, \qquad LER_b = \frac{Y_{ba}}{Y_{bb}}.$$

The CR for species *a* and species *b*, respectively, was calculated as follows:

$$CR_a = \frac{LER_a}{LER_b} \cdot \frac{Z_{ba}}{Z_{ab}}, \qquad CR_b = \frac{LER_b}{LER_a} \cdot \frac{Z_{ab}}{Z_{ba}}$$

(Dhima et al. 2007, Willey and Rao 1980),

where: Y_{aa} – species *a* yield in pure sowing; Y_{bb} – species *b* yield in pure sowing; Y_{ab} – species *a* yield when sown in a mixture with species *b*; Y_{ba} – species *b* yield when sown in a mixture with species *a*; proportion of sowing species *a* in cultivation with species *b*; Z_{ab} – proportion of sowing for species *a* in cultivation with the species *b*; Z_{ba} – proportion of sowing for species *b* in cultivation with the species *a*.

Statistics. The one-way ANOVA and the Tukey's test were used to verify significant differences among treatments, with significance levels: α = 0.05, α = 0.01, and α = 0.001.

RESULTS AND DISCUSSION

Dry matter yield. The yields of mixtures differed and the analysis of variance with low probability value showed the existence of significant differences between the average values for the following factors: species × year of study and year of study × fertilisation (Table 2). Furthermore, there were interactions shown between the factors: species × fertilisation × year of study. The highest yield in relation to pure sowing was observed for the mixtures: *L. multiflorum* + *T. repens* and *L.* × *boucheanum* + *T. repens* sown in the proportion 50:50, fertilised with $N_{50}S_{15}$. During the years of the study, yield increase in these objects was on average 3.29–3.40 t/ha. So at maximum NS fertilisation, higher yields of dry matter were obtained, when the above-mentioned mixture components were sown in the proportion 50:50 than for 75:25. These differences were 0.12–1.17 t/ha, in relation to pure sowing.

At the dose of $N_{30}S_{15}$ in relation to N_{30} fertilisation, the increase in dry matter yield for the years of the study was 0.83–2.72 t/ha. In the $N_{50}S_{15}$ fertilisation, the yield increased by 1.09–2.67 t/ha compared to the N_{50} treatment (Figure 1); however, in relation to the $N_{30}S_{15}$ dose, the $N_{50}S_{15}$ dose showed the highest yield increase in the years of the study, on average 3.73–4.12 t/ha for the LB + TR mixture sown in a 50:50 weight ratio of seeds. During the study period, the highest yield increase was obtained after the application of N_{50} for mixtures LB + TR and LM + TR (50:50) – 3.00–3.43 t/ha, in relation to the plots fertilised with N_{30}. The mean DM yield was higher in the 1^{st} year of the study, compared to the 2^{nd} year in all the fertilisation treatments and types of

Table 2. ANOVA *F*-test probabilities for the effects of dry matter (without control)

	Cut		
	I	II	III
Mixture	***	***	***
Year	***	***	***
Fertilisation	***	***	***
Mixture × year			
Mixture × fertilisation	**		
Year × fertilisation		*	
Mixture × year × fertilisation			

*P < 0.05; **P < 0.01; ***P < 0.001

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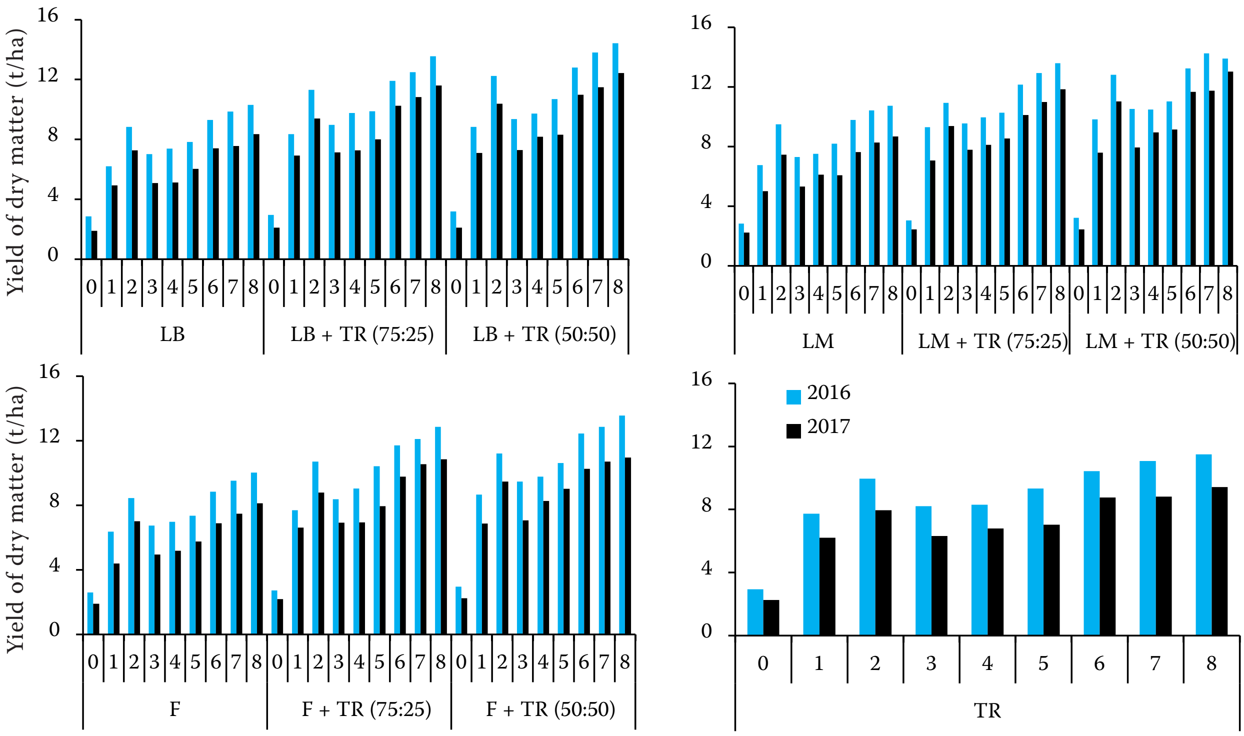

Figure 1. Yield of dry matter (t/ha). 0 – control; 1 – N_{30}; 2 – N_{50}; 3 – $N_{30}S_5$; 4 – $N_{30}S_{10}$; 5 – $N_{30}S_{15}$; 6 – $N_{50}S_5$; 7 – $N_{50}S_{10}$; 8 – $N_{50}S_{15}$. LB – *Lolium* × *boucheanum*; LM – *L. multiflorum*; F – *Festulolium*; TR – *Trifolium repens*; proportion of grasses to white clover in mixtures: 75:25, 50:50

mixtures. The difference in yield ranged from 1.71 (N_{30}) to 2.09 t /ha ($N_{50}S_{10}$) on average.

Dry matter yield of mixtures with *Lolium* sp. is mostly impacted by the course of precipitation and temperatures in spring (Kim and Sung 2019). Meteorological data for 2016–2017 showed that both average annual and the growing season temperatures were favourable for the yield. Despite lower precipitation in relation to the average multiannual period, no unfavourable effects on yield were observed. That was due to the specific type of soil, with the ability to accumulate rainfall. The DM results obtained in the experiment indicate that N fertilisation has improved the competitive ability of grasses in relation to TR. Such low competitiveness of TR compared to grass species was the most noticeable in the 1st year. It was related to the dynamic growth of grass components in mixtures, with better use of light by these species, and to N fertilisation. Such a suppressive effect of LM on growth and development of TR was also confirmed by de Hass et al. (2019); it was explained by high DM production. Similarly high DM production was observed in LB and F, which have high requirements for NS fertilisation and show high competitiveness against TR. The relative yield of grass species clearly depends both on the absolute NS availability and their relative ratio. It also means that the optimal N:S ratio in different species is varied; hence, when one component is deficient, the other may not be fully used for plant growth.

A similar regularity was reported in a study by Neugschwandtner and Kaul (2014), where a less competitive legume component was dominated by species from the Poaceae family. In the 2nd year of the study, the grass species were observed less competitive when compared to TR, which should be linked to the short-term use of these species in a moderate climate. Futhermore, Ghaley et al. (2005) and Corre-Hellou et al. (2006) confirm that cereals cultivated in intercrops with legumes can use more N than in pure sowing, which improves the competitiveness of this component. However, it was presumed that the share of components from the Fabaceae family can be significantly reduced by N fertilising, especially when both mixture components are harvested at one time. Bedoussac and Justes (2011) reported that grasses and legumes are more suitable for the organic system that equals low N fertilisation, in comparison with conventional ones. Moreover, reduced mineral fertilisation with

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simultaneous S fertilisation, results in such plants in bigger DM yields (Grygierzec et al. 2015).

Land equivalent ratio and competitive ratio. Calculated values of the LER index differed for species in individual research objects, cuts and years. However, most often they were higher than 1.0 (Figure 2). Yet, it was also observed that in a few cases, LER was slightly lower than 1.0. The lowest values for that index were calculated for F + TR mixture sown in the proportion 75:25, in research object N_{30} (cut I, 1st year of study) – 0.962. In contrast, for plants sown in the proportion 50:50, the lowest LER was observed for the control objects LB + TR, in cut III, 2nd year – 0.914. The greatest differentiation in LER was observed for plants from cut III, sown in the proportion 75:25, in both years, wherein in the 2nd year it was observed that differentiation of LER was nearly twice as high for LB and more than twice as high for F, when compared to the 1st year. For plants sown in the proportion 50:50, the greatest differences in LER were observed for LB in cut III, in 2nd year.

When analysing the impact of NS fertilisation on LER with reference to the same fertilisation with N only, higher values of LER were obtained for the analysed grass species sown 75:25 mixtures, with N_{50} and S in each dose, only in cut I in each year. On the other hand, when comparing the impact of NS on LER to different dose of N fertilisation, it was observed that all grass species sown in mixtures in the proportion 50:50, obtained a higher LER only in the 2nd year, in cuts I and II, under the influence of N_{30} + S and in cut I with N_{50} + S. In other treatments, an increase or decrease in LER was observed after the application of combined NS in comparison to fertilisation with N in the identical dose. A higher N dose combined with any dose of S, when compared to a lower dose of N and the respective dose of S, only caused an increase in LER for LB sown in mixtures in the proportion 50:50 in cuts I and II in subsequent years, and for LB sown in mixtures in the proportion 75:25 in cut I, 1st year, in cuts I and II in the 2nd year, and for F in cut I in 2nd year of the study.

When LER is equal to 1.0, it means there is no difference between species in mixture (Mazaheri and Oveysi 2004). The calculated values of LER differed for species in individual objects, cuts and study years,

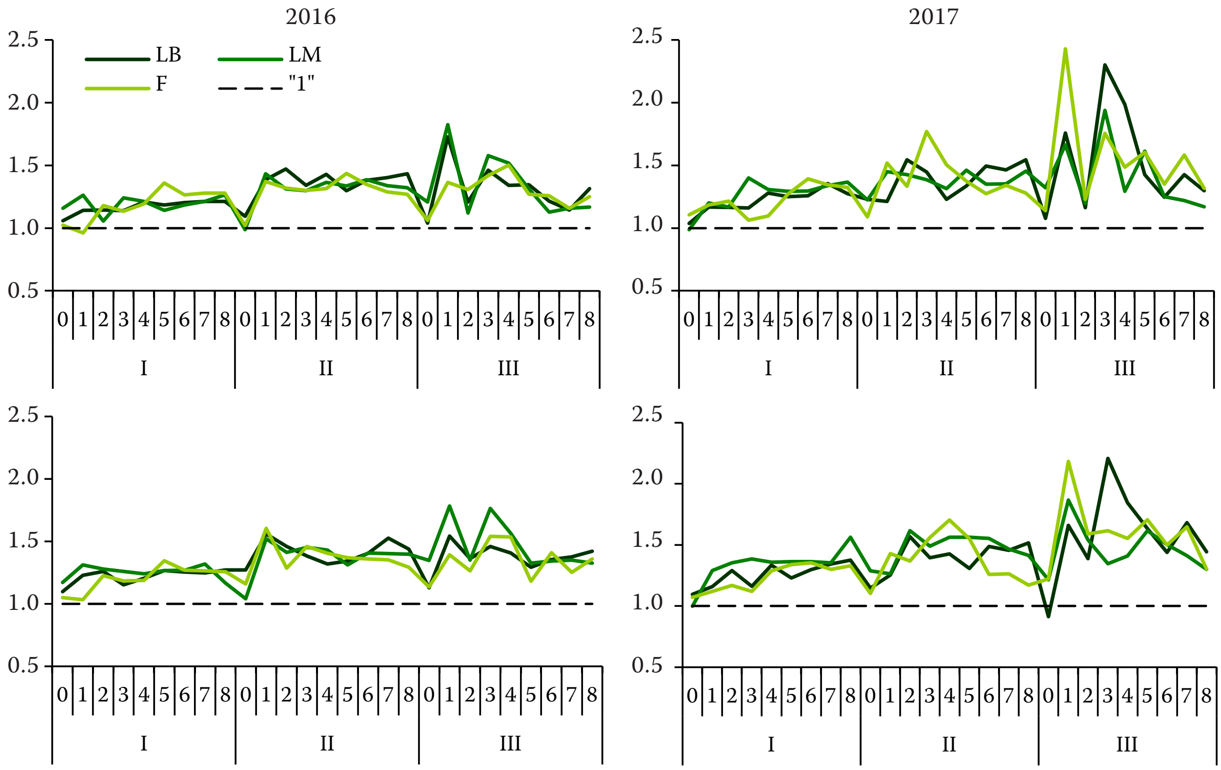

Figure 2. Shaping of land equivalent ratio (LER) for analysed grasses sown in mixtures with *Trifolium repens* (TR) in subsequent cuts and years of the study. 0 – control; 1 – N_{30}; 2 – N_{50}; 3 – $N_{30}S_5$; 4 – $N_{30}S_{10}$; 5 – $N_{30}S_{15}$; 6 – $N_{50}S_5$; 7 – $N_{50}S_{10}$; 8 – $N_{50}S_{15}$. Cuts: I, II, III; proportion of grasses to white clover in mixtures: a – 75:25; b – 50:50; LB – *Lolium* × *boucheanum*; LM – *L. multiflorum*; F – *Festulolium*

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and were most often higher than 1.0. This points to a positive interference between individual components of the mixtures (Dariush et al. 2006). The LER increased under the influence of N for such mixtures sown in short-term grasslands. Also, Morgado and Willey (2003, 2008) noted similar results for intercrops. Thus, LER points to the effectiveness of sowing species in mixtures to make the best of natural resources offered by the environment, as compared to pure sowing of individual species.

The CR represents the ratio of LER for individual species, considering the proportion of sowing (Dhima et al. 2007). The calculated values of CR index differed for the species in individual research objects, in specific cuts and years (Figure 3). In most cases, during the 1st year it was higher than 1.0, which means that grasses were more competitive than TR. Yet, in the 2nd year it was most often lower than 1.0 for LB and LM sown in mixtures with TR in the proportion 75:25. It should be noted that CR calculated for F sown in mixtures with TR in the proportion 75:25 was always higher than 1.0, and it was the highest in subsequent years of study and cuts in control objects, namely 2.409–3.508. The CR values for the species sown in the proportion 75:25 were more diverse compared to the species sown in mixtures in the proportion 50:50. The greatest CR differentiation was observed for F sown in mixtures with TR in the proportion 75:25, in subsequent cuts and years, and for LB, for both proportions, in cut III, in 2nd year of study.

When analysing the impact of NS fertilisation on the CR with reference to the same fertilisation with N only, higher values of CR were obtained for LB and LM sown in mixtures, in the proportion 75:25 in cuts I and II. It was also higher for LM in cut III using N_{50} and S in each dose, and for LB in cut III using N_{30} and S in each dose as well. However, the above conclusions may not have references to biennials and three-year-old plants. In some objects with LB and LM sown with TR (75:25), the results show that the CR index in the 2nd year was lower than 1.0. This means that grasses were sown in a 3:1 ratio and their density was greater than TR, whereas at the same time they were less competitive than TR. The exception in this respect were grasses in $N_{50}S_{10}$ and $N_{50}S_{15}$ treatments where CR > 1.0. This clearly indicates that mineral fertilisation in the above-

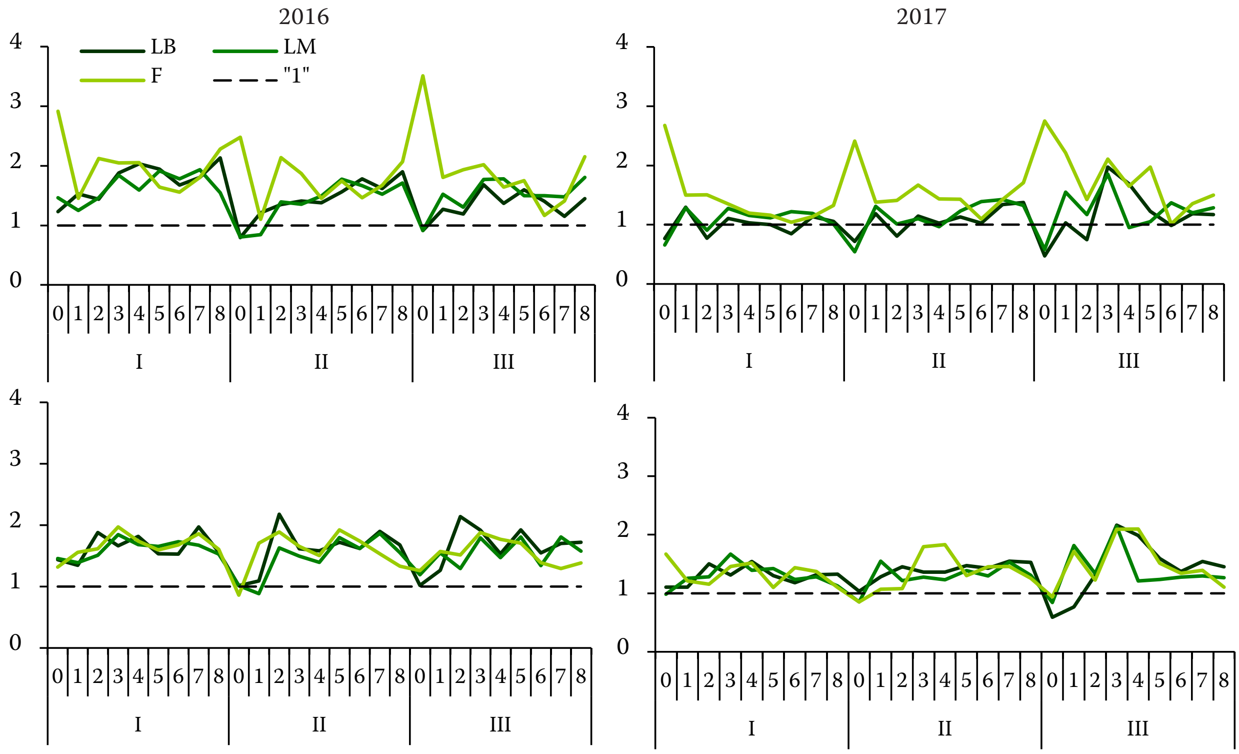

Figure 3. Shaping of competitive ratio (CR) index for analysed grasses sown in mixtures with *Trifolium repens* (TR) in subsequent cuts and years of the study. 0 – control; 1 – N_{30}; 2 – N_{50}; 3 – $N_{30}S_5$; 4 – $N_{30}S_{10}$; 5 – $N_{30}S_{15}$; 6 – $N_{50}S_5$; 7 – $N_{50}S_{10}$; 8 – $N_{50}S_{15}$. Cuts – I, II, III; proportion of grasses to white clover in mixtures: a – 75:25; b – 50:50; LB – *Lolium* × *boucheanum*; LM – *L. multiflorum*; F – *Festulolium*

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mentioned amounts had a positive impact on the competitiveness of the analysed species.

Competition for N between species stimulates its binding by bacteria coexisting with Fabaceae (Ndakidemi 2006, Davis et al. 2015). Therefore, to obtain high production, it is necessary to account for the optimum share of individual components of the mixture, which proves quite difficult in practice (Annicchiarico and Proietti 2010). In the 2nd year, it was confirmed that grasses were less competitive in relation to TR, which should be combined with their short-term use in temperate climates, while at the same time TR can effectively use other sources of N than mineral fertilisation if only S is available. According to Tallec et al. (2008), higher S availability for TR results in increased binding of N_2, while high fertilisation and high N availability can suppress N_2 binding. Moreover, Habtemichial et al. (2007) showed that the S supply in *Vicia faba* increases N_2 binding in an environment rich in N, and the effects of S deficiency may result from the constant loss of N. Also, Tallec et al. (2008) suggest that a similar mechanism may occur in grass mixtures with TR, giving it a competitive advantage, and at the same time affecting the durability of this species under conditions of fertilisation with a higher amount of N.

Fertilising with NS accounted for considerable differences in the yield of grasses mixtures with TR. The calculated LER and CR for grass species with short-term use (LB, LM, F) should be presented in the annual cycle rather than average values for the years of the study. Based on the calculated LER values, a positive interaction was shown for the mixtures of grasses and TR sown in both proportions, 75:25 and 50:50. The CR calculated for grass species pointed to the high competitiveness of these species compared to TR, especially in the 1st year of the study. During this period of time, the highest yield increase was obtained after the application of $N_{50}S_{15}$, for mixtures LB + TR and LM + TR, sown in proportions of weight ratio of seeds 50:50. The highest ratio of the LER was for Festulolium from cut III in the 2nd year of the study with fertilisation N_{30}, regardless of the amount of the sowing ratio. The greatest differentiation of the CR was observed for F sown in mixtures with TR in proportion 75:25, in subsequent cuts and years. Our studies have shown that the highest yield for a specific mixture and level of NS fertilisation were not positively correlated with the highest competitive interactions of that mixture.

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