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THE EFFECT OF VARIETIES AND FERTILIZATION ON YIELD AND SOIL MICROBIOLOGICAL DIVERSITY UNDER LUCERNE

SUMMARY

Lucerne represents one of the most important perennial forage legumes in Bosnia and Herzegovina, recognized for its high yield and nutritional value. It is characterized by a high content of crude protein, almost all essential vitamins, and is a rich source of mineral elements. The yield and quality of lucerne are largely determined by agroecological conditions, the genetic potential of the cultivar, soil fertility, as well as the phenological stage of the plant at the time of mowing. The aim of this study was to determine the effects of cultivar and fertilization on dry matter yield, crude protein content, and microbial diversity under alfalfa in the agroecological conditions of the Butmir locality, near Sarajevo. The study included two lucerne cultivars (Banat VS and Soča), two fertilization treatments (N0 - control, application of 50 kg N/ha, and incorporation of straw into the soil at 5.0 t/ha), as well as the abundance of systematic and physiological groups of microorganisms. Mowing was carried out at the flowering stage of lucerne. The results showed that the application of nitrogen fertilizer had a positive effect on almost all analyzed parameters. The highest dry matter yield, crude protein content, and total number of bacteria were recorded in treatments with applied fertilizer at a rate of 50 kg N/ha, regardless of cultivar. In contrast, the total number of ammonifiers and actinomycetes was highest in the control treatment. The Banat VS cultivar exhibited a more diverse soil microbial community, with higher numbers of bacteria, fungi, and actinomycetes compared to the other cultivar.

Keywords: Lucerne, cultivars, fertilization, dry matter, crude protein, microbial diversity

INTRODUCTION

Lucerne (*Medicago sativa* L.) represents the most important perennial legume in modern livestock and crop production, primarily due to its high yield

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and exceptional forage quality. The nutrients of lucerne are characterized by high digestibility, which decreases as the plant advances in its developmental stage at the time of mowing. Owing to its high nutritional value, particularly its high content of proteins, vitamins, and mineral elements, lucerne is rightly regarded as a leading forage crop (Lukić, 2000; Katić, 2002; Stjepanović, 2009). Under optimal agroecological conditions, lucerne exhibits strong regenerative capacity, enabling four to six cuts per year, and up to seven under irrigated conditions. Green mass yields may reach 60–80 t/ha, while dry matter yields exceed 20 t/ha (Katić *et al.*, 2002). However, in Bosnia and Herzegovina, achieved yields are significantly lower, averaging around 4 t/ha of dry matter, indicating suboptimal agronomic practices and considerable potential for improving production. Lucerne requires carefully planned agronomic management, including appropriate placement in crop rotation, high-quality soil tillage, proper fertilization, and optimal utilization of cuts. Earlier perceptions of its modest requirements are no longer sustainable in modern production systems, as high yields can only be achieved through the application of a complete technological package. In addition to its direct production importance, lucerne also has a positive effect on soil fertility. After cultivation, it leaves substantial amounts of organic matter in the soil, equivalent to 50–80 t of high-quality manure, as well as 200–300 kg/ha of biologically fixed nitrogen (Halagić, 2005), thereby contributing to improved crop performance in subsequent seasons. As a perennial crop with multiple cuts during the growing season, lucerne meets a large proportion of its nitrogen requirements through symbiotic fixation of atmospheric nitrogen by root nodule bacteria, making it an important component of environmentally sustainable and economically efficient forage production. Monitoring agronomic practices, including cultivar selection and fertilization strategies, is essential for achieving high yields and stable forage quality, as well as for preserving and enhancing soil microbial diversity (Vučković, 2019; Yaoquan *et al.*, 2025). The types and amounts of mineral fertilizers that satisfy crop requirements also positively influence the abundance and enzymatic activity of soil microorganisms (Govedarica and Jarak 1995). The aim of this study was to determine the effects of cultivar and fertilization on dry matter yield, crude protein content, and microbial diversity under alfalfa in the agroecological conditions of the Butmir locality, near Sarajevo.

MATERIAL AND METHODS

The study was conducted at the Butmir experimental field (520 m a.s.l.), where the experiment was arranged in a randomized complete block design with four replications. The size of the basic plot was 6 m² (3×2 m). The experiment included two lucerne cultivars (Banat VS and Soča) and two plant nutrition treatments: N0 (control) and fertilization with 50 kg N/ha combined with the incorporation of 5 t/ha of straw into the soil. Sowing was performed in early June 2021 using a seed drill at an inter-row spacing of 12 to 15 cm. Total nitrogen was determined using the Kjeldahl method. Ground cover was assessed based on the percentage of plot surface covered by plants. Dry matter yield was calculated based on green mass yield and its dry matter percentage. In the samples, dry matter content was determined by drying to 13–15% moisture. Total nitrogen was determined by the Kjeldahl method, and crude protein content was calculated using a conversion factor of 6.25. Crude protein yield was obtained based on dry matter yield and crude protein content. The main systematic groups of microorganisms were analyzed, including the total number of microorganisms, fungi, and actinomycetes, using appropriate culture media. Isolated fungi were identified to the genus level. The results were statistically processed using analysis of variance (ANOVA), and differences between means were tested using Tukey's test. Soil chemical properties were analyzed at the beginning and at the end of the experiment.

Table 1. Chemical properties of the soil at the Butmir experimental field

Soil layer	Depth (cm)	pH value		Humus (%)	P_2O_5/100g tla	K_2O/100 g tla
		H_2O	KCl			
Layer I	0-30	5.98	4.91	2.24	17.28	22.59
Layer II	30-60	6.08	4.97	2.20	15.26	19.62

Soil pH was determined electrometrically, humus content colorimetrically, and available phosphorus and potassium using the AL method. Chemical analysis showed that the soil of the Butmir experimental field (Table 1) had an acidic reaction, with pH values ranging from 4.91 to 4.97. The humus content amounted to 2.24% in the surface layer and 2.20% in the deeper layer, indicating a low to moderately humic soil. Available phosphorus was present at levels of 17.28 and 15.26 mg/100 g of soil, suggesting low to moderate availability. Potassium was present at levels of 22.59 and 19.62 mg/100 g of soil, corresponding to good potassium availability.

RESULTS AND DISCUSSION

Weather conditions during the study. Weather conditions during the study year were unfavorable. They were characterized by high air temperatures and a lack of precipitation during the vegetation period.

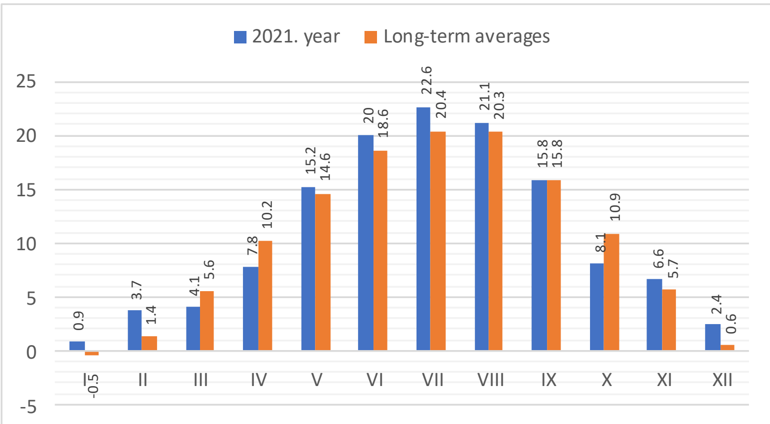

Figure 1. Mean monthly air temperatures for 2021 and long-term averages for the Butmir area

In 2021, the average annual air temperature (Figure 1) was 10.7 °C, which is 0.4 °C higher than the long-term average (10.3 °C). The lowest mean monthly temperature was recorded in January (0.9 °C), while the highest was recorded in July (22.6 °C). The total annual precipitation amounted to 501.8 mm, which is 309.4 mm lower than the long-term average, clearly indicating a pronounced moisture deficit (Figure 2). In June, only 11.7 mm of precipitation was recorded, which is 60.9 mm below the average, while in July precipitation increased to 25.8 mm but remained significantly below typical values.

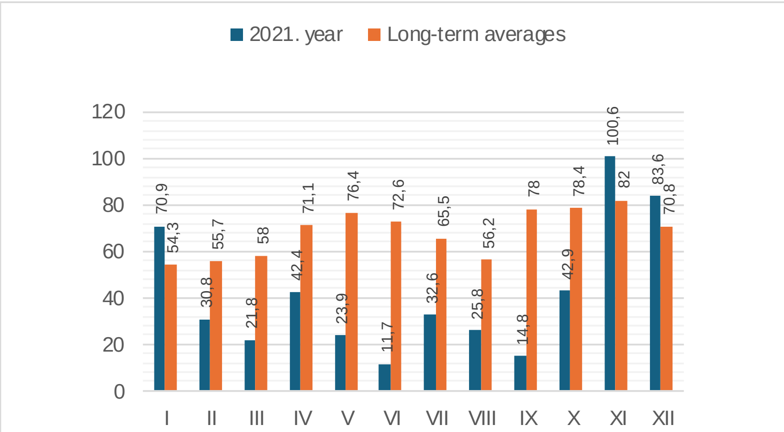

Figure 2. Average precipitation amounts (mm) for the Butmir locality

Effect of weather conditions on lucerne emergence and ground cover

The results of the study showed that unfavorable weather conditions in the year of sowing, particularly moisture deficit and elevated temperatures, had a pronounced effect on the germination, emergence, and early development of alfalfa. These findings are consistent with the reports of Hayat *et al.*, (2010), who emphasize that optimal soil moisture and moderate temperatures are key factors for successful emergence and stand establishment of alfalfa, whereas drought and high temperatures significantly reduce ground cover and biomass accumulation. During the research year, a pronounced lack of precipitation was recorded in the post-sowing period, accompanied by unfavorable temperature conditions. Such agroecological conditions negatively affected germination energy, uniformity of emergence, and final crop ground cover in both tested cultivars. For the cultivar Banat VS, the highest ground cover (Table 2) was recorded in the control treatment (36.2%), while treatments with the application of 50 kg N/ha and straw incorporation achieved 33.7% and 29.2%, respectively. Analysis of variance (ANOVA) showed that differences among treatments were not statistically significant ($p=0.673$; $p>0.05$). The obtained results indicate that neither nitrogen fertilization nor straw incorporation had a statistically significant effect on the ground cover of the Banat VS cultivar. The observed differences were numerical in nature and can be attributed to variability caused by unfavorable weather factors. For the Soča cultivar, the highest ground cover was recorded in the nitrogen fertilization treatment (53.7%), followed by the straw incorporation treatment (51.2%), while the lowest value was observed in the control treatment (45.0%). Analysis of variance showed that, for this cultivar as well, differences among treatments were not statistically significant (p=0.714; p>0.05).

Table 2. Alfalfa plant cover (%) depending on treatment and variety

Variants	Cultivars	
	Banat VS	Soča
Control	36.2^{b}	45.0^{a}
Straw (5,0 t/ha)	29.2^{b}	51.2^{a}
50 kg N/ha	33.7^{b}	53.7^{a}
Average	$33{,}0^{b}$	$49{,}9^{a}$

[ab]Values followed by different letters differ significantly (p<0.05). Letters within columns indicate comparisons among treatments within the same cultivar, while letters next to the mean indicate significant differences between cultivars

He obtained results indicate that, for both the Banat VS and Soča cultivars, the application of nitrogen fertilizer and straw incorporation did not have a statistically significant effect on ground cover. However, in the Soča cultivar, a slight increasing trend in ground cover was observed in treatments with nitrogen application and straw incorporation. When compared by cultivars, the average ground cover of the Soča cultivar was 49.9%, which was 16.9% higher than that of the Banat VS cultivar. Comparison of cultivars within individual treatments revealed statistically significant differences in all variants (control p=0.035; straw p=0.041; nitrogen fertilizer p=0.016; p<0.05), with the Soča cultivar achieving significantly higher ground cover than the Banat VS cultivar. These results indicate pronounced genotypic differences in the ability of early growth and stand establishment. The obtained data are consistent with the findings of Miletić and Radulović (2005), who emphasize that genotypic differences among alfalfa cultivars may result in faster and more uniform early plant growth under the same agroecological conditions, which is directly reflected in the degree of crop ground cover.

Dry matter yield and crude protein content

Results of the study showed that dry matter yield and crude protein yield were numerically higher in treatments with mineral fertilization (50 kg N/ha) and straw incorporation (5 t/ha); however, within cultivars, these differences were not statistically significant (Table 3). Analysis of variance for dry matter yield revealed no statistically significant differences among treatments (p=0.913; p>0.05), indicating that the applied treatments did not have a significant effect on this parameter in the research year. Additionally, no statistically significant difference was found between the Banat VS and Soča cultivars in average dry matter yield (p=0.459; p>0.05). At the cultivar level, the average dry matter yield of the Soča cultivar was 0.50 t/ha, which was approximately 0.01 t/ha higher than that of the Banat VS cultivar. Although this difference suggests a slight genotypic advantage of the Soča cultivar, it was not statistically confirmed. A similar pattern was observed for crude protein yield. No statistically significant differences were detected among treatments (p=0.890; p>0.05), and no significant differences were found between the average values of the Banat VS and Soča cultivars (p=0.765; p>0.05).

Table 3. Dry matter yield (t/ha) and crude protein yield (kg/ha)

Variants	Dry matter yield		Crude protein yield	
	Banat VS	Soča	Banat VS	Soča
Control	0.47^{ns}	0.49^{ns}	95.5^{ns}	96.1^{ns}
Straw 5,0 t/ha	0.50^{ns}	0.51^{ns}	97.2^{ns}	97.3^{ns}
50 kg N/ha	0.51^{ns}	0.52^{ns}	97.8^{ns}	98.8^{ns}
Average	0.49^{ns}	0.50^{ns}	96.8^{ns}	97.4^{ns}

Values marked with "ns" indicate no significant differences (p > 0.05). Letters within columns compare treatments within the same cultivar, while letters next to the mean compare treatments between cultivars

The average crude protein yield of the Soča cultivar was slightly higher than that of the Banat VS cultivar; however, the difference was minimal and not statistically significant. The obtained results are partially consistent with the findings of Aon and Colaneri (2001), who reported that nitrogen addition or the incorporation of organic matter may increase alfalfa yield and quality, but that under stress conditions, such as moisture deficit and high temperatures, the effects of fertilization may be limited. In the research year, unfavorable climatic conditions represented the dominant limiting factor, reducing the potential effects of the applied treatments. Furthermore, although the Soča cultivar showed a tendency toward slightly higher dry matter and crude protein yields, statistical analysis did not confirm a significant genotypic advantage under the given conditions. This indicates that climatic stress had a stronger influence on yield and quality than genetic differences between cultivars, which is consistent with the findings of Stamenov (2013) regarding the variable efficiency of cultivars in protein accumulation depending on environmental conditions.

Soil microbial community under Lucerne

The results of the study indicate that different treatments (control, fertilization with 50 kg N/ha, and straw incorporation at 5 t/ha) and lucerne cultivars (Banat VS and Soča) significantly influenced the soil microbial community, including bacteria, ammonifiers, fungi, and actinomycetes. Bacteria were the most abundant group of microorganisms, which is consistent with general knowledge that their abundance reflects overall soil biological activity and ecosystem quality (Miletić and Radulović, 2005; Hayat *et al.*, 2010). In our study (Table 4), the highest number of bacteria was recorded in the treatment with mineral nitrogen, while the lowest was observed in the straw incorporation treatment. These results confirm the findings of Li *et al.*, (2020), who demonstrated that the addition of mineral nitrogen can stimulate bacterial populations in the lucerne rhizosphere, potentially enhancing biological processes that support plant growth. The number of ammonifiers was highest in the control treatment, whereas fertilization with 50 kg N/ha resulted in a reduction in their abundance. This trend can be explained by the fact that the availability of mineral nitrogen reduces the soil's need for the decomposition of organic nitrogen, which is consistent with the basic principles of the soil nitrogen cycle (Stamenov, 2013; Aon and Colaneri, 2001). Fungi, on the other hand, showed the highest abundance in the straw incorporation treatment and the lowest in the mineral nitrogen treatment. This confirms their role in the decomposition of complex organic matter and soil humification, in accordance with previous studies by Yudiarti *et al.,* (2010).

Table 4. Total number of bacteria and ammonifiers, fungi and actinomycetes

Cultivar	Variants	Total number of bacteria CFU x 10^4/g	Total number of ammonifiers CFU x 10^3/g	Total number of fungi CFU x 10^2/g	Total number of actinomycetes CFU x 10^2/g
Banat VS	Straw 5,0 t/ha	33.7 x 10^4	25.1 x 10^3	12.5 x 10^2	10.2 x 10^2
	50 kg N/ha	59.5 x 10^4	22.5 x 10^3	7.1 x 10^2	18.4 x 10^2
	Control	45.3 x 104	34.5 x 10^3	9.3 x 10^2	24.8 x 10^2
Soča	Straw 5,0 t/ha	39.4 x 10^4	21.2 x 10^3	11.2 x 10^2	8.5 x 10^2
	50 kg N/ha	47.5 x 10^4	13.5 x 10^3	6.7 x 10^2	15.5 x 10^2
	Control	40.8 x 10^4	25.5 x 10^3	8.2 x 10^2	22.3 x 10^2

CFU/g stands for colony-forming units per gram of sample.

Organic amendments such as straw stimulate the development of fungal populations, thereby contributing to long-term soil fertility. Actinomycetes exhibited the highest abundance in the control treatment, slightly lower abundance in the mineral nitrogen treatment, and the lowest abundance in the straw incorporation treatment. This indicates their sensitivity to changes in the availability of organic and mineral substrates and highlights their role in the decomposition of complex organic compounds and soil humification (Miletić and Radulović 2005). Overall, the results confirm that agronomic practices, such as cultivar selection and fertilization strategy, significantly influence the soil microbial community. The Banat VS and Soča cultivars exhibited similar trends, although Banat VS generally showed slightly higher numbers of bacteria and actinomycetes, while Soča had higher fungal abundance in certain treatments. These findings are consistent with the literature emphasizing the interaction between cultivar and soil microbial activity (Jarak *et al.*, 2005). The application of mineral nitrogen stimulates bacterial populations, whereas organic amendments (straw) increase fungal abundance, indicating the potential for targeted manipulation of the soil microbial community to improve soil fertility. These results contribute to the understanding of the long-term effects of different treatments on soil and support the need for an integrated approach to soil management in lucerne production.

CONCLUSIONS

The results of the study on the effects of cultivar and fertilization on yield and soil microbial diversity under lucerne showed the following. Late sowing and unfavorable weather conditions, particularly moisture deficit and high temperatures, negatively affected plant growth, ground cover, and the yields of dry matter and crude protein in both the Banat VS and Soča cultivars, and also contributed to a reduction in microbial activity. Cultivars differed significantly in ground cover, with Soča achieving statistically significantly higher values compared to the cultivar Banat VS. Mineral fertilization increased dry matter yield, while straw incorporation partially enhanced soil microbial activity, particularly fungal abundance. Overall microbial activity was higher in the Banat VS cultivar, indicating different interactions between cultivars and the soil microbial community. Since lucerne sowing was carried out later than the optimal period, this factor had a decisive negative impact on all examined parameters. Delayed sowing, in combination with unfavorable weather conditions, limited plant development and reduced the effectiveness of applied agronomic measures. This highlights the importance of timely sowing as a fundamental prerequisite for achieving stable yields and maintaining soil microbial activity. Further research should focus on optimizing sowing dates and combinations of organic and mineral fertilizers in order to improve productivity and biological soil activity.

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