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Influence of macro-mineral and biological fertilization on the botanical composition and quality of natural grassland in the Central Balkan Mountains

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Summary: The aim of this study was to evaluate the effects of mineral and biological fertilizers on the botanical composition, structural fibre characteristics, and in *vitro dry* matter digestibility (IVDMD) of forage biomass from two mountain grassland communities: an *Agrostis capillaris–Festuca fallax* meadow and a *Nardus stricta* pasture. Mineral fertilization consisted of calcium ammonium nitrate combined with phosphorus, whereas the biological treatments included Panamin Agro and the sapropel-derived products Blago, Blago 3 and Blago 5. Botanical composition was expressed as the proportion of grasses, legumes and forbs in total fresh biomass. Mineral fertilization increased the proportion of grasses in both grassland types, with the strongest response observed in the meadow, where the highest N–P–Ca treatment increased grass proportion from 60.0% to 79.1%. Biological fertilizers generally increased legume participation and reduced the proportion of forbs, particularly under Blago treatments and the lowest Panamin Agro rate. Fertilization also modified several forage-quality parameters. In both grassland types, the combined Blago 5 + Blago treatment resulted in the lowest NDF, ADF and cellulose concentrations and the highest numerical IVDMD values. Compared with the corresponding controls, IVDMD increased by 19.6% in the meadow and 22.9% in the pasture. The results demonstrate that fertilization effects depended on grassland type, fertilizer product and application rate. Mineral fertilization primarily promoted grass development, whereas selected biological fertilizer treatments, particularly the combined Blago 5 + Blago application, produced a broader response involving greater legume participation, reduced forb contribution, and improved measured forage-quality characteristics.

Key words: *Agrostis–Festuca*, *Nardus stricta*, biostimulants, botanical composition, forage quality, mountain grasslands, sustainable fertilization

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Introduction

Natural grasslands are multifunctional ecosystems that provide forage resources while supporting biodiversity conservation, soil protection, and a wide range of ecosystem services. Their productivity and nutritive value are strongly influenced by botanical composition, which affects biomass yield, chemical characteristics, and the feeding value of forage (Dudek et al., 2020; Churkova & Churkova, 2024). The relative contribution of grasses, legumes and forbs is particularly important because these functional groups differ in nutrient acquisition, chemical

composition, digestibility, and their contribution to livestock nutrition (Hejcman et al., 2010; Wróbel et al., 2025). Grasses generally provide a major source of structural carbohydrates and energy, whereas legumes may increase forage protein supply through biological nitrogen fixation and contribute to nitrogen cycling within grassland ecosystems (Castro-Montoya & Dickhoefer, 2020).

In Bulgaria, natural meadows and pastures occupy approximately 1.41 million hectares and are mainly distributed in mountainous and semi-mountainous regions (National Statistical Institute, 2024). In addition to their importance for extensive livestock production, these ecosystems contribute to erosion control, landscape conservation, and the maintenance of plant diversity (Pergola et al., 2024). Sustainable management of mountain grasslands is therefore important for both agricultural production and biodiversity conservation.

Fertilization is one of the management practices used to increase forage productivity and improve nutritive value in semi-natural grasslands (Petković et al., 2025). Mineral fertilizers rapidly increase the availability of essential nutrients and may stimulate the growth of productive grasses, whereas biological fertilizers and biostimulants may influence nutrient availability and plant physiological processes through different mechanisms (Iliev, 2018; Samuil et al., 2018; Dindová et al., 2019; Iliev et al., 2020; Vranić et al., 2020). The response of grassland vegetation to fertilization, however, depends strongly on initial environmental conditions, including soil acidity, nutrient availability, altitude, and the dominant plant species.

Mountain grasslands dominated by *Nardus stricta* are commonly associated with acidic, nutrient-poor soils. Low phosphorus availability and unfavourable soil reaction may restrict the development of productive forage species and legumes. Previous studies have shown that appropriate nutrient management can improve the nutritive value of *Nardus*-dominated grasslands by modifying nutrient availability, structural fibre fractions, and forage digestibility (Tarcău et al., 2012; Nazare et al., 2024). In more productive meadow communities dominated by *Agrostis capillaris* and *Festuca fallax*, fertilization may instead modify competition among established plant groups, potentially increasing grass dominance depending on the amount and type of nutrients supplied.

Recent research has also highlighted the potential of biological fertilizers and related products to improve plant growth, nutrient utilization and forage quality through effects on root development, nutrient mobilization and plant physiological performance (Karpenko et al., 2020; Mackiewicz-Walec & Olszewska, 2023). Nevertheless, comparative information on the effects ofmineral and biological fertilizers in different mountain grassland communities remains limited.

We hypothesized that biological fertilization would promote a more balanced botanical composition, characterized by greater legume participation and a lower proportion of forbs, while reducing structural fibre concentrations and increasing forage digestibility. We further hypothesized that mineral fertilization would primarily stimulate grass development as a result of increased mineral nutrient availability.

Therefore, the objective of this study was to assess the effects of calcium ammonium nitrate combined with phosphorus, Panamin Agro, and the sapropel-derived biological fertilizers Blago, Blago 3 and Blago 5 on the botanical composition, structural fibre characteristics, and *in vitro* dry matter digestibility of forage biomass from two representative mountain grassland communities of the Central Balkan Mountains: an *Agrostis capillaris–Festuca fallax* meadow and a *Nardus stricta* pasture.

Materials and methods

Experimental site and design

Field experiments were conducted during three consecutive growing seasons (2021–2023) in the Central Balkan Mountains, Bulgaria. Two semi-natural grassland differing in altitude, dominant vegetation and soil constraints were investigated: an *Agrostis capillaris* and *Festuca fallax* meadow located at 520 m a.s.l. and a *Nardus stricta* pasture located at 1379 m a.s.l.

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For each grassland type, three independent fertilization experiments were established using a randomized complete block design with four replications. The harvested area of each experimental plot was 5 m². The experiments evaluated three groups of treatments: macro-mineral fertilization, foliar application of Panamin Agro, and foliar application of Blago products. Each experiment included an unfertilized control.

The initial botanical composition differed between the two grasslands. In the meadow, the corresponding control treatments contained 60.0–65.8% grasses, 2.7–6.1% legumes and 28.1–37.0% forbs. In the *Nardus stricta* pasture, the corresponding control treatments contained 49.3–64.0% grasses, 2.0–17.8% legumes and 32.8–38.6% forbs. These differences were considered when interpreting the fertilization response.

Climatic and soil characteristics

The experimental sites are located in the Central Balkan Mountains and are influenced by altitude and topography, which determine differences in temperature and precipitation regimes. The experimental region has a temperate continental climate, with annual precipitation generally increasing with elevation.

The *Agrostis capillaris–Festuca fallax* meadow was established on Planosols (pseudopodzolic soils), characterized by an acidic reaction, relatively low natural fertility and restricted nutrient availability. The *Nardus stricta* pasture was located in the higher mountain zone on strongly acidic soils, with $pH_{(H_2O)}$ values of 4.2–4.6. These soils had high organic matter content but limited availability of phosphorus, potassium and several micronutrients. The acidic soil reaction and low nutrient availability were considered important ecological constraints for the establishment and persistence of forage legumes.

Fertilization treatments

Three fertilization experiments were conducted separately at each grassland site.

Experiment I: Macro-mineral fertilization

The macro-mineral treatments consisted of calcium ammonium nitrate (CAN) combined with phosphorus:

- Control – unfertilized;
- $P_6N_5Ca_{2.8}$ – 185 kg ha^{-1} CAN + phosphorus;
- $P_6N_{10}Ca_{5.6}$ – 370 kg ha^{-1} CAN + phosphorus;
- $P_6N_{15}Ca_{8.3}$ – 556 kg ha^{-1} CAN + phosphorus.

Calcium ammonium nitrate was used as the nitrogen source and supplied both nitrogen and calcium. The treatment codes indicate the applied amounts of phosphorus, nitrogen and calcium, respectively, according to the experimental formulation.

Experiment II: Panamin Agro

Panamin Agro was applied at three rates:

- Control – unfertilized;
- Panamin Agro – 1.5 kg ha^{-1};
- Panamin Agro – 3.0 kg ha^{-1};
- Panamin Agro – 4.5 kg ha^{-1} .

Panamin Agro is a mineral-based biological fertilizer containing finely ground rock materials, natural minerals and limestone. The product is intended to improve mineral availability and support plant development and soilremineralization.

Experiment III: Blago products

The Blago treatments were:

- Control – unfertilized;
- Blago 5 – 1.5 L ha^{-1};

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- Blago 3 – 3.0 L ha^{-1};
- Blago 5 – 4.5 L ha^{-1} + Blago – 1.0 L ha^{-1}.

The products differed in their nutrient and organic-matter composition.

Blago contained 10.0–11.0% N, 3.2–5.0% P_2O_5 and 10.4–11.7% K_2O, together with MgO, Mn, Zn, Cu, B, Mo and Co, at the concentrations specified by the product formulation. It also contained ≥1.6–1.7% humic acids and ≥35% organic matter, with pH 6.5–7.5.

Blago 3 contained 11.0% N, 20.0% P_2O_5 and 11.0% K_2O, together with 0.56% MgO, 0.56% Mn, 0.65% Zn, 1.12% Cu, 0.50% B, 0.09% Mo and 0.15% Co. It contained ≥2% humic and fulvic acids and ≥54% organic matter derived from lake sapropel, with pH 6.5–7.5.

Blago 5 contained 0.17% N, 15.5% P_2O_5 and 9.75% K_2O, together with 0.43% MgO, 0.60% Mn, 0.30% Zn, 0.17% Cu, 0.17% B, 0.90% Mo and 0.15% Co. It contained ≥2% humic and fulvic acids and ≥70% organic matter derived from lake sapropel, with pH 6.5–7.5.

Mineral fertilizers were broadcast-applied in spring when vegetation reached approximately 8-10 cm in height. Panamin Agro and Blago were applied as foliar treatments using the respective recommended spray application procedure. The liquid Blago products were diluted in water before application and sprayed uniformly over the vegetation. All fertilization treatments were applied twice annually during the experimental period.

Harvesting and botanical composition analysis

The *Agrostis capillaris* and *Festuca fallax* meadow was harvested at the heading to early flowering stage of the dominant grasses, whereas the *Nardus stricta* pasture was harvested at the heading stage.

Botanical composition was determined immediately before harvesting using the weight separation method. A representative fresh biomass samples from each plot was manually separated into three functional botanical groups: grasses, legumes and forbs. The proportion of each group was calculated as a percentage of the total fresh biomass. Thus, the reported percentages describe the relative contribution of grasses, legumes and forbs to the above-ground fresh biomass of the sward.

Laboratory analyses

Forage samples were analysed for structural fiber fractions using the detergent fibre method of Van Soest and Robertson (1979). The following parameters were determined:

- neutral detergent fibre (NDF, g kg^{-1} DM);
- acid detergent fibre (ADF, g kg^{-1} DM);
- acid detergent lignin (ADL, g kg^{-1} DM).

Hemicellulose and cellulose were calculated as:

Hemicellulose = NDF – ADF

Cellulose = ADF – ADL

In vitro dry matter digestibility (IVDMD) was determined using the two-stage pepsin–cellulase enzymatic method described by Aufrère (1982). Higher IVDMD values were interpreted as indicating greater potential digestibility of the forage dry matter.

Statistical analysis

The effects of fertilization treatment and experimental year were evaluated separately for each grassland type and fertilizer experiment using analysis of variance (ANOVA). Differences among treatment means were evaluated using the least significant difference (LSD) test at the 5% probability level. Statistical significance was accepted at $P \leq 0.05$. Statistical analyses were performed using Microsoft Excel 2010 with the Analysis ToolPak.

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Results and discussion

Botanical composition of the Agrostis capillaris–Festuca fallax *meadow and* Nardus stricta *pasture:* Fertilization modified the relative contribution of grasses, legumes and forbs in both grassland types, but the magnitude and direction of the changes depended on the initial botanical composition and the characteristics of the fertilizer product. The *Agrostis capillaris–Festuca fallax* meadow initially had a relatively high grass proportion and a low contribution of legumes, whereas the *Nardus stricta* pasture showed greater variation among the corresponding controls. The observed responses therefore indicate that nutrient addition affected competitive relationships among already established functional groups rather than producing a uniform botanical response.

Agrostis capillaris–Festuca fallax meadow

The unfertilized controls contained 60.0–65.8% grasses, 2.7–6.1% legumes and 28.1–37.0% forbs (Figure 1). The relatively low legume contribution indicates limited participation of nitrogen-fixing species in the meadow sward, whereas grasses constituted the dominant functional group.

Macro-mineral fertilization

Macro-mineral fertilization strongly increased the contribution of grasses. Relative to the corresponding control, grass participation increased from 60.0% to 73.1%, 72.1% and 79.1% under $P_6N_5Ca_{2.8}$, $P_6N_{10}Ca_{5.6}$ and $P_6N_{15}Ca_{8.3}$, respectively. This agrees with observations that increased nitrogen inputs can promote grass dominance while reducing plant functional diversity in managed grasslands (Sbrissia et al., 2026). At the same time, the contribution of forbs declined from 37.0% in the control to 13.3–16.8% in the fertilized treatments.

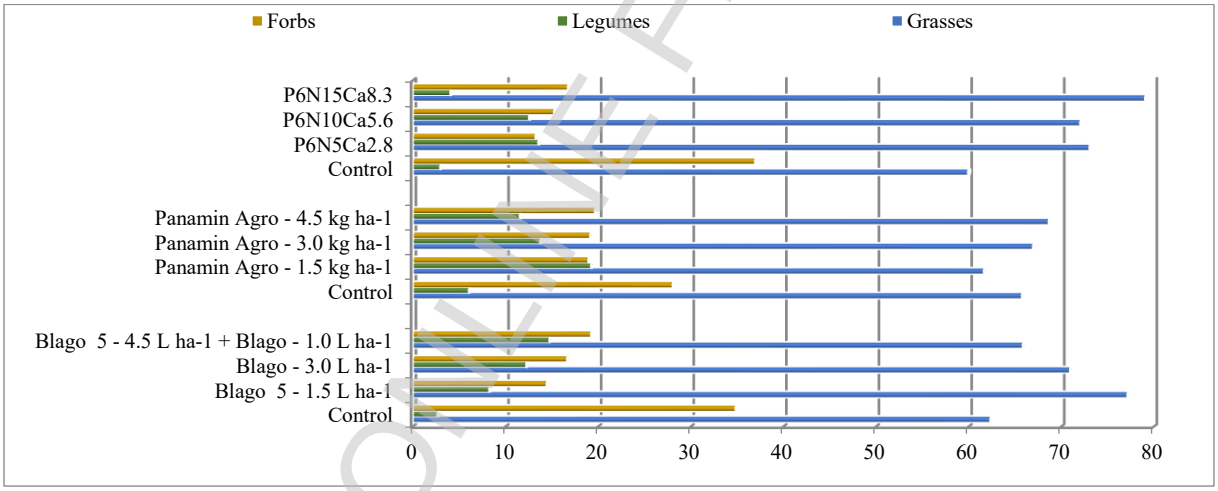

Figure 1. Botanical composition (%) of an *Agrostis capillaris–Festuca fallax* meadow grassland under mineral and biological fertilization, expressed as the proportions of grasses, legumes and forbs

The response of legumes was dose-dependent. Legume participation increased from 3.0% in the control to 13.6% and 12.6% at the two lower macro-mineral rates but decreased to 4.1% at the highest rate. Thus, moderate nutrient addition was associated with an increased relative contribution of legumes, whereas the highest nitrogen rate favoured grasses more strongly. This pattern is consistent with the generally greater competitive response of fast-growing grasses to increased nitrogen availability, which can reduce the relative contribution of legumes when nitrogen supply becomes high.

The results therefore indicate that macro-mineral fertilization was particularly effective in increasing the contribution of grasses and reducing the relative contribution of forbs. However, the highest N rate did not maintain the increase in legumes observed at the lower rates. This suggests that the botanical response cannot be described simply as an increase in forage species; rather, it depended on the balance between nutrient stimulation and interspecific competition.

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Panamin Agro

Panamin Agro produced a different response from macro-mineral fertilization. The strongest increase in legume participation occurred at 1.5 kg ha^{-1}, where legumes increased from 6.1% in the control to 19.3%. At 3.0 and 4.5 kg ha^{-1}, legume proportions were 13.8% and 11.6%, respectively.

Grass participation was 61.7% at 1.5 kg ha^{-1} and increased to 67.0% and 68.7% at the two higher rates. The contribution of forbs was reduced from 28.1% in the control to approximately 19% in all Panamin Agro treatments.

The results suggest that the lowest application rate produced the most pronounced shift towards legumes, whereas higher rates favoured grasses to a greater extent. The response may be associated with improved nutrient availability and plant physiological performance, but the present experiment did not directly measure nutrient uptake or root activity. Therefore, these mechanisms should be regarded as possible explanations rather than demonstrated causal processes. The observed reduction in the relative contribution of forbs indicates that Panamin Agro changed competitive relationships within the sward. Similar effects of biological products on plant stress tolerance and nutrient utilization have been reported by Karpenko et al. (2020) and Mackiewicz-Walec and Olszewska (2023).

Blago products

Blago treatments also altered botanical composition. The proportion of grasses increased from 62.4% in the control to 77.2% under Blago 5 at 1.5 L ha^{-1}, 71.0% under Blago 3 at 3.0 L ha^{-1} and 65.9% under the combined Blago 5 + Blago treatment.

The legume proportion increased progressively from 2.7% in the control to 8.3%, 12.3% and 14.8% in these treatments, respectively. At the same time, the contribution of forbs declined from 34.9% to 14.5–19.3%.

The combined Blago 5 + Blago treatment therefore produced the largest increase in the relative contribution of legumes and a substantial reduction in forbs while maintaining a grass proportion close to that of the control. This response differed from the highest macro-mineral rate, which strongly increased grass dominance but resulted in only 4.1% legumes.

The differences among Blago products should be interpreted in relation to their distinct formulations. Blago 5 contained high organic matter and P_2O_5, whereas Blago 3 contained a substantially higher P_2O_5 concentration and a different micronutrient profile. Consequently, the response cannot be attributed to a generic "Blago effect"; it reflects the particular nutrient and organic-matter composition of each product and its application rate.

***Nardus stricta* pasture**

The unfertilized controls of the *Nardus stricta* pasture contained 49.3–64.0% grasses, 2.0–17.8% legumes and 32.8–38.6% forbs (Figure 2). The broad variation among control values reflects differences among the independent fertilizer experiments and indicates that the initial botanical composition was not identical among experimental series.

The *Nardus stricta* pasture developed under strongly acidic soil conditions, with pH(H_2O) of 4.2–4.6. Such conditions are generally associated with restricted availability of several nutrients and can constrain the establishment of less acid-tolerant forage species.

Macro-mineral fertilization

Macro-mineral fertilization increased the contribution of grasses in all treatments relative to the corresponding control. Grass participation increased from 50.3% in the control to 69.5%, 69.3% and 63.7% at the three N–P–Ca rates, respectively. The contribution of forbs decreased from 38.6% to 20.8–25.3%.

In contrast to the meadow, legumes increased at the two higher nutrient rates, reaching 10.0% at $P_6N_{10}Ca_{5.6}$ and 15.4% at $P_6N_{15}Ca_{8.3}$, compared with 11.1% in the control. The highest

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treatment therefore combined a substantial increase in grass contribution with a moderate increase in legumes and a reduction in forbs.

The response indicates that nutrient addition was associated with increased participation of productive species in the *Nardus stricta* pasture. The presence of calcium in the macro-mineral treatment may also have contributed to the response under acidic soil conditions; however, soil pH was not measured after fertilization, and therefore a direct liming effect cannot be established from the present data.

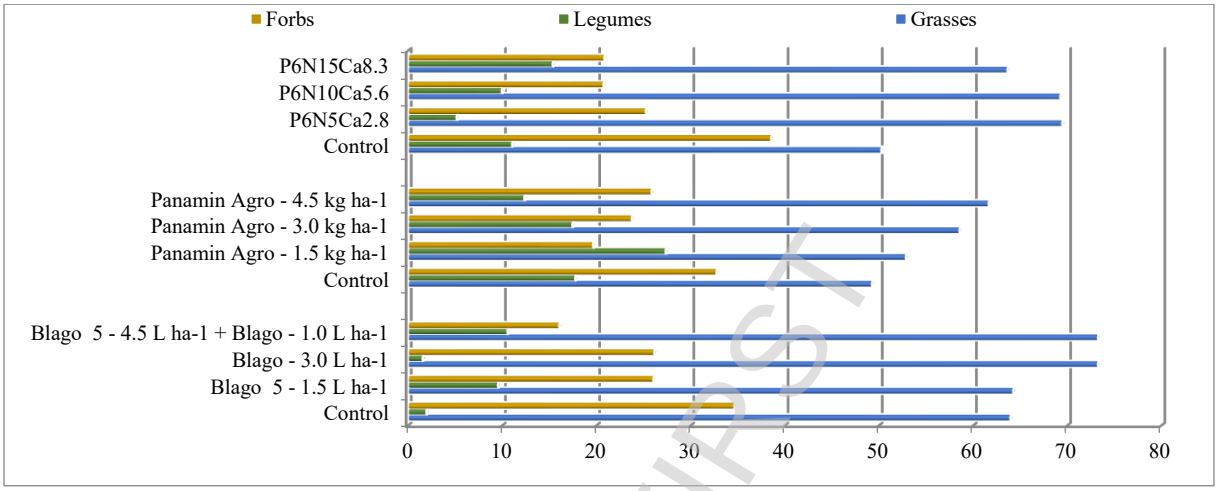

Figure 2. Botanical composition (%) of a *Nardus stricta* pasture grassland under mineral and biological fertilization, expressed as the proportions of grasses, legumes and forbs..

Panamin Agro

Panamin Agro produced the clearest increase in legumes at the lowest rate. Legume participation increased from 17.8% in the control to 27.4% at 1.5 kg ha^{-1}. At 3.0 and 4.5 kg ha^{-1}, the corresponding values were 17.5% and 12.4%.

Grass participation increased progressively from 49.3% in the control to 52.9%, 58.6% and 61.7% with increasing application rate. The contribution of forbs decreased from 32.8% to 19.7% at the lowest rate but increased slightly at the two higher rates.

The results show that the lowest Panamin Agro rate produced the largest relative increase in legumes, whereas increasing the rate progressively favoured grasses. This dose-dependent response indicates that a higher application rate did not necessarily provide a greater improvement in the botanical composition of the pasture.

Blago products

Blago treatments increased grass and legume participation in the *Nardus stricta* pasture, although the magnitude of the response differed among products and rates. Blago 5 at 1.5 L ha^{-1} increased legumes from 2.0% to 9.6%, while the combined Blago 5 + Blago treatment increased them to 10.6%.

The combined treatment produced the greatest reduction in forbs, from 34.7% in the control to 16.1%, while grass participation increased from 64.0% to 73.3%. Blago 3 at 3.0 L ha^{-1} also increased grass participation to 73.3%, but legume participation was only 1.6%.

Thus, the combined Blago treatment produced the most favourable simultaneous change in the three functional groups: greater participation of grasses and legumes and a marked reduction in forbs. However, the response of Blago 3 demonstrates that the botanical effect was strongly dependent on product formulation rather than being a uniform response to all Blago applications.

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Comparison of botanical responses between grassland types

The two grassland types responded differently to fertilization. In the *Agrostis capillaris–Festuca fallax* meadow, macro-mineral fertilization primarily increased grass dominance, while Panamin Agro and Blago treatments generally produced larger increases in legume participation. In the *Nardus stricta* pasture, fertilization also increased grass participation, but several treatments increased legumes while reducing the contribution of forbs.

These differences are consistent with the different initial conditions of the grasslands. The meadow already had a high proportion of grasses, whereas the *Nardus stricta* pasture was characterized by strongly acidic soil and nutrient limitations. Consequently, the same fertilizer input could produce different competitive responses depending on the initial composition and environmental constraints of the sward.

The results also show that biological fertilization should not be treated as a single uniform category. Panamin Agro and the different Blago formulations produced distinct botanical responses, demonstrating the importance of product composition and application rate.

Fiber fractions and forage quality

Fertilization modified several structural fibre fractions and IVDMD in both grasslands (Tables 1 and 2). The responses were generally characterized by lower NDF and ADF concentrations and higher numerical IVDMD values in selected fertilized treatments. Because NDF and ADF represent major components of the structural carbohydrate fraction of forage and lignin restricts fibre degradation, reductions in these parameters are generally associated with improved forage nutritive value (Huhtanen & Krizsan, 2023; Wróbel et al., 2025).

***Agrostis capillaris–Festuca fallax* meadow**

Macro-mineral fertilization

Macro-mineral fertilization significantly affected NDF ($P < 0.01$), ADL ($P = 0.001$) and cellulose ($P < 0.05$), whereas the effects on ADF and IVDMD were not statistically significant according to the ANOVA results.

The highest N–P–Ca treatment produced NDF of 664.7 g kg^{-1} DM, ADF of 326.3 g kg^{-1} DM and ADL of 61.3 g kg^{-1} DM. Cellulose decreased to 264.9 g kg^{-1} DM, while IVDMD reached 615.0 g kg^{-1} DM, compared with 550.8 g kg^{-1} DM in the corresponding control. Thus, the numerical increase in IVDMD was 11.7%.

The reduction in several fibre fractions at the highest nutrient rate indicates that improved mineral nutrition was associated with a less fibrous forage profile. However, because the treatment effect on IVDMD was not statistically significant, the numerical increase should not be interpreted as a statistically confirmed treatment effect.

Panamin Agro

Panamin Agro significantly affected NDF, ADL, hemicellulose and cellulose in the meadow. The 3.0 kg ha^{-1} treatment produced the lowest NDF (563.1 g kg^{-1} DM), ADF (294.0 g kg^{-1} DM) and cellulose (235.0 g kg^{-1} DM), together with the highest IVDMD (657.1 g kg^{-1} DM).

Compared with the corresponding control, NDF decreased by 18.3%, ADF by 25.8% and cellulose by 29.6%, while IVDMD increased by 14.7%. The 4.5 kg ha^{-1} treatment did not produce a further improvement, indicating a non-linear response to application rate.

The results suggest that the intermediate Panamin Agro rate was more effective than the highest rate in modifying forage fibre characteristics. This response is consistent with the botanical results, where the lower rate also produced a relatively greater increase in legumes.

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Table 1. Structural fibre fractions and in vitro dry matter digestibility (IVDMD) of forage from the *Agrostis capillaris–Festuca fallax* meadow under macro-mineral and biological fertilization, mean values for 2021–2023 (g kg^{-1} DM)

Fertilization treatment	NDF	ADF	ADL	Hemicellulose	Cellulose	IVDMD
Macro-mineral fertilization						
Control	701.8	420.4	72.5	281.4	347.9	550.8
$P_6N_5Ca_{2.8}$	659.1	391.9	72.1	267.1	319.8	576.3
$P_6N_{10}Ca_{5.6}$	659.0	366.9	76.0	292.1	290.9	603.4
$P_6N_{15}Ca_{8.3}$	664.7	326.3	61.3	338.5	264.9	615.0
$LSD_{0.05}$	***43.1***	***59.2***	***20.5***	***96.4***	***58.2***	***40.4***
Panamin Agro						
Control	688.9	396.3	62.3	292.6	334.0	572.7
Panamin Agro – 1.5 kg ha^{-1}	644.8	330.2	64.6	314.6	265.6	626.1
Panamin Agro – 3.0 kg ha^{-1}	563.1	294.0	59.0	269.1	235.0	657.1
Panamin Agro – 4.5 kg ha^{-1}	607.3	346.4	52.6	260.9	293.8	606.7
$LSD_{0.05}$	***45.1***	***53.2***	***14.9***	***64.2***	***63.0***	***42.3***
Blago products						
Control	663.8	407.3	71.7	256.5	335.6	560.9
Blago 5 – 1.5 L ha^{-1}	614.8	328.9	54.3	285.9	274.6	637.8
Blago 3 – 3.0 L ha^{-1}	592.7	321.1	45.8	271.6	275.4	642.1
Blago 5 – 4.5 L ha^{-1} + Blago – 1.0 L ha^{-1}	571.6	282.5	47.2	289.1	235.3	670.6
$LSD_{0.05}$	***24.3***	***45.7***	***18.1***	***42.4***	***45.7***	***49.5***

Note: Treatment effects were significant for NDF ($P < 0.01$), ADL ($P = 0.001$) and cellulose ($P < 0.05$) under macro-mineral fertilization; for NDF ($P < 0.001$), ADL ($P < 0.001$), hemicellulose ($P < 0.01$) and cellulose ($P < 0.05$) under Panamin Agro; and for NDF ($P < 0.001$), ADL ($P < 0.01$) and hemicellulose ($P = 0.01$) under Blago products. Year effects were significant for selected parameters ($P \leq 0.05$).

Blago products

The Blago treatments produced the strongest improvement in the fibre profile of the meadow. The combined Blago 5 at 4.5 L ha^{-1} + Blago at 1.0 L ha^{-1} treatment resulted in the lowest NDF (571.6 g kg^{-1} DM), ADF (282.5 g kg^{-1} DM), ADL (47.2 g kg^{-1} DM) and cellulose (235.3 g kg^{-1} DM), together with the highest numerical IVDMD (670.6 g kg^{-1} DM).

Relative to the corresponding control, NDF decreased by 13.9%, ADF by 30.6%, ADL by 34.2% and cellulose by 29.9%, while IVDMD increased by 19.6%.

The treatment effect was statistically significant for NDF ($P < 0.001$), ADL ($P < 0.01$) and hemicellulose ($P = 0.01$), whereas the effect on IVDMD was not statistically significant. Therefore, the high IVDMD value represents a strong numerical improvement in forage quality, but it should not be described as a statistically confirmed treatment effect.

The stronger response of the combined Blago treatment may be related to its combined nutrient and organic-matter inputs. Nevertheless, because several chemical constituents differed among Blago, Blago 3 and Blago 5, the present experiment does not allow the individual contribution of a particular component to be separated.

Nardus stricta pasture

The *Nardus stricta* pasture generally exhibited larger reductions in fibre fractions than the meadow, particularly under the biological fertilizer treatments.

Macro-mineral fertilization

Macro-mineral fertilization significantly affected NDF ($P < 0.001$), ADF ($P < 0.001$), cellulose ($P < 0.01$) and IVDMD ($P < 0.001$). The highest N–P–Ca treatment reduced NDF from 704.9 g kg^{-1} DM in the control to 618.1 g kg^{-1} DM and ADF from 426.5 to 341.8 g kg^{-1} DM.

IVDMD increased from 553.6 to 626.8 g kg^{-1} DM, corresponding to a numerical increase of 13.2%. The reduction in NDF and ADF indicates a less fibrous forage profile under the highest nutrient rate.

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ADL declined numerically from 107.6 to 72.4 g kg^{-1} DM, but the treatment effect was not statistically significant. Therefore, the numerical reduction in ADL should be interpreted cautiously.

The stronger response of the *Nardus stricta* pasture compared with the meadow is consistent with its lower initial nutrient availability and strongly acidic soil conditions. Nutrient addition may have alleviated part of the nutrient limitation and altered biomass development, although changes in soil nutrient status were not measured directly in this experiment.

The response indicates that nutrient addition improved plant growth conditions and reduced the accumulation of highly structural tissues typically associated with slow-growing species such as *Nardus stricta* (Iliev et al., 2022)

Table 2. Structural fibre fractions and in vitro dry matter digestibility (IVDMD) of forage from the *Nardus stricta* pasture under macro-mineral and biological fertilization, mean values for 2021–2023 (g kg^{-1} DM)

Fertilization treatment	NDF	ADF	ADL	Hemicellulose	Cellulose	IVDMD
Macro-mineral fertilization						
Control	704.9	426.5	107.6	278.4	318.9	553.6
$P_6N_5Ca_{2.8}$	689.4	400.0	76.5	289.4	323.5	575.1
$P_6N_{10}Ca_{5.6}$	643.3	377.0	61.5	266.3	315.5	602.6
$P_6N_{15}Ca_{8.3}$	618.1	341.8	72.4	276.3	269.4	626.8
$LSD_{0.05}$	***46.1***	***27.3***	***36.6***	***53.2***	***47.3***	***19.1***
Panamin Agro						
Control	706.8	437.1	66.4	269.7	370.7	549.9
Panamin Agro – 1.5 kg ha^{-1}	614.1	269.8	76.1	298.0	256.7	655.8
Panamin Agro – 3.0 kg ha^{-1}	634.4	329.3	77.9	271.8	261.3	632.4
Panamin Agro – 4.5 kg ha^{-1}	662.6	332.4	76.0	303.5	263.0	630.7
$LSD_{0.05}$	***90.5***	***41.2***	***35.9***	***97.7***	***45.7***	***73.8***
Blago products						
Control	711.5	433.9	89.0	277.5	345.0	552.7
Blago 5 – 1.5 L ha^{-1}	631.7	373.0	78.1	258.7	294.9	600.1
Blago 3 – 3.0 L ha^{-1}	619.3	358.3	70.0	261.0	288.3	612.2
Blago 5 – 4.5 L ha^{-1} + Blago – 1.0 L ha^{-1}	595.2	263.0	80.1	332.2	182.9	679.2
$LSD_{0.05}$	***54.8***	***66.5***	***40.1***	***25.0***	***95.1***	***39.0***

Note: Treatment effects were significant for NDF ($P < 0.001$), ADF ($P < 0.001$), cellulose ($P < 0.01$) and IVDMD ($P < 0.001$) under macro-mineral fertilization; for ADF ($P < 0.01$) and cellulose ($P < 0.001$) under Panamin Agro; and for NDF ($P = 0.01$), ADF ($P = 0.01$), hemicellulose ($P < 0.05$) and IVDMD ($P < 0.01$) under Blago products. Year effects were significant for selected parameters ($P \leq 0.05$).

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Panamin Agro

Panamin Agro significantly affected ADF ($P < 0.01$) and cellulose ($P < 0.001$). The lowest application rate, 1.5 kg ha^{-1}, produced the lowest ADF (269.8 g kg^{-1} DM) and NDF (614.1 g kg^{-1} DM) and the highest IVDMD (655.8 g kg^{-1} DM) among the Panamin Agro treatments.

Compared with the control, NDF decreased by 13.1%, ADF by 38.3% and cellulose by 30.7%, while IVDMD increased by 19.3%. Increasing the application rate did not produce further improvement in these parameters.

The response was therefore clearly dose-dependent. The lowest rate produced the strongest numerical improvement in forage quality, paralleling the botanical response in which the lowest rate produced the largest increase in legume participation.

Blago products

The combined Blago 5 at 4.5 L ha^{-1} + Blago at 1.0 L ha^{-1} treatment produced the strongest improvement in forage quality in the *Nardus stricta* pasture. NDF decreased from 711.5 to 595.2 g kg^{-1} DM, ADF from 433.9 to 263.0 g kg^{-1} DM, and cellulose from 345.0 to 182.9 g kg^{-1} DM. IVDMD increased from 552.7 to 679.2 g kg^{-1} DM.

These changes correspond to reductions of 16.3% in NDF, 39.4% in ADF and 47.0% in cellulose, together with a 22.9% increase in IVDMD.

Treatment effects were significant for NDF ($P = 0.01$), ADF ($P = 0.01$), hemicellulose ($P < 0.05$) and IVDMD ($P < 0.01$), whereas the effect on ADL was not significant. The strong reduction in ADF and cellulose together with the increase in IVDMD indicates a substantial improvement in the measured forage-quality characteristics.

Comparative interpretation of forage quality

The forage-quality response differed between the two grassland types and among fertilizer products. In the meadow, the largest numerical improvement in IVDMD was obtained with the combined Blago treatment (670.6 g kg^{-1} DM), followed by Panamin Agro at 3.0 kg ha^{-1} (657.1 g kg^{-1} DM). In the *Nardus stricta* pasture, the combined Blago treatment again produced the highest IVDMD (679.2 g kg^{-1} DM), while Panamin Agro at 1.5 kg ha^{-1} produced 655.8 g kg^{-1} DM.

The combined Blago treatment therefore produced the highest IVDMD in both grassland types. It also resulted in some of the lowest NDF, ADF and cellulose concentrations. This consistent response identifies the combined Blago application as the most promising treatment in the present experiment for improving the measured forage-quality characteristics.

However, the results should not be interpreted as evidence that all biological fertilizers are superior to mineral fertilization under all conditions. Panamin Agro showed a strong response at different rates, and macro-mineral fertilization was particularly effective in increasing grass participation and improving several fibre characteristics. Rather, the data indicate that fertilizer source and application rate should be matched to the initial ecological conditions and management objectives of the grassland.

The statistical results also demonstrate that year affected several forage-quality parameters. This confirms that climatic variation among growing seasons contributed to differences in forage composition. Therefore, treatment effects should be interpreted within the context of year-to-year environmental variability.

Overall, the results support a differentiated management approach. Where the principal objective is to increase the contribution of grasses, macro-mineral fertilization was effective. Where improvement of forage quality and greater participation of legumes are also desired, selected biological fertilizer treatments, particularly the combined Blago 5 + Blago application, showed greater potential in the present study.

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Conclusions

Fertilization altered both botanical composition and forage quality in the two investigated mountain grasslands, but the response depended on grassland type, fertilizer product and application rate.

In the *Agrostis capillaris–Festuca fallax* meadow, macro-mineral N–P–Ca fertilization primarily increased the contribution of grasses and reduced the relative contribution of forbs. The highest N–P–Ca rate increased grass participation from 60.0% to 79.1% in the corresponding control comparison, whereas the legume proportion at this rate was only 4.1%. In contrast, Panamin Agro and Blago treatments generally produced greater increases in legume participation. The highest legume proportion in the meadow, 19.3%, was recorded with Panamin Agro at 1.5 kg ha^{-1}, while the combined Blago 5 + Blago treatment increased legumes to 14.8%.

In the *Nardus stricta* pasture, fertilization increased the contribution of grasses and reduced the relative contribution of forbs in most treatments. Panamin Agro at 1.5 kg ha^{-1} produced the highest legume proportion within the Panamin Agro experiment (27.4%), whereas the combined Blago 5 + Blago treatment increased legumes from 2.0% to 10.6% and reduced forbs from 34.7% to 16.1%. These responses demonstrate that the effect of fertilization on botanical composition was strongly dependent on the initial sward and fertilizer formulation.

The fertilizer treatments also modified forage fibre characteristics. In the meadow, the combined Blago 5 at 4.5 L ha^{-1} + Blago at 1.0 L ha^{-1} treatment produced the lowest NDF, ADF, ADL and cellulose concentrations and the highest numerical IVDMD (670.6 g kg^{-1} DM). In the

Nardus stricta pasture, the same combined treatment again produced the lowest NDF, ADF and cellulose concentrations and the highest IVDMD (679.2 g kg^{-1} DM). Relative to the corresponding controls, IVDMD increased by 19.6% in the meadow and 22.9% in the pasture.

Among the tested biological fertilizers, the combined Blago 5 + Blago treatment produced the most consistent improvement in the measured forage-quality characteristics in both grassland types. Panamin Agro also improved fibre characteristics, but its most favourable numerical response differed between the two ecosystems: 3.0 kg ha^{-1} in the meadow and 1.5 kg ha^{-1} in the *Nardus stricta* pasture.

The results indicate that fertilizer selection should be based on the ecological characteristics and management objectives of the grassland. Macro-mineral fertilization was particularly effective for increasing grass participation, whereas selected biological fertilizer treatments, especially the combined Blago 5 + Blago application, showed greater potential for improving forage digestibility and several structural fibre characteristics while also increasing legume participation in the sward.

Overall, the study demonstrates that the management of nutrient-limited mountain grasslands requires consideration of both productivity and botanical composition. The differentiated responses of the two grassland types indicate that fertilizer rate and product formulation are important determinants of the resulting botanical and forage-quality characteristics.

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Uticaj makromineralnog i biološkog đubrenja na botanički sastav i kvalitet prirodnih travnjaka na planinama centralnog Balkana

Tatyana Bozhanska · Biser Bozhanski

Sažetak: Cilj ovog istraživanja bio je da se procene efekti mineralnih i bioloških đubriva na botanički sastav, strukturne karakteristike vlakana i *in vitro* svarljivost suve materije (IVDMD) krmne biomase iz dve planinske travnate zajednice: livade sa vrstama *Agrostis capillaris* i *Festuca fallax*, kao i pašnjaka sa vrstom *Nardus stricta*. Mineralno đubrenje sastojalo se od primene kalcijum-amonijum-nitrata u kombinaciji sa fosforom, dok su biološki tretmani uključivali preparat *Panamin Agro*, kao i proizvode dobijene od sapropela – *Blago*, *Blago 3* i *Blago 5*. Botanički sastav je izražen kao udeo trava, leguminoza i ostalih zeljastih biljaka u ukupnoj svežoj biomasi. Mineralno đubrenje je povećalo udeo trava u oba tipa travnatih zajednica, pri čemu je najizraženiji efekat zabeležen na livadi, gde je primena najveće doze N–P–Ca dovela do povećanja udela trava sa 60,0% na 79,1%. Biološka đubriva su uglavnom povećala zastupljenost leguminoza i smanjila udeo ostalih zeljastih biljaka, naročito pri primeni preparata *Blag** i najniže doze preparata *Panamin Agro*. Đubrenje je takođe izmenilo nekoliko parametara kvaliteta krme. U oba tipa travnatih zajednica, kombinovana primena preparata *Blago 5* i *Blago* rezultirala je najnižim koncentracijama NDF-a, ADF-a i celuloze,

kao i najvišim numeričkim vrednostima IVDMD-a. U poređenju sa odgovarajućim kontrolnim varijantama, IVDMD je povećan za 19,6% na livadi i za 22,9% na pašnjaku. Rezultati pokazuju da su efekti đubrenja zavisili od tipa travnate zajednice, vrste đubriva i primenjene doze. Mineralno đubrenje je prvenstveno podstaklo razvoj trava, dok su odabrani biološki tretmani – posebno kombinovana primena *Blago 5* i *Blago* – doveli do šireg spektra promena, uključujući veću zastupljenost leguminoza, smanjen udeo ostalih zeljastih biljaka i poboljšanje merenih karakteristika kvaliteta krme.

Ključne reči: *Agrostis–Festuca*, *Nardus stricta*, biostimulatori, botanički sastav, kvalitet krme, planinske travnate zajednice, održivo đubrenje

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