Original scientific paper
Journal of Central European Agriculture, 2024, 25(2), p.542-553

DOI: /10.5513/JCEA01/25.2.4208

# NPK fertilisation of natural grassland for the development of a sustainable management system

---

# Đubrenje prirodnog travnjaka sa NPK u cilju razvoja održivog sistema upravljanja

Dalibor TOMIĆ[1] (✉), Vladeta STEVOVIĆ[1], Dragan ĐUROVIĆ[1], Milomirka MADIĆ[1], Miloš MARJANOVIĆ[1], Nenad PAVLOVIĆ[1], Duško BRKOVIĆ[1], Mirjana PETROVIĆ[2], Vladimir ZORNIĆ[2], Đorđe LAZAREVIĆ[2]

1 University of Kragujevac, Faculty of Agronomy, Cara Dušana, 34, +38132000 Čačak, Serbia
2 Institute of Forage Crops, +38137251 Globoder, Kruševac, Serbia

✉ Corresponding author: dalibort@kg.ac.rs

Received: December 13, 2023; accepted: January 28, 2024

## ABSTRACT

In this study, the effect of NPK fertilization with different nitrogen rates on the dry matter yield and botanical composition of a *Festucetum pratense* natural grassland was analysed in a 10-year field trial. The experiment was conducted in the period 2011–2020 in central Serbia, 1000 m above sea level. The fertiliser treatments included: control without fertilisers, N60:P40:K40, N100:P40:K40, and N140:P40:K40 kg/ha per year. The mineral fertilisation treatments had a significant effect on the dry matter yield and botanical composition of the grassland, especially in the first cut, resulting in a lower total number of plant species in the grassland. The effect of fertilization on dry matter yield and botanical composition was largely dependent on weather conditions. In the last years of the trial, the dry matter yield gradually decreased under the fertiliser treatments, which is a consequence of the decrease in diversity. Satisfactory results on this grassland can be achieved with low nitrogen fertiliser applications combined with the maintenance of plant diversity as a basis for sustainable grassland management in temperate latitudes.

**Keywords:** botanical composition, dry matter yield, fertilisation, natural grassland

## ABSTRAKT

Cilj rada je analiza efekta đubrenja NPK đubrivima sa različitim nivoima azota na prinos suve materije i botanički sastav prirodnog travnjaka *Festucetum pratense* u 10-godišnjem poljskom ogledu. Eksperiment je sproveden u periodu 2011–2020. godine u centralnoj Srbiji, 1000 m nadmorske visine. Tretmani đubrenja su obuhvatali: kontrolu – bez đubriva, N60:P40:K40, N100:P40:K40 i N140:P40:K40 kg/ha godišnje. Tretmani mineralnim đubrivima su značajno uticali na prinos suve materije i botanički sastav travnjaka, posebno u prvom otkosu, što je rezultiralo smanjenjem ukupnog broja biljnih vrsta na travnjaku. Uticaj đubrenja na prinos suve materije i botanički sastav je u velikoj meri zavisio od vremenskih uslova. U poslednjih nekoliko godina eksperimenta, prinos suve materije je postepeno opadao u tretmanima đubrenja, što je posledica smanjenja raznovrsnosti. Zadovoljavajući rezultati na ovom travnjaku se mogu postići pri niskim stopama azota u kombinaciji sa održavanjem raznovrsnosti biljaka kao osnove za održivo upravljanje travnjacima umerenog pojasa.

**Ključne riječi:** botanički sastav, prinos suve materije, prirodni travnjaci, đubrenje

## INTRODUCTION

Grasslands are important worldwide for the production of sufficient amounts of high-quality food and are economically important as the basis for milk and meat production in many regions of the world (Dasselaar et al., 2021). Natural grasslands account for 23% of the world's land area and 40% of the total utilised agricultural area in Europe (Huyghe et al., 2014). In the European Union, there is a permanent grassland area of around 60 million ha (Eurostat, 2023). Grasslands cover one third to one half of the total utilised agricultural area in south-eastern and central European countries (Cirebea et al., 2020). Although intensive grassland management was practised in industrialised countries in the twentieth century, an extensive approach is still practised in some places, such as the Balkans. These extensively managed secondary grasslands are one of the most species-rich habitats in Europe (Török et al., 2020).

To recommend appropriate cultural practises that increase biomass yields in such grasslands, the focus should be on investigating the effects of climate drivers and functional floral diversity on the yield and biomass composition of grasslands subjected to different treatments (Simić et al., 2020). The diversity and productivity of grasslands depend on the availability of water and nutrients (Grašič et al., 2023). Nitrogen is one of the most important nutrients and the most common limiting factor for achieving high yields from natural grasslands (You et al., 2017). In common practise, farmers apply NPK fertilisers, which, depending on the amount, can cause a significant decline in plant biodiversity (Rotar et al., 2016a; Cirebea et al., 2020).

Despite the great interest in understanding the impact of fertilization on the yield and composition of natural grasslands, it is not known how a particular plant community will respond. A three-year research period is generally not sufficient to make long-term predictions about plant community development, and there are potential differences in grassland management recommendations between short- and long-term experiments (Lepš, 2014). Medium-term experiments, which last about ten years, create a balance and provide enough data to create a management plan.

Meadow fescue (*Festuca pratensis* Huds.) is an edifying species in the model community where the trial was conducted. This medium-term trial aims to study yield variation over time with a small investment of time and capital, which is the basis of the rural way of life. In this respect, the present study aimed to analyse and quantify the effects of NPK fertilisation with different nitrogen levels on the yield and botanical composition of a natural grassland over a 10-year period in order to develop sustainable management systems.

## MATERIALS AND METHODS

### *Location and soil properties*

The experiment was conducted from 2011 to 2020 (10 years) on the northern slopes of Mount Kopaonik (central Serbia) in the village of Rakovac (43°23'35.89'N; 20°48'29.24'E, 1000 m above sea level), near Jošanička Banja, on natural grassland classified as *Festucetum pratense*. The experiment was conducted on a ranker with a pH of 4.8, 3.64% organic matter, 1.0% $CaCO_3$, 0.317 % N, 2.33 mg P/100 g soil, and 10.1 mg K/100 g soil. The grassland was located on the north-western slope.

### *Weather conditions*

During the period analysed, the mean annual air temperature for the specified area was 7.9 °C, with monthly averages ranging from -3.2 °C to 15.9 °C for January and July respectively. The average annual precipitation was 795 mm. The largest amounts of precipitation fell during the year in the period May–June (Table 1). The annual precipitation totals were highest in 2014 and 2016 (over 1000 mm), and lowest in 2011, 2012, and 2013 (Figure 1).

### *Experimental design and analyses*

The experiment was designed as a completely randomised block system with three replicates. The plot size was 5 x 1.5 metres and the distance between plots was 1 metre. The fertilisation treatments included:

**Table 1.** Monthly precipitation distribution for the trial period compared to the 10-year average (2011–2020)

| Month | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2011 | 24.4 | 26.2 | 56.4 | 19.7 | 90.7 | 63.2 | 61.1 | 2.1 | 47.8 | 46.6 | 2.0 | 58.9 |
| 2012 | 110.9 | 44.3 | 29.7 | 62.5 | 94.0 | 32.3 | 53.4 | 1.1 | 20.5 | 68.8 | 5.8 | 78.6 |
| 2013 | 67.0 | 63.7 | 72.9 | 32.8 | 116.8 | 81.8 | 69.3 | 14.1 | 46.7 | 52.4 | 47.0 | 21.5 |
| 2014 | 17.5 | 8.2 | 78.0 | 165.9 | 159.1 | 100.6 | 89.3 | 87.8 | 150.0 | 71.1 | 53.1 | 70.4 |
| 2015 | 78.9 | 70.4 | 98.8 | 52.5 | 93.4 | 141.4 | 11.3 | 58.6 | 58.9 | 70.5 | 51.8 | 9.7 |
| 2016 | 74.8 | 55.5 | 147.6 | 28.1 | 133.8 | 123.9 | 70.6 | 80.7 | 72.5 | 87.6 | 119.4 | 25.8 |
| 2017 | 30.3 | 20.1 | 66.8 | 82.3 | 81.9 | 71.8 | 61.8 | 46.7 | 64.0 | 116.6 | 31.6 | 67.8 |
| 2018 | 65.6 | 50.9 | 95.7 | 35.9 | 87.4 | 105.3 | 268.3 | 23.7 | 10.8 | 17.6 | 31.0 | 54.4 |
| 2019 | 85.6 | 59.7 | 29.2 | 68.4 | 99.5 | 108.5 | 64.2 | 78.9 | 22.3 | 11.6 | 71.1 | 102.0 |
| 2020 | 33.9 | 52.6 | 52.1 | 25.1 | 121.1 | 200.5 | 56.2 | 154.6 | 32.3 | 100.6 | 5.8 | 71.4 |

A0 – no fertiliser control, A1 – N60:P40:K40, A2 – N100:P40:K40, and A3 – N140:P40:K40 kg/ha per year. During fertilisation, the entire amount of phosphorus and potassium as well as most of the nitrogen was applied immediately before the beginning of the growing season (in March), while the remaining 20 kg/ha of nitrogen in each fertilised treatment was used for additional fertilisation after the first cut. The fertilisers were applied every year.

The grassland was mowed twice a year in 2011 and 2013–2020. There was no second cut in 2012 due to the drought. The mowing took place during the inflorescence formation phase of *Festuca pratensis* Hud. The forage yield (t/ha) was determined by measuring the total biomass of the plot immediately after mowing. After the measurement, samples (1000 g) were taken to analyse the moisture content of the forage and the botanical composition. The plants were categorised into three functional groups: Grasses (*Fam. Poaceae*), legumes (*Fam. Fabaceae*) and other herbs (plant species belonging to other plant families in the sample). The material was dried at 65 °C to determine the dry matter yield (t/ha). The proportion of the individual plant groups in the dry sample was then calculated.

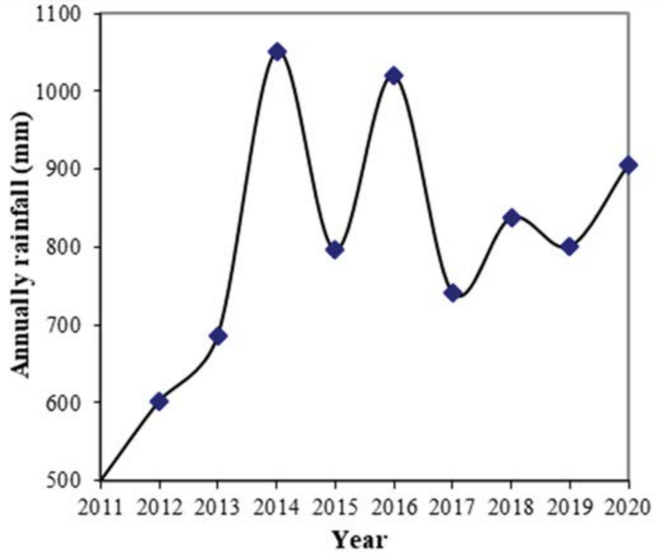


**Figure 1.** Precipitation distribution over the trial years (2011–2020)

The results obtained were subjected to a mixed analysis of variance model (year as a random effect, fertilization as a fixed effect) using STATISTICA 8 (StatSoft Inc., 2007). The differences between the mean values were tested using the LSD test. The total dry matter yield for both cuts was analysed using the AMMI ANOVA model.

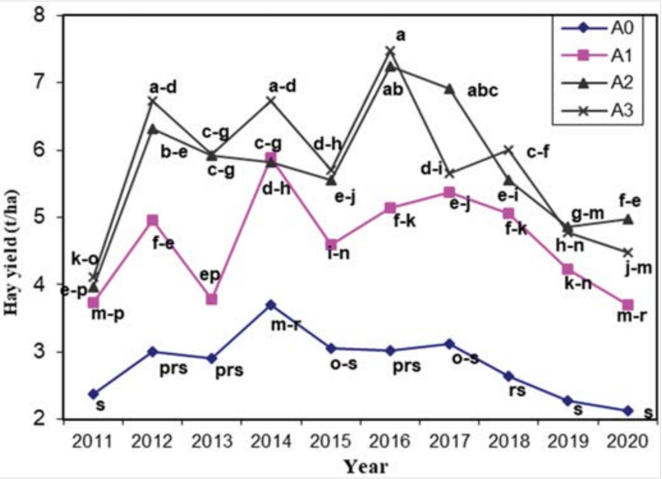
## RESULTS

The results of the soil analysis show that the soil properties have changed over time. The initial state is reflected in the control values (Table 2). It was found that the management measures had a greater influence on the amount of potassium compared to the initial level. The most accessible potassium was measured in the N100 and N140 treatments, which means that up to 20 times more K is extracted from the soil over the course of 10 years. The amount of accessible P was almost doubled in treatment N140, while the nitrogen content did not increase to the same extent as P and K after 10 years.

In almost all years between 2011 and 2020, all treatments with mineral fertiliser led to a significant increase in dry matter yield in the first cut compared to the control treatment – A0 (Table 3). Only in treatment A1 in 2013 was the increase not significant (significance of the fertiliser/year interaction; Figure 2). However, there were significant differences in dry matter yield between years and fertiliser treatments. In 2011, 2014, 2018 and 2019, the dry matter yields at first cut did not differ significantly between the fertiliser treatments (A1, A2 and A3). In 2012, 2015, 2016, 2017 and 2020, however, the dry matter yield tended to increase with increasing fertilisation from treatment A1 to A3.

Regardless of fertilisation, the dry matter yield of the first cut showed differences between years, being highest in 2014 and 2016 and lowest in 2019 and 2020.

The dry matter yield of the second cut was significantly lower than that of the first cut, regardless of fertilisation. There was no second cut in 2012 due to the drought (Table 3). The effects of the different mineral fertiliser rates on the dry matter yield of the second cut were not pronounced in contrast to the effects on the dry matter yield of the first cut (Figure 3). In 2011, 2015, 2017, 2019 and 2020, the mineral fertiliser treatments had no significant effect on dry matter yield. In contrast, the dry matter yield was higher for all fertiliser treatments in 2013 and 2014 and for certain treatments in 2016 and 2018 than for the control (interaction fertilisation/year).



Values labelled with different lower case letters differ significantly ($P \leq 0.05$) according to the LSD test

**Figure 2.** Dry matter yield in the first cut over the years as a function of mineral fertilisation

**Table 2.** Results of the chemical soil analysis after 10 years of the experiment

| | pH($H_2O$) | pH (KCl) | N (%) | $P_2O_5$ (mg/100 g) | $K_2O$ (mg/100 g) | Organic matter (%) | $CaCO_3$ (%) |
|---|---|---|---|---|---|---|---|
| A0 | 4.81 | 3.63 | 0.317 | 2.33 | 10.1 | 3.64 | 1 |
| A1 | 5.1 | 3.79 | 0.378 | 2.87 | 72.7 | 3.55 | 0.8 |
| A2 | 5.08 | 3.81 | 0.378 | 3.15 | 199.0 | 3.68 | 1 |
| A3 | 4.85 | 3.91 | 0.445 | 4.01 | 144.8 | 3.65 | 1.2 |

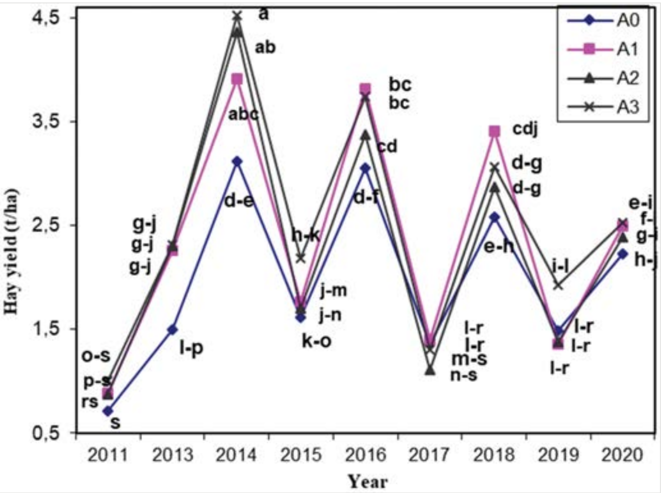
**Table 3.** Average values of dry matter yield depending on treatment

| | | Dry matter yield (t/ha) ± SD | |
|---|---|---|---|
| | | I cut | II cut |
| Fertilisation (F) | A 0 | 2.823 ± 0.51$^{c}$ | 1.764 ± 0.62$^{c}$ |
| | A 1 | 4.644 ± 0.79$^{b}$ | 2.129 ± 0.49$^{ab}$ |
| | A 2 | 5.708 ± 0.90$^{a}$ | 2.037 ± 0.38$^{b}$ |
| | A 3 | 5.759 ± 1.12$^{a}$ | 2.258 ± 0.41$^{a}$ |
| Year (Y) | 2011 | 3.546 ± 0.76$^{d}$ | 0.870 ± 0.13$^{i}$ |
| | 2012 | 5.252 ± 1.60$^{ab}$ | - |
| | 2013 | 4.636 ± 1.33$^{c}$ | 2.096 ± 0.37$^{e}$ |
| | 2014 | 5.532 ± 1.27$^{a}$ | 3.977 ± 0.65$^{a}$ |
| | 2015 | 4.727 ± 1.15$^{c}$ | 1.816 ± 0.30$^{f}$ |
| | 2016 | 5.717 ± 1.86$^{a}$ | 3.495 ± 0.41$^{b}$ |
| | 2017 | 5.264 ± 1.28$^{ab}$ | 1.291 ± 0.18$^{h}$ |
| | 2018 | 4.811 ± 1.42$^{bc}$ | 2.983 ± 0.49$^{c}$ |
| | 2019 | 4.032 ± 1.13$^{d}$ | 1.535 ± 0.27$^{g}$ |
| | 2020 | 3.818 ± 1.18$^{d}$ | 2.409 ± 0.21$^{d}$ |
| ANOVA | F | ** | ** |
| | Y | ** | ** |
| | F x Y | * | * |

Values followed by different small letters within columns are significantly different ($P \leq 0.05$) according to the LSD test; *F test significant at $P \leq 0.05$; **F test significant at $P \leq 0.01$

Regardless of fertilisation, the dry matter yield in the second cut showed significant differences between the years, with the highest value being achieved in 2014 and 2016 and the lowest in 2011.

Regardless of the year, the proportion of grasses in the first cut was significantly higher in all fertilizer treatments than in the control (Table 4). In all years, the proportion of grasses was significantly higher under treatments A2 and A3 than under A1. Regardless of the year, the proportion of legumes in the first cut was significantly lower in all treatments than in the control, while there were no significant differences between the fertilizer treatments. The effect of fertilisation on the proportion of other herbs in the first cut was inverse to the effect on the proportion of grasses. In all years, the proportion of other herbs was significantly lower in all treatments than under the control, and significantly lower in A2 and A3 than in A1.



Values labelled with different lower case letters differ significantly ($P \leq 0.05$) according to the LSD test

**Figure 3.** Dry matter yield in the second cut over several years depending on mineral fertilisation

Irrespective of fertilisation, the proportions of grasses, legumes and other herbs in the first cut differed significantly between years. The proportion of grasses was highest in 2016 and lowest in 2013, 2019 and 2020. The proportion of legumes was highest in the first year and then decreased until 2017, when it increased slightly. The proportion of other herbs was highest in 2013 and lowest in 2016.

In all years, the proportion of grasses was significantly higher and the proportion of legumes and other herbs lower in the second cut in all fertiliser treatments than in the control. Under A2 and A3, a significantly higher proportion of grasses and a significantly lower proportion of other herbs was found than under A1. Treatment A1 resulted in a significantly higher proportion of legumes compared to A2.

**Table 4.** The botanical composition (%) of natural grassland depending on treatment

| | | I cut | | | II cut | | |
|---|---|---|---|---|---|---|---|
| | | Grasses | Legumes | O. herbs | Grasses | Legumes | O. herbs |
| Fertilisation | A 0 | $58.0^{c}$ | $5.11^{a}$ | $36.9^{a}$ | $41.6^{c}$ | $2.93^{a}$ | $45.5^{a}$ |
| (F) | A 1 | $75.3^{b}$ | $2.57^{b}$ | $22.2^{b}$ | $69.4^{b}$ | $1.32^{b}$ | $19.3^{b}$ |
| | A 2 | $84.7^{a}$ | $2.54^{b}$ | $12.7^{c}$ | $79.9^{a}$ | $0.63^{c}$ | $9.5^{c}$ |
| | A 3 | $89.5^{a}$ | $1.06^{b}$ | $9.5^{c}$ | $78.2^{a}$ | $0.84^{bc}$ | $11.0^{c}$ |
| Year | 2011 | $74.5^{bc}$ | $9.54^{a}$ | $16.0^{ef}$ | $83.7^{a}$ | $2.48^{b}$ | $13.8^{c}$ |
| (Y) | 2012 | $79.2^{b}$ | $2.96^{bc}$ | $17.8^{ef}$ | - | - | - |
| | 2013 | $69.0^{c}$ | $0.86^{c}$ | $30.1^{a}$ | $70.6^{bc}$ | $0.61^{d}$ | $28.8^{ab}$ |
| | 2014 | $80.6^{ab}$ | $0.12^{c}$ | $19.3^{c\text{-}f}$ | $80.1^{ab}$ | $0.60^{d}$ | $19.3^{bc}$ |
| | 2015 | $81.2^{ab}$ | $0.60^{c}$ | $18.2^{def}$ | $86.7^{a}$ | $0.50^{d}$ | $12.8^{c}$ |
| | 2016 | $87.8^{a}$ | $0.99^{c}$ | $11.2^{g}$ | $78.6^{ab}$ | $1.77^{bc}$ | $19.6^{bc}$ |
| | 2017 | $80.6^{ab}$ | $5.00^{b}$ | $14.4^{f}$ | $77.1^{abc}$ | $0.62^{d}$ | $22.2^{bc}$ |
| | 2018 | $76.1^{bc}$ | $1.97^{bc}$ | $22.0^{b\text{-}e}$ | $69.4^{bc}$ | $2.04^{bc}$ | $28.5^{bc}$ |
| | 2019 | $71.0^{c}$ | $2.88^{bc}$ | $26.2^{abc}$ | $58.8^{d}$ | $1.15^{c}$ | $39.6^{a}$ |
| | 2020 | $68.6^{c}$ | $3.26^{bc}$ | $28.1^{ab}$ | $67.5^{cd}$ | $4.11^{a}$ | $28.4^{b}$ |
| ANOVA | F | ** | ** | ** | ** | ** | ** |
| | Y | ** | ** | ** | ** | ** | ** |
| | F x Y | ns | ns | ns | ns | ns | ns |

Values labelled with different lower case letters differ significantly ($P \leq 0.05$) according to the LSD test; *F test significant at $P \leq 0.05$; **F test significant at $P \leq 0.01$; ns – non-significant

A total of 50 plant species from 44 genera and 20 families were identified on the control grassland. The Poaceae family was most frequently represented with 7 species, with *Festuca pratensis* being the most dominant and forming a typical community *Festucetum pratense*. In addition, six species of legumes (Fabaceae) and 37 other herbs were identified. In treatment A1 there were 31 species. In treatments A2 and A3, there were 20 plant species, i.e. 4 species from the Poaceae family, 3 species of Fabaceae and 13 other herbs.

For the trait total dry matter yield, a significant interaction for IPCA1 was found for both cuts (Figure 4).

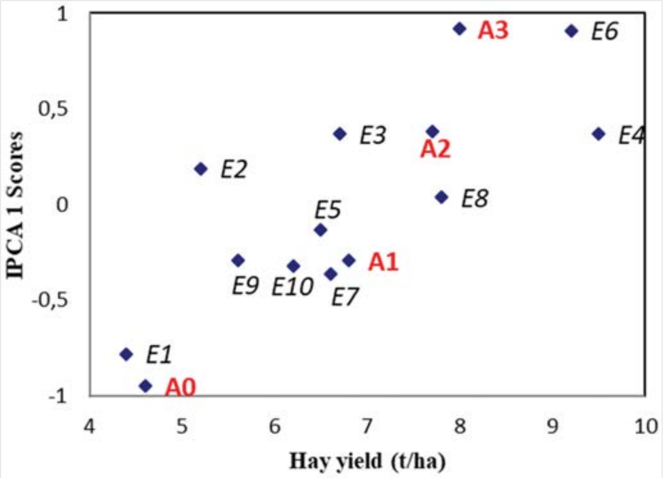


**Figure 4.** AMMI biplot showing the main effects of mineral nitrogen fertilisation of natural grassland ($A_0$ – control, $A_1$ – N60:P40:K40, $A_2$ – N100:O40:K40, $A_3$ – N140:P40:K40 kg/ha per year) in the period 2011–2020 ($E_1$–$E_{10}$) and the values of the first interaction principal component IPCA1

Since the first principal component explains more than 94% of the interaction, the values for IPCA2 were not shown. The results show that the interaction fertilisation/year was more pronounced in treatments A0 and A3. The treatment with the highest amount of nitrogen had the highest effect on dry matter yield in 2014 and 2016 and treatment A2 in 2013 and 2018. The effect of treatment A1 on dry matter yield was highest in 2015, 2017 and 2020. Overall, the lowest effect of fertilisation on dry matter yield was recorded in 2011 and 2012.

## DISCUSSION

### *Effect of fertilisation rate on dry matter yield*

Mineral fertiliser treatments had a major impact on both the dry matter yield and the botanical composition of the grassland, especially in the first cut. On average over the years, the dry matter yield increased by 64.5% in treatment A1 and by more than 100% in treatments A2 and A3 compared to the control. In the second cut, the effect of fertilisation was less pronounced, which is mainly due to the low rainfall and high air temperatures in the second part of the vegetation period. In the first cut in 2011, 2014, 2018 and 2019, all fertiliser treatments produced significantly higher dry matter yields compared to the control, but there was no significant difference between the treatments. In the other trial years, the dry matter yield increased with increasing fertiliser application. This was also the result of the influence of weather factors, especially rainfall. When the plants received more moisture, they were more efficient in absorbing mineral nutrients; under these conditions, an increased amount of fertiliser had an increased effect and the opposite. A similar effect was found on the *Agrostietum capillaries* grassland (Simić et al., 2020). This was also confirmed by the results of these condcut in this experiment, which showed a significantly lower effect of different nitrogen fertiliser rates. Compared to the control, significantly higher dry matter yields were achieved in the second cut under the fertiliser treatments in 2013, 2014, 2016 and 2018, while fertilisation had no significant effect on dry matter yield in other years. The experimental region typically experienced a period of dry weather and high air temperatures during second growth in July and August, which led to little or no effect of fertilisation following the same pattern. The results showed that the overall effect of mineral fertilisation and different nitrogen application rates on dry matter yield was largely dependent on weather conditions, particularly rainfall. Lin et al. (2018) found that weather and soil conditions on grasslands strongly influence the carbon content of the soil. Under favourable conditions, fertilisation on most grasslands areas lead to decrease in soil carbon content, and the micronutrients required for plant nutrition are supplied from the humus. The long-term effects on soil C content may be partly due to the positive effects of N fertilisation on the amount of microbially processed C content in the soil (Fornara et al., 2020).

In some other climates and soils, soil carbon may increase due to unfavourable conditions for microbial activity (Sulman et al., 2014) or even due to increased microbial biomass and metabolites that can subsequently accumulate carbon in the soil through organomineral compounds (Cotrufo et al., 2015). The yield and composition of a natural grassland can largely depend on these processes. In the present experiment, there was no change in the humus content of the soil over time.

The AMMI analysis showed that treatment A3 had the greatest effect on dry matter yield in the years with the highest rainfall (2014 and 2016) (Figure 4). In these years, the soil moisture content, which is necessary for the dissolution and uptake of fertilisers, was not a limiting factor for dry matter yield throughout the growing season. In 2013 and 2018, soil moisture conditions were also favourable with the exception of a short period in summer. However, the lower yields in these years were also due to the pronounced autumn drought in previous years, which left the plants less well prepared for winter and with lower reserves of organic matter for the first growth in spring. In these years, the highest yield was achieved with treatment A2, as a further increase in the fertiliser application rate was unable to increase the dry matter yield. The years 2015, 2017 and 2020 also generally had extremely dry summer periods, but they followed the rainy years 2014, 2016 and 2019, so that the plants were well prepared for the following season, in which the treatment with the lowest amount of nitrogen (A1) had a positive effect. The years 2011 and 2012 were characterised by the lowest rainfall and long periods of pronounced drought during the summer. These conditions and the fact that these were the first years of the experiment were the main reason for the lowest fertiliser effect in all treatments throughout the experiment. This result was confirmed by Cirebea et al. (2020), who reported a lower fertilising effect of the grassland in the first year than in the second and third years of the experiment. Under humid conditions with an annual rainfall of 1769 mm, the nitrogen content in the soil after fertilisation was directly correlated with the dry matter yield (Viégas et al., 2017).

### *Effect of the climate factors*

The results showed that the dry matter yield was positively correlated with the amount of precipitation during plant growth. An increase in dry matter yield of the natural grassland, in dependent of fertilisation in both cuttings, was obtained in years with increased rainfall during the growing season (2014 and 2016). Under these conditions, the plants were supplied with sufficient amounts of water so that the efficiency of nutrient uptake was improved. Similar results were reported for an *Agrostietum capillaries* grassland (Simić et al., 2020), where the differences in yield were primarily attributed to an increased tillering rate under favourable weather conditions not only during the growing season, but also in the autumn of the previous season when the plants were preparing for winter dormancy. The yield of grassland is strongly influenced by the amount of precipitation at the beginning of the growing season (March–April), when the grasses undergo intensive tillering, as the results of the present experiment confirm. Low rainfall and an abrupt increase in air temperature during this period led to a shorter tillering stage, an earlier onset of internode elongation and accelerated senescence (death) of the lower leaves. Favourable weather conditions (without drought) were particularly beneficial for the development of Poaceae plants. Due to their height, these plants were more competitive with other plants, so that their share in the grassland increased in some cases. As the yield of grass species was by far the highest, the total dry matter yield also increased. On the other hand, when rainfall was low during plant growth, a lower dry matter yield was accompanied by a lower proportion of grasses, i.e. a higher proportion of legumes and especially other herbs. This phenomenon was mainly due to the less intensive growth of grasses, which left more space for the development of other plants.

### *Effect of the fertilisation rate on the botanical composition*

Fertilisation had a significant influence on the floristic composition of the grassland in both cuts. Already in treatment A1, the average proportion of grasses increased by 29.8% and 66.8% in the first and second cuts, respectively; the proportion of legumes decreased by 49.7% in the first cut and 54.8% in the second cut; and the proportion of other herbs decreased by 39.9% and 57.6% in the first and second cuts, respectively, compared to the control. When the nitrogen application rate (A2) was further increased, the proportion of grasses continued to increase significantly, while the proportion of other plants decreased significantly in the first cut and the proportion of legumes and other herbs decreased in the second cut. A further increase in the amount of nitrogen (A3) did not lead to any significant change in the botanical composition of the grassland in either cut compared to treatment A2. Numerous studies lasting up to three years have shown similar results. The biomass production of the natural rangeland over-seeded with ryegrass was strongly dependent on the N rate (Ávila et al., 2019), and the botanical composition was altered. The highest species diversity and uniformity were achieved in the control treatment where no nitrogen was added. Similar results were obtained on the *Agrostietum capillaries* grassland (Simić et al., 2020). On the natural grassland *Festuca rubra – Agrostis capillaries* (1250 m a.s.l.), fertilisation with N100P50K50 resulted in a dry matter yield of 4.86 t/ha, which corresponded to an increase of 110.8% compared to the control, and an increase in the proportions of grasses and legumes by 14.9% and 28.8% respectively, and a reduction in the proportion of other herbs by 23.2% (Rotar et al., 2016a). Similar reports were published by Cirebea et al. (2020). Nitrogen fertilisation of grassland in Turkey had a negative effect on crude protein concentration in animal feed, as it reduced the proportion of legumes from 47% in untreated plots to 5% in plots treated with high nitrogen levels (Aydin and Uzun, 2005). There are different opinions on critical levels and economically viable NPK ratios for grassland fertilisation, depending on the type of grassland, management of grassland, and local weather conditions (Simić et al., 2020). The use of low inputs, i.e. a low amount of fertiliser, can be a solution that leads to higher yields, the preservation of biodiversity and good forage quality, as the results of Samuil et al. (2018) show. A two-year study recommended up to 100 kg/ha UAN (30 kg N) for *Festuca rubra* L. with *Agrostis capillaries* L. natural grassland (Rotar et al., 2016b). The long-term application of PK or NPK in mountain grassland primarily increased the P and K concentrations in the forage as well as the DM yield, while the nutrient and phenolic composition remained largely unaffected by the fertilisation (Ineichen et al., 2020).

In this experiment, however, there was a gradual decrease in total yield under the fertiliser treatments over the last 4 years. This can be explained by the fact that the decrease in plant diversity with increasing nitrogen fertilisation in treatments A1 and A2 could have a detrimental effect on the arbuscular mycorrhizal fungi in the soil, which is confirmed by numerous research results. Longterm fertilisation with nitrogen (100 kg/ha) and phosphorus (50 kg/ha) in a natural grassland reduced the number and altered the structure of arbuscular mycorrhizal fungi in the soil (which help plants to take up nutrients such as phosphorus, sulphur, nitrogen and micronutrients from the soil) (Chen et al., 2014). Low levels of plant diversity had a negative effect on soil microorganisms in semi-natural grassland, as rhizodeposition, plant productivity, and soil moisture were altered (Strecker et al., 2015). In this respect, a high level of plant diversity has a positive effect on the microsystemic functions of the ecosystem, such as decomposition and material cycling in nature. As Kotas et al. (2016) found, long-term annual NPK fertilisation of oligotrophic species-rich grassland had a negative effect on the entire system. Aboveground, plant species richness decreased, belowground, the soil became acidified and cation depleted, and although C mineralisation decreased, total soil organic matter storage was reduced. In 12 managed semi-natural temperate grasslands, natural fertilisation influenced soil microbial activity both directly and indirectly through its effects on soil and plant community characteristics, and plant species diversity had positive effects on soil microbial biomass (Dietrich et al., 2017).

In all treatments in this experiment, the Poaceae family was the most represented in terms of number of species, followed by Asteraceae and Fabaceae, with the proportion of Poaceae plants being significantly higher, especially in the fertilisation treatments (Table 4). Nitrogen fertilisation of natural grasslands reduces plant diversity and the presence of legumes and C4 plants, while the presence of C3 plants increases (Stevens et al., 2004). This phenomenon is the result of increased competition between plants for light (Xia and Wan, 2008). In terms of the number of species, the control grassland consisted of 14% grasses, 12% legumes and 74% other herbs, while their proportion in fertiliser treatments A2 and A3 was 20%, 15% and 65% respectively. The comparison of the number of species present in the grassland and their percentages indicates that long-term mineral fertilisation led to an increase in the proportion of grasses present, while the number of other species decreased over time. These results are consistent with numerous findings obtained under other conditions. In their four-year study, Simić et al. (2020) found 29 plant species in an unfertilised *Agrostietum capillaris* grassland, including grasses (17%), legumes (7%) and other herbs (76%). With fertilisation, the total number of species was lower, but the percentages of grasses and legumes increased, and the number of other herbs decreased. Jing et al. (2017) reported that long-term fertilisation of a natural grassland with urea in spring at 100 kg/ha/year under semi-arid climate conditions with a mean annual temperature of 5 °C reduced species diversity by 16.8% and increased the percentage of perennial grasses. Jaurena et al. (2020) pointed out that ten years of phosphorus fertilisation of a natural grassland led to a significant reduction in species diversity and a decrease in the total number of species present by more than 50 %. These trends were confirmed in long-term experiments by Ávila et al. (2019) and Ineichen et al. (2020).

Schaub et al. (2020) suggested that increased plant diversity in grassland is a strategy to improve sustainable systems and that the on going challenge is to develop management systems that ensure the maintenance of high plant diversity even in fertilised grassland. As the authors found, this can be achieved on *Arrhenatherion elatioris* grassland that is only lightly fertilised. Moderate PK fertilisation has the potential to maintain species richness and forage productivity of mown grasslands that are harvested with low frequency; PK fertilisation can increase the proportion of legumes on upland pastures (Ineichen et al., 2020). The plant diversity of grasslands at different fertiliser rates depends on species complementarity, i.e. the coexistence of conservation species (Niu et al., 2014). In a fifteen-year experiment on natural grassland, frequent mowing had a positive effect on species richness (Lepš, 2014). Moderate fertilisation and harvest intensity, as carried out in the mountain grassland studied over decades, made it possible to maintain both forage productivity and species-rich swards (Ineichen et al., 2020). These results are promising, as grasslands with species-rich swards tend to have higher resilience to environmental stress, so further research should lead in this direction.

## CONCLUSIONS

The mineral fertiliser treatments had a significant effect on the dry matter yield at the first cut and the botanical composition of the grassland. On average over the entire trial, the dry matter yield increased by 64.5 % under treatment N60:P40:K40 kg/ha and by over 100% under treatments N100:P40:K40 and N140:P40:K40 kg/ha compared to the control. The effect of fertilisation on dry matter yield was largely determined by weather conditions. In years with sufficient rainfall, any increase in nitrogen application rate led to a significant increase in dry matter yield. In the years with low rainfall and the year following the extremely dry autumn of the previous year, all fertilisation treatments led to an increase in dry matter yield compared to the control, although the treatments did not differ significantly from each other. In the second cut, the fertiliser effect was significantly lower than in the first cut due to the lower rainfall. The ten-year mineral fertilisation led to a decrease in the total number of plant species on the grassland. High rainfall was particularly favourable for Poaceae plants, whose percentage share increased due to their higher competitiveness. Under dry conditions, the less intensive growth of grasses favoured the development of other plants, whose percentage share increased, and the total yield decreased. Fertilization with N60:P40:K40 kg/ha affected the increase in the percentage of grasses in both cuts, while the percentage of legumes and other herbs decreased compared to the control treatment. When the nitrogen application rate was further increased (N100:P40:K40 kg/ha), the percentage of grasses continued to increase significantly, while the percentage of other plants decreased significantly in the first cut and that of legumes and other herbs decreased in the second cut. An additional increase in the amount of nitrogen (N140:P40:K40 kg/ha) did not lead to any significant change in the botanical composition in either cut compared to treatment N100:P40:K40 kg/ha. In the last four years of the trial, there was a gradual decline in dry matter yields under the fertiliser treatments, which is probably due to the detrimental effect of the fertiliser and the reduced plant diversity on the arbuscular mycorrhizal fungi in the soil.

The results of this study indicate that dry matter yields of *Festucetum pratense* on natural grassland can be increased if low nitrogen fertilisation and adequate phosphorus and potassium fertilisation are used (N60:P40:K40 kg/ha). As the decline in plant diversity has a negative impact on the ecosystem, the challenge remains to develop sustainable systems that ensure the maintenance of plant diversity in combination with frequent mowing. This challenge should be addressed in future research.

## ACKNOWLEDGMENTS

The research presented in this article is part of the projects ref. no. 451-03-66/2024-03/200088 and 451-03-66/2024-03/200217 was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia.

## REFERENCES

Ávila, M.R., Nabinger, C., Schneider-Canny, Fedrigo, J.K. (2019) Botanical composition of a natural rangeland overseeded with annual ryegrass under N fertilization. Scientia Agropecuaria, 10 (2), 303–305. DOI: https://doi.org/10.17268/sci.agropecu.2019.02.16

Aydin, I., Uzin, F. (2005) Nitrogen and phosphorus fertilization of rangelands affects yield, forage quality and the botanical composition. European Journal of Agronomy, 23, 8–14. DOI: https://doi.org/10.1016/j.eja.2004.08.001

Chen, Y.L., Zhang, X., Ye, J.S., Han, H.Y., Wan, S.Q., Chen, B.D. (2014) Six-year fertilization modifies the biodiversity of arbuscular mycorrhizal fungi in a temperate steppe in Inner Mongolia. Soil Biology and Biochemistry, 69, 371–381. DOI: https://doi.org/10.1016/j.soilbio.2013.11.020

Cirebea, M., Rotar, I., Vidican, R., Pleșa, A., Morea, A., Ranta, O. (2020) Impact of organo-mineral fertilization upon phytocoenosis and feed quality of the grasslands in the region of Transylvania. Romanian Agricultural Research, 37, 179–188. Available at: https://www.incda-fundulea.ro/rar/nr37/rar37.21.pdf [Accessed 12 October 2023].

Cotrufo, M.F., Ngao, J., Marzaioli, F., Piermatteo, D. (2010) Intercomparison of methods for quantifying above-ground leaf litter decomposition rates. Plant and Soil, 334, 365–76. DOI: https://doi.org/10.1007/s11104-010-0388-0

Dasselaar, A.V.P., Becker, T., Botana, F.A., Peratoner, G. (2021) Societal and economic options to support grassland-based dairy production in Europe. Irish Journal of Agricultural and Food Research, 59 (2), 258-269. DOI: https://doi.org/10.15212/ijafr-2020-0128

Dietrich, P., Buchmann, T., Cesarz, S., Eisenhauer, N., Roscher, C. (2017) Fertilization, soil and plant community characteristics determine soil microbial activity in managed temperate grasslands. Plant and Soil, 419, 189–199.DOI: https://doi.org/10.1007/s11104-017-3328-4

Eurostat (2023) Available online: https://ec.europa.eu/eurostat/web/main/data/ database [Accessed 05 November 2023].

Fornara, A.D., Flynn, D., Caruso, T. (2020) Effects of nutrient fertilization on root decomposition and carbon accumulation in intensively managed grassland soils. Ecosphere, 11 (4), 1–17. DOI: https://doi.org/10.1002/ecs2.3103

Grašič, M., Šabić, A., Lukač, B. (2023) A review of methodology for grassland restoration and management with practical examples. Acta Biologica Slovenica, 66 (1), 52-77. DOI: https://doi.org/10.14720/abs.66.1.13230

Huyghe, C., De Vliegher, A., Van Gils, B., Peeters, A. (2014) Grasslands and Herbivore production in Europe and Effects of Common Policies. Synthesis, Versailes, Cedex, France, 320. DOI: https://doi.org/10.35690/978-2-7592-2157-8

Ineichen, S., Marquardt, S., Kreuzer, M., Reidy, B. (2020) Forage quality of species-rich mountain grasslands subjected to zero, PK and NPK mineral fertilization for decades. Grass and Forage Science, 75(4), 385-397. DOI: https://doi.org/10.1111/gfs.12488

Jaurena, M. Ayala, W., Terra, J., Bermudez, R., Barrios, E., Lagomarsino, X. (2020) Impacts of Long-Term Phosphorus Fertilization and Addition of Perennial Legumes on a Temperate Natural Grassland: I. Changes in Species Biodiversity and Stability. Proceedings of International Grassland Congress “Ecology of grasslands/rangelands”. University of Kentucky, USA, 1, pp. 80–82. Available online: https://uknowledge.uky.edu/cgi/viewcontent.cgi?article=2185&context=igc [Accessed 12 December 2023].

Jing, G., Li, W., Yu, K., Ratajczak, Z., Kallengach, R., Cheng, J. (2017) Effects of fertilization, burning, and grazing on plant community in the long term fenced grasslands. Plant Soil and Environment, 63 (4), 171–176. DOI: https://doi.org/10.17221/64/2017-PSE

Kotas, P., Choma, M., Šantručková, H., Lepš, J., Třiška, J., Kaštovská, E. (2016) Linking Above- and Belowground Responses to 16 Years of Fertilization, Mowing, and Removal of the Dominant Species in a Temperate Grassland. Ecosystems, 20, 354–367. DOI: https://doi.org/10.1007/s10021-016-0031-x

Lepš, J. (2014) Scale- and time-dependent effects of fertilization, mowing and dominant removal on a grassland community during a 15-year experiment. Journal of Applied Ecology, 51, 978–987. DOI: https://doi.org/10.1111/1365-2664.12255

Lin, Y., Slessarev, W.E., Yehl, T.S., Antonio, M.C., King, Y.J. (2018) Long-term Nutrient Fertilization Increased Soil Carbon Storage in California Grasslands. Ecosystems, 22, 754–766. DOI: https://doi.org/10.1007/s10021-018-0300-y

Niu, K., Choler, P., Mirotchnick, N., Sun, S. (2014) Fertilization decreases species diversity but increases functionaldiversity: A three-year experiment in a Tibetan alpine meadow. Agriculture Ecosystems and Environment, 182, 106–112. DOI: https://doi.org/10.1016/j.agee.2013.07.015

Rotar, I., Cirebea, M., Păcurar, F., Vidican, R., Malinas, A., Ranta, O. (2016a) Mineral and organic fertilization influence on *Festuca rubra*–Agrostis capillaris natural meadow. Romanian Journal of Grassland and Forage Crops, 13, 39–46. Available at: https://sropaj.ro/documente/ro/revista/articole/RJGFC-13-2016_art-5.pdf [Accessed 12 November 2023].

Rotar, I., Cirebea, M., Vidican, R., Păcurar, F., Malinaş, A., Ranta, O. (2016b) Mineral Fertilization with UAN on Natural Grassland *Festuca rubra* L. with *Agrostis capillaries* L.. Bulletin UASVM Cluj-Napoca Agriculture, 73 (2), 300–305. DOI: https://doi.org/10.15835/buasvmcn-agr:12449

Samuil, C., Stavarache, M., Sîrbu, C., Vîntu, V. (2018) Influence of Sustainable Fertilization on Yield and Quality Food of Mountain Grassland. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 46 (2), 410–417. DOI: https://doi.org/10.15835/nbha46210660

Schaub, S., Finger, R., Leiber, F., Probst, S., Kreuzer, M., Weigelt, A., Buchmann, N., Scherer-Lorenzen, M. (2020) Plant diversity effects on forage quality, yield and revenues of semi-natural grasslands. Nature Communications, 11, 768. DOI: https://doi.org/10.1038/s41467-020-14541-4

Simić, A., Mandić, V., Vučković, S., Bijelić, Z., Stanisavljević, R., Štrbanović, R., Sokolović, D. (2020) Assessment of yield, quality and nitrogen index of *Agrostietum capillaris* grassland as affected by fertilizations. Biotechnology in Animal Husbandry, 36 (1), 101–113. DOI: https://doi.org/10.2298/ BAH2001101S

StatSoft Inc. (2007) STATISTICA for Windows (data analysis software system), version 8.0., Tulsa, OK, USA.

Stevens, C.J., Dise, N.B., Moutford, J.O., Gowing, D.J. (2004) Impact of nitrogen deposition on the species richness of grasslands. Science, 303, 1876–1879. DOI: https://doi.org/10.1126/science.1094678

Strecker, T, Barnard, RL, Niklaus, PA, Scherer-Lorenzen, M, Weigelt, A, Scheu, S, Eisenhauer, N. (2015) Effects of Plant Diversity, Functional Group Composition, and Fertilization on Soil Microbial Properties in Experimental Grassland. PLoS ONE, 10 (5), e0125678.
DOI: https://doi.org/10.1371/journal.pone.0125678

Sulman, B.N., Phillips, R.P., Oishi, A.C., Shevliakova, E., Pacala, S.W. (2014) Microbe-driven turnover offsets mineral-mediated storage of soil carbon under elevated $CO_2$. Nature Climate Change, 4, 1099–1102. DOI: https://doi.org/10.1038/NCLIMATE2436

Török, P., Dembicz, I., Dajić-Stevanović, Z., Kuzemko, A. (2020) Grasslands of Eastern Europe. In: Goldstein, M.I., DellaSala, D.A., eds. Encyclopedia of the World's Biomes. Amsterdam: Elsevier, pp. 703-713.
DOI: https://doi.org/10.1016/B978-0-12-409548-9.12042-1

Viégas, J., de Oliveira, L.B., de Souza, I.B., Cruz, P., de Quadros, F.L.F., Tiecher, T. (2017) Fertilization response and nitrogen nutrition diagnosis of anatural grassland in Southern Brazil. Acta Iguazu, 6(2), 25–36. DOI: https://doi.org/10.48075/actaiguaz.v6i2.17539

Xia, J.Y.,Wan, S.Q. (2008) Global response patterns of terrestrial plant species to nitrogen addition. New Phytologist, 179, 428–439.
DOI: https://doi.org/10.1111/j.1469-8137.2008.02488.x

You, C., Wu, F., Gan, Y., Yang, W., Hu, Z., Xu, Z., Tan, B., Liu, L.,Ni, X. (2017) Grass and forbs respond differently to nitrogen addition: a meta-analysis of global grassland ecosystems. Scientific Reports 7, 1563 DOI: https://doi.org/10.1038/s41598-017-01728-x