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AGRONOMIC POTENTIAL OF LEGUMES AND GRASS MIXTURES FOR RIPARIAN GRASSLANDS RENOVATION - FORAGE YIELD AND QUALITY

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Abstract

The objective of this study was to evaluate the forage yield and chemical composition of six variants including two alfalfa cultivars, a bird's-foot trefoil cultivar, and three grass mixtures selected as valuable biological material for potential riparian grasslands' renovation. The experiments were carried out on plots in Dragomiresti village located near Dambovita River riparian area. One level of fertilization (120 g m^{-2}) was considered for each variant. The plant material had high concentrations of dry matter, crude protein, neutral detergent fiber, and phosphorus, respectively. All the fertilized samples exhibited higher concentrations of dry matter, nitrates, and phosphorus than the corresponding non-fertilized samples. High concentrations of water-soluble carbohydrates were determined for alfalfa which showed also the smallest amount of neutral detergent fiber. The maximum ADF content was found in the fertilized Valahia alfalfa cultivar and the non-fertilized complex grass mixture. Regarding the potential for riparian grassland renovation, we can conclude that the Valahia alfalfa cultivar and the complex grass mixture with five species performed better in terms of forage yield and overall quality.

Key words: *alfalfa, bird's-foot trefoil, grass mixtures, Dry matter, ADF, NDF, crude protein, renovation.*

INTRODUCTION

Assessing grassland productivity depends on a good knowledge of the interactions of environmental factors, vegetation type and properties, and human activity (Dunea and Dincă, 2014).
Vegetation settled spontaneously on many grasslands, being quite diverse due to their dynamic nature, having seasonal fluctuations in the dynamics of the species that make up that floristic composition, which generates various aspects during the vegetation period, depending on the phenology of the plants (Peter et al., 2008).
Annually, phenological changes occur in the vegetation of natural grasslands, depending on weather conditions, flowering periods, and other phenological phases, but a series of essential characteristics to define the phytocenosis, for example: the structure of the canopy, the floristic composition, the efficiency rates are more or less constant (Gosse et al., 1987).
The predominant vegetation of natural grasslands consists mostly of perennial plants. They are associated in complex functional groups that give grasslands specific features both regarding the relationships between the component species and between them and the biotope in which they are formed. Perennial species also show specific traits regarding economic value, improvement possibilities, and multifunctional use.
To address the new challenges arising from climate change and increasingly significant human disturbances, an increasing number of studies have been conducted on topics such as biodiversity conservation, grassland restoration, as well as the sustainable use and management

of grassland resources and the enhancement of their multifunctional role.
Among the 38 major themes identified by Zhao (2023), ecology and environment themes such as biodiversity conservation, land use and soil erosion, climate change, and paleoenvironment have developed rapidly. The increase in biodiversity conservation ranking during the study period (from 1900-) was the fastest.
The ranking of technology-related topics such as remote sensing and numerical modeling has also increased in recent decades. The ranks of traditional themes (e.g., biological nitrogen fixation, grazing, plant nutrition, germplasm and breeding, forage cultivation, and animal production) showed a sharp downward trend.
However, due to the climate variability impact on floristic composition, new experiments should be performed to support grassland renovation for pastoral value improvements (Dincă and Dunea, 2018).
The nutrient and biologically active content of forage is strongly related to animal health and welfare, thus having an impact on people's nutrition. The conversion of fodder into livestock production is one of the main agricultural production processes, and the problems of the rational utilization of fodder in animal nutrition acquire a key value (Cosman, 2018).
Various forages differ according to their technological properties, botanical composition, content of nutrients, and influence on the animal body.
The effectiveness of the feed will be higher the more it corresponds to the requirements of the domestic animals, in terms of physico-mechanical properties and content of nutrients, in agreement with their productive genetic potential (Cosman, 2018).
Apart from ensuring the traditional indices of food rations with protein, fat, cellulose, ash, calcium, phosphorus, carotene, the ratio between energy and protein, sugar and protein, the amount and correlation of amino acids, the amount and correlation of microelements, as well as of the vitamins are of great importance. In many cases, the evidence of these indices is decisive for increasing the assimilation of the essential nutritional elements of the basic forage (Cosman, 2018).
Increasing resilience through the renovation of the riparian grassland is one of the measures of implementing the direction of action "*Assessing the vulnerability of species and ecosystems to the effects of climate change*", sustaining the objective "*Supporting the development of a coherent, connected and representative network of protected areas which implements the adaptive management*" of The National Strategy on Adaptation to Climate Change for the period 2022-2030 with the perspective of 2050.
In this direction, the experimental approaches have considered the location of a field experiment for testing some mixtures of grasses, but also some valuable legumes in pure culture, in the riparian area or immediately close to the Dâmbovița River, monitoring the established pilot areas through in situ assessments, but also at the hydrographic basin level by using remote sensing means (case study - Argeș Hydrographic Basin).
This paper aims to characterize the agronomic potential of grass mixtures (three types of various complexity) and legumes (two alfalfa cultivars and 1 cultivar of bird's-foot trefoil) for riparian grassland renovation based on forage yield and quality.
The degree of novelty of our study lies in the fact that the renovation of riparian grasslands is not commonly studied despite the multifunctional valences of these areas and the selected biological material considered for screening is novel.

MATERIALS AND METHODS

The experiments were carried out on plots in Dragomiresti village located near Dambovita River riparian area, (Lat. N44°53'7.40", Long. E 25°24'6.89"). The soil type is luvic brown. For potential grassland renovation in riparian areas, we have selected species that almost naturally occur when proper conditions are met (Table 1). Alfalfa was considered with two valuable cultivars: Valahia - V1, a new Romanian variety (Dincă et al., 2022), and Italian Pomposa - V2, often cultivated by the farmers. Another legume was the bird's-foot trefoil (Leo cultivar) - V3. The grass mixtures were considered with three degrees of complexity: perennial ryegrass and red fescue - V4, perennial ryegrass, tall fescue, and smooth meadow-grass - V5, and red fescue, perennial ryegrass, smooth meadow-grass, tall fescue, and annual ryegrass - V6. One level of fertilization (120 g m^{-2}) was considered for each variant i.e., 16% (N) total nitrogen, 4.7% (N) nitric nitrogen, 11.3% (N) ammoniacal nitrogen, 5.2% (P_2O_5) phosphorus pentaoxide soluble in neutral ammonium citrate solution and water, 3.4% (P_2O_5) soluble phosphorus pentaoxide, 5.2% (K_2O) soluble potassium oxide, 3% (MgO) total magnesium oxide, 26% (SO_3) total sulfur trioxide, 4% (Fe) total iron.

Consequently, 36 plots were grouped into 6 randomized blocks, 3 fertilized and 3 unfertilized with 3 repetitions (6 × 2 × 3). The sowing was performed on 24 March 2023. Sampling of the vegetal material was performed on 27 June 2023 (94 DAS - days after sowing) using a quadrat of 50 × 50 cm in two points of each parcel on the diagonal. The harvested material was dried in an oven to assess the dry matter content.

Table 1. Biological material used in the experiment (percentage of participation in the seeding material)

V1	
MEDICAGO SATIVA VALAHIA	100%
V2	
MEDICAGO SATIVA POMPOSA	100%
V3	
LOTUS CORNICULATUS - LEO	100%
V4	
LOLIUM PERENNE 1	45%
FESTUCA RUBRA	25%
LOLIUM PERENNE 2	30%
V5	
FESTUCA ARUNDINACEA 1	37%
POA PRATENSIS	9%
LOLIUM PERENNE	9%
FESTUCA ARUNDINACEA 2	37%
FESTUCA ARUNDINACEA 3	8%
V6	
FESTUCA RUBRA	10%
LOLIUM PERENNE 1	25%
LOLIUM PERENNE 2	10%
LOLIUM PERENNE 3	5%
POA PRATENSIS	8%
FESTUCA ARUNDINACEA 1	3,90%
FESTUCA ARUNDINACEA 2	1,10%
LOLIUM MULTIFLORUM 1	4,60%
LOLIUM MULTIFLORUM 2	32,40%

The fresh samples were dried in an oven (SLW 53, POL-EKO, Poland) at 60°C for 72 h. The dried samples were then ground using a vibratory disc mill (Retsch RS 200, Germany) and sifted through a 1-mm mesh sieve.

The dry matter (DM) content was determined by drying the samples at 105°C for 3 h, until the constant mass. The ash content was measured by calcination at 550°C in a lab furnace (STC 411.06, Sweden). The crude protein (CP) content was calculated by multiplying the nitrogen content determined using the Kjeldahl method by 6.25. Water-soluble carbohydrates (WSC) were analyzed using the method with 3,5-dinitrosalicylic acid, measuring the absorbance of the solution at 540 nm (UV/VIS spectrophotometer UV 1720). Acid detergent fiber (ADF) was determined according to Van Soest et al. (1991) and ISO 13906:2008. Neutral detergent fiber (NDF) was determined using heat-stable amylase and sodium sulfite (Van Soest et al., 1991).

The nitrates content of the samples was determined spectrophotometrically by measuring the intensity of the color of the yellow-orange nitroderivatives resulting from the reaction of NO_3^- with phenol 2,4-disulfonic acid at 470 nm. The phosphorus content was calculated by multiplying with 0.436 the amount of the phosphoric anhydride determined spectrophotometrically, based on the formation of a colored complex with the ammonium molybdate, whose absorbance was read at 720 nm.

All the chemical assays were performed in triplicate and the results were expressed on DM basis.

The data were reported as the mean ± standard deviation and analyzed using SPSS 26.0 (SPSS, USA) for descriptive, comparative, and associative analyses.

RESULTS AND DISCUSSIONS

The **dry matter** (DM) is an important characteristic of the forage quality. It includes the macronutrients (i.e., proteins, carbohydrates, fat), but also the micronutrients (i.e., minerals, vitamins) and the compounds important in animal digestion (i.e., fibers). The preservation of the forage is strongly related to the amount of dry matter.

Figure 1 shows the dry matter accumulation at 94 DAS both for non-fertilized and fertilized variants. The dry matter content ranged between 95.2 g m^{-2} (V5NF) and 594.2 g m^{-2} (V6F). All the fertilized samples exhibited higher quantities of dry matter than the non-fertilized ones except the bird's foot trefoil (V3). It can be emphasized that within the tested variants, higher amounts of dry matter were determined in the fertilized samples V1F, V2F, V4F, V5F, and V6F compared to the non-fertilized ones.

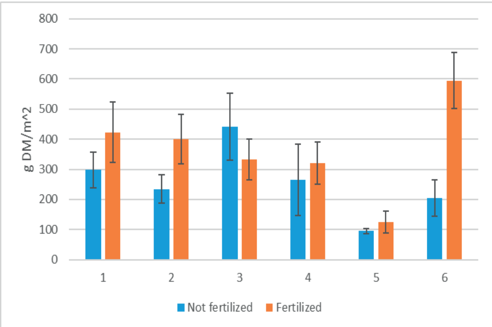


Figure 1. Dry matter accumulation for each variant at 94 DAS

Alfalfa cultivars and the V6 grass mixture presented significant DM gains following the fertilization.
The general image of the chemical data reveals differences between samples both in terms of the plant species and applied treatment (fertilized vs. non-fertilized).
The dry matter content of the experimental samples (Figure 2) ranged between 219.35 g/kg FW (V5NF) and 224.97 g/kg FW (V5F). Practically, the fertilization treatment had a higher influence on the dry matter content of the sample V5, determining the increase of this parameter with 2.56%. In the fertilized samples, an increase of the dry matter content was also determined in V6 with 1.56% comparatively with the non-treated variant.
The **ash** of the samples (Figure 2) was different within the group of the samples, being significantly greater in V4F (122.59 g/kg DM), while V6NF was situated to the inferior limit of the determined values (103.93 g/kg DM) for this parameter. If we discuss the fertilized samples *vs*. the unfertilized ones in terms of their mineral content, it can be remarked that all the experimental samples, except V1, had a smaller amount of ash than their corresponding fertilized variants. This aspect is important in the context in which no nutritional benefit to livestock is brought by high levels of ash content. The concentration of ash in the green mass (*Lotus corniculatus* 'Doru') was high at the third cut (10.66% DM), the smallest value, of 9.14% DM, being determined at the fourth cut (Cosman et al., 2020).
The ash content of the first-cut alfalfa cultivar Banat harvested at the initial flowering stage and wilted 24 h, until a dry matter of 419.9 g/kg, was 86.48 g/kg DM (Đorđević et al., 2016).

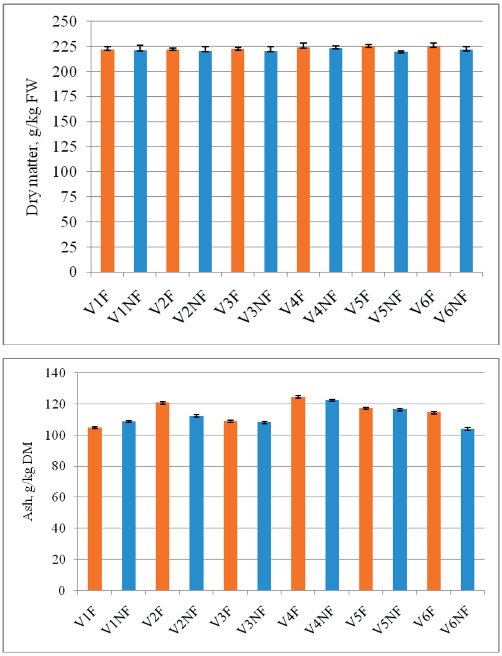


Figure 2. Dry matter content (a) and ash content (b) for each variant

The nutritive value of white clover (*Trifolium repens* L.) and Kentucky bluegrass (*Poa pratensis* L.) was evaluated by (Stojanović et al., 2022). The authors reported a significant effect of the consecutive cuts during the spring growing season on their quality. The DM of the white clover was 18.07% at the first cut, respectively by 20.93% at the third cut. The dry matter content of *Poa pratensis* ranged between 21.61% (cut I) and 22.42% (cut III), the value determined at the second cut being 22.43%.

The ash content of the same species ranged between 8.97% (first cut) and 8.70% (third cut) in the case of the legume, respectively between 7.12% (first cut) and 6.84% (third cut) for the grass herbage.

Forage nutritive value is directly related to the content of essential nutrients (McDonald et al., 2021), and being affected by different factors. It was reported that it depends on the year of cultivation, but also, even if they are cultivated in the same field, similar plants can exhibit varying nutritive values.

The quality of proteins is determined by their amino-acid composition. Alfalfa does not require nitrogen fertilization (Dien et al., 2006), its leaves being a valuable supplemental protein feed for livestock.

Excepting the V5 sample, in all the non-fertilized samples, higher quantities of **crude protein** (Figure 3) were determined than in the corresponding fertilized ones. The crude protein content ranged between 83.76 g/kg DM (V6F) and 190.61 g/kg DM (V4NF). Increased values of this nutrient, by 61.57% in V3NF than in V3F, respectively by 46.62% in V6NF than in V6F, were determined.

The crude protein content of the freshly chopped alfalfa wilted to 300 and 400 g DM/kg fresh weight, respectively, was determined by Zhang et al. (2021), recording values of 256 and 264 g/kg of DM respectively. Li et al. (2021) evaluated the chemical composition of fresh alfalfa with about 30% DM and determined an average value of 247 g CP/kg DM. According to Đorđević et al. (2016), the crude protein content of the first-cut alfalfa cultivar Banat harvested at the initial flowering stage and wilted 24-h was only 165.94 g/kg DM.

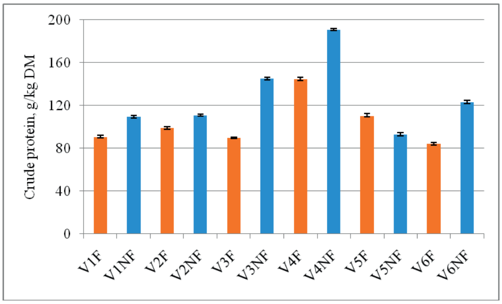
The CP concentration in cuts of two alfalfa and grasses as pure cultures and legume-grass mixtures was found from 65 to 199 g/kg DM (McDonald et al., 2021). Referring to this parameter, there was no interaction between species treatments and fertilizer applications in all cuts of two years.

The CP values for white clover and Kentucky bluegrass significantly decreased during the spring growing season (Stojanović et al., 2022). For clover, the CP content decreased from 26.25% DM (first cut) to 18.91% DM (third cut). CP of the second harvest of grass herbage, of 8.51% DM, was lower compared with the first and third ones.

An important factor that determines the quality and the feeding value of the forage is its content of sugar and starch as easily assimilable energy sources.

Alfalfa is typically difficult to ensile due to a low **water-soluble carbohydrates** (WSC) (Figure 3) concentration at harvest (Li et al., 2021). The concentration of WSC was only 18.37 g/kg DM in V6F, while an amount of 1.94 times higher was determined in the V6NF variant. Within the experiment, V1NF, V2NF, and V3NF exhibited a content of WSC greater than 50 g/kg DM. The WSC content of the variants without fertilization was higher comparatively with those of the fertilized samples, except the V5NF sample, as it has been determined for CP content.

The concentration of WSC was 45.9 and 46.2 g/kg of DM respectively in fresh chopped alfalfa wilted to 300 and 400 g dry matter/kg fresh weight (Zhang et al., 2021). An average value of 62.5 g WSC/kg DM was determined by Li et al. (2021) before the ensiling of fresh alfalfa wilted naturally to 312 g DM/kg FW.



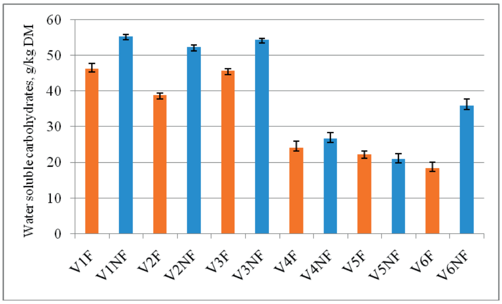


Figure 3. Crude protein content and water-soluble carbohydrates for each variant

The chemical composition in various alfalfa varieties, namely six species commonly found in northern China, was significantly different (Jianyu et al., 2023). The wilted forages were characterized by a dry matter content from 463.66 to 513.53 g/kg FM. The crude protein content ranged between 166.62 and 222.34 g/kg DM, while the WSC was found within the interval 38.37 and 66.06 g/kg DM.

Three forage species, namely alfalfa, reed canarygrass, and switchgrass were evaluated at different maturity stages by Dien et al. (2006) for their bioconversion potential as energy crops. The compositional analysis revealed that the concentration of ash and protein declined with the maturity of all three herbaceous species. Thus, the ash content of alfalfa bud was 81 g/kg DM, while the full flower showed 58 g/kg DM. The crude protein content ranged between 127 g/kg DM (alfalfa bud) and 88 g/kg DM (alfalfa full flower). The vegetative reed canarygrass had the highest concentration of crude protein, 88 g/kg DM, with this concentration decreasing to 45 g/kg DM in ripe seeds. The ash content of vegetative reed canarygrass was the highest of all the three analyzed biomass species, with 128 g/kg DM. The pre-boot switchgrass contained 65 g crude protein/kg DM and 89 g ash/kg DM.

The quality of the green mass of bird's-foot-trefoil cv. Doru varied depending on the harvest time (Cosman et al., 2020), as follows: crude protein 143.5-194.5 g/kg DM, soluble sugars 37.5-61.0 g/kg DM, and phosphorus 2.2-3.3 g/kg DM. It was reported that the concentration of CP in the green mass was high after the second harvest and very low after the fourth harvest.

The fiber composition of forage influenced to a large extent its biological value (Bozhanska, 2020). Cellulose plays an important physiological role not only as a source of energy but also as a factor that ensures the normalization of the digestive processes (Cosman, 2018). Determination of the cell wall polysaccharides is of vital importance when animal nutrition is discussed. Numerous methods for the determination of different fractions (cellulose, hemicelluloses, lignin) are known, the detergent fiber system being widely applied.

The **neutral detergent fiber** (NDF) content of the experimental samples ranged between 250.51 g/kg DM (V1NF) and 377.65 g/kg DM (V4NF) (Figure 4). The **acid detergent fiber** (ADF) recorded the smallest value, 168.64 g/kg DM for the sample V2NF, while the highest amount (312.99 g/kg DM) was determined also in a sample without fertilization, namely V6NF (Figure 4). Values close to this upper limit of ADF content were determined in V1F, too.

The non-fertilized variants V3, V4, and V6 exhibited higher amounts of NDF and ADF than the corresponding fertilized samples, respectively. The acid detergent fiber (ADF) of the freshly chopped alfalfa wilted to two dry matter contents of approximately 300 and 400 g/kg fresh weight recorded values of 220 and 293 g/kg of DM respectively (Zhang et al., 2021). Average values of 222 g ADF/kg DM and 296 g NDF/kg DM were determined by Li et al. (2021) through analysis of the chemical composition of the fresh alfalfa wilted naturally to about 30% DM, while You et al. (2022) determined a percent of acid detergent fiber by 31.1% of DM in alfalfa with 47.8% DM and 41.6% of DM neutral detergent fiber.

The ADF and NDF content of the first-cut alfalfa cultivar Banat harvested at the initial flowering stage and wilted 24 h were 351.78 g/kg DM and 408.61 g/kg DM, respectively (Đorđević et al., 2016).

The ADF and NDF content of the six samples of alfalfa wilted in the field for 12 h ranged between 257.24 and 345.87 g/kg DM, and 364.79 and 419.94 g/kg DM respectively (Jianyu et al., 2023).

The concentration of ADF in cuts of two alfalfa and grasses as pure cultures and legume-grass mixtures recorded values of 309 g/kg DM (roundup ready RR tonica alfalfa) - 431 g/kg DM (conventional alfalfa + tall fescue), while the NDF concentration ranged between 460 g/kg DM (low-lignin Hi-Gest 360 LL alfalfa) and 614 g/kg DM (smooth bromegrass) (McDonald et al., 2021). The authors established that among the legume-grass mixtures and alfalfa pure cultures were no differences in ADF. Also, in all cuts, no significant effect of nitrogen application on ADF concentration was determined. On the contrary, nitrogen fertilizer application significantly increased the NDF (McDonald et al., 2021).

Testing two biofertilizers (Lumbrical and Lumbrex) on a grassland with bird's-foot-trefoil of 'Leo' cultivar (Bozhanska, 2020) determined a higher amount of NDF and ADF in the dry feed mass in the variants with soil treatment compared to foliar. The average values reported were 404.11 g/kg DM for NDF and 289.18 g/kg DM for ADF, respectively.

An increase in the fiber content (NDF and ADF) of white clover and Kentucky bluegrass was observed by Stojanović et al. (2022) during the spring grazing period. Thus, in the second cut, the highest content of NDF was determined with 36.06% in legume and 60.61% respectively in grass herbage. In the same cut, the ADF content was 29.03% (*Trifolium repens* L.) and 36.57% (*Poa pratensis* L.).

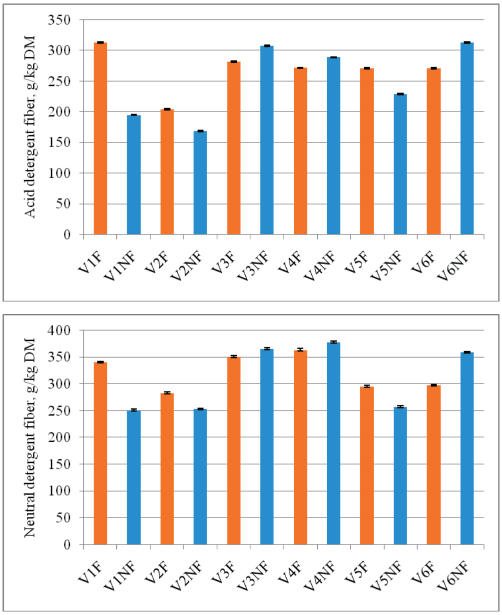


Figure 4. ADF and NDF content for each variant

The potential of the Romanian cultivars of tall fescue *Festuca arundinacea* 'Adela', 'Brio', and 'Măgurele 5' respectively for the restoration of degraded permanent grasslands in the Republic of Moldova was reported by Titei et al. (2019). The study of the quality of the freshly harvested biomass revealed that its dry matter contained 114-136 g/kg CP, 582-593 g/kg NDF and 392-396 g/kg ADF. The crude ash content ranged between 74 and 89 g/kg DM.

Nitrate is a naturally occurring form of nitrogen (Santamaria, 2006) that is formed from different sources, including fertilizers. The content of nitrate can vary within species and cultivars, the plants having different capacities to accumulate nitrate. This plant feature seemed to be correlated with the nitrate reductase activity, as well as to the degree of nitrate absorption and transfer in the plant (Santamaria, 2006).

The increasing use of synthetic nitrogen fertilizers and livestock manure in intensive agriculture raises the issue of the potential high content of nitrate in plants. Nitrate *per se* is relatively non-toxic, but part of the ingested nitrate is converted into the more toxic nitrite.

Because the nitrate metabolites are responsible for several health effects, the maximum levels for nitrates in foodstuffs (i.e., green leaf vegetables and baby food) are settled by the Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food.

The nitrate content was by far the highest in the sample V2, both in fertilized and non-fertilized variants. This parameter ranged in large limits within the set of samples, from 11.45 mg/kg DM (V5NF) to 110.93 mg/kg DM (V2F). Mean values were determined for V1F, V1NF, and V3F respectively, while the other samples contained smaller amounts of nitrates at the moment of the plant harvesting. In all the experimental samples, higher quantities of nitrates were determined in the fertilized samples compared to the non-fertilized ones.

Phosphorus fertilization increases plant yield, so it is compulsory to know the changes in the chemical composition of plants as they are affected by the level of fertilization. The content of phosphorus in samples ranged in rather lower limits compared to nitrates, i.e., between 215.89 mg/kg DM (V3NF) and 250.18 mg/kg DM (V4F), respectively. The amount of phosphorus in fertilized variants was 12.86% higher in V3F and only 3.32% higher in V5F. In all the experimental samples, higher quantities of phosphorus were determined in the fertilized samples compared to the non-fertilized ones. Fertilizer treatments with different ammonium nitrate doses increased the nitrogen and phosphorus content of three commonly cultivated grass varieties (*Lolium perenne* TOPGUN, *Festuca rubra* SERGEİ, and *Poa pratensis* AVALANCHE) (Alkan et al., 2022). The authors showed that the grasses absorb the phosphorus contents of the soil as a result of nitrogen-containing fertilization.

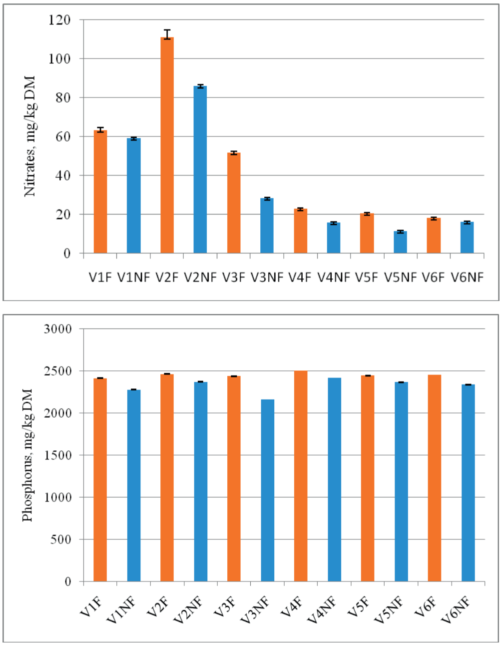


Figure 5. Nitrates and phosphorous content for each variant

A great impact of two growth stimulants (Ti and amino acids) on the chemical composition of *Poa pratensis*, including the phosphorus content, was also reported by Radkowski et al. (2022). Thus, compared to the control plants, the weighted average of P content ranged from 1.586 to 2.424 g/kg DM. A significant correlation between the dry matter yield and the P content was established.

The correlation between the crude protein content and the remanent concentration of the chemicals involved in increasing the plants' yield is mentioned below (Figure 6). The graphical representation emphasizes that a high amount of phosphorus is generally inversely correlated with crude protein concentration. The sample V4 (with and without fertilization) exhibited both high content of phosphorus and CP. If the nitrates level is discussed, the data scattering is large in the interval 0-40 mg nitrates/kg DM. Concentration of nitrates higher than 40 mg/kg DM led to average and small values of crude protein content.

The cumulative box plots of macronutrients (CP and WSC) and fibers (NDF and ADF) (Figure 7) emphasize the influence of fertilization on their concentration within the group of the experimental variants.

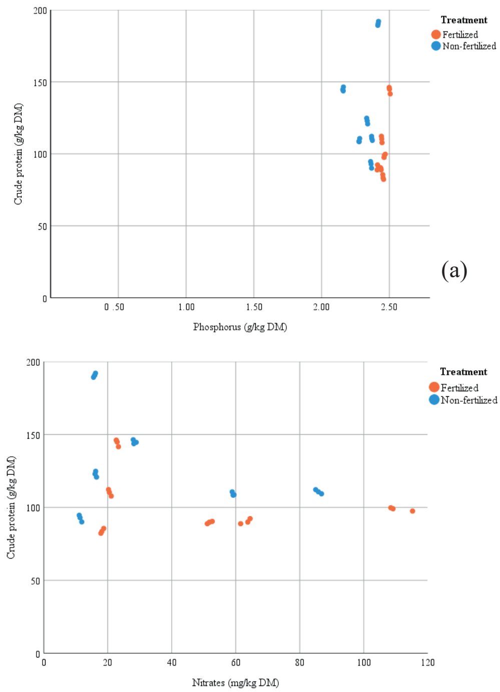


Figure 6. Scatter plots of the crude protein content and phosphorus (a), and nitrates (b)

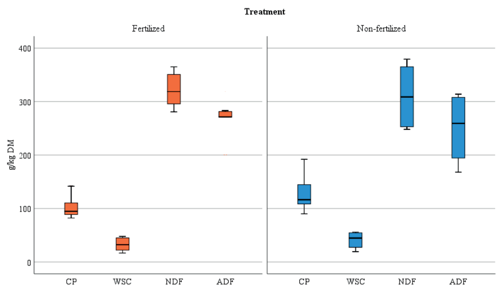


Figure 7. Box plots of CP, WSC, NDF, and ADF in experiments with and without fertilization

Thus, at the first cut of the plants, the fertilization type and level applied seemed to have a positive effect on the CP and WSC levels only in the case of V5, constituted from perennial ryegrass, tall fescue, and smooth meadow grass. To deepen the knowledge referring to this, a supplementary analysis is needed. A possible explanation is that at the moment of harvesting the entire process of transformation of the monomers (i.e. amino acids and monosaccharides respectively) under the action of the corresponding enzymes was not completed.

A different situation may emerge if the neutral and acid fibers are discussed. The fertilization treatment led to an increasing amount of fibers both in alfalfa cultivars and V5. On the contrary, V3, V4, and V6 exhibited a higher content of fiber in samples without fertilization (Figure 7).

One-way ANOVA analysis was applied to establish if the applied treatment determined significant effects on the dependent variables. The plant fertilization produced significant effects on the dependent variables i.e., DM, CP, and phosphorus.

Table 2. Pearson correlation coefficient values and type of association for the analyzed parameters; *correlation is significant at the 0.05 level (2-tailed); **correlation is significant at the 0.01 level (2-tailed)

-	Dry matter	Ash	CP	WSC	NDF	ADF	Nitrates	Phosphorus
Dry matter	1	0.245	0.034	-0.311	0.142	0.161	-0.132	**0.374***
Ash	-	1	**0.379***	**-0.612****	-0.029	-0.260	-0.073	**0.574****
CP	-	-	1	-0.058	**0.580****	0.267	**-0.345***	-0.184
WSC	-	-	-	1	-0.070	-0.210	**0.597****	**-0.610****
NDF	-	-	-	-	1	**0.855****	**-0.404***	0.011
ADF	-	-	-	-	-	1	**-0.589****	-0.050
Nitrates	-	-	-	-	-	-	1	0.094
Phosphorus	-	-	-	-	-	-	-	1

The correlation analysis (Table 2) shows an expected direct and strong correlation between NDF and ADF. An indirect correlation was established between WSC (a nutritional component) and ash content (that influences negatively animal nutrition), while between ash content and phosphorus, it was underlined also a moderate but positive correlation.

Weak and indirect correlations were established between (CP and nitrates) and (NDF and nitrates), respectively.

The Principal Component Analysis (PCA) was used to analyze the data for the dependent factors (Dunea et al., 2015). The relevant factor loadings (0.55) were considered for each component and the component relationships were plotted in Figure 8.

Table 3. Factor loadings (Varimax normalized) using the principal component extraction

Factor	Eigenvalue	Cum var (%)	DM	Ash	CP	WSC	NDF	ADF	NO3	P
Component 1	3.08	38.57	**0.789**	**0.688**	-0.091	**-0.855**	-0.014	0.032	-0.230	**0.889**
Component 2	2.35	67.96	0.286	-0.277	0.370	-0.223	**0.855**	**0.971**	**-0.713**	-0.126
Component 3	1.14	82.20	-0.011	0.634	**0.901**	-0.120	0.269	-0.093	-0.179	-0.107

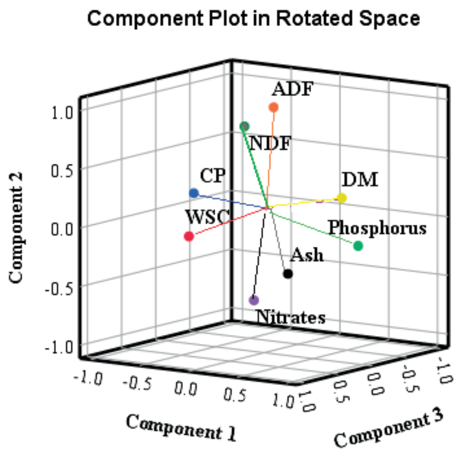


Figure 8. Relationships of components in PCA

Three PCs were extracted, which accounted for 82.20% of the total variance. PC1 explained 38.57% of the total variance, being formed by DM, ash, and phosphorus in its positive part, respectively WSC in its negative part. PC2 was highly positively associated with NDF and ADF and negatively with the nitrates concentration, while PC3 contained only the crude protein content (Table 3).

Hierarchical Cluster analysis allowed the grouping of the investigated parameters of the experimental samples into homogeneous

groups based on their common characteristics. This analysis intended to find out if, in the set of the eight investigated indicators of the forage, there are identifiable groups with similar characteristics.
The method used in clustering was the Ward method on variables of type interval, by applying the Squared Euclidean distance (Figure 9). The Hierarchical Cluster Analysis proved to be statistically significant at a significance threshold of 5%.

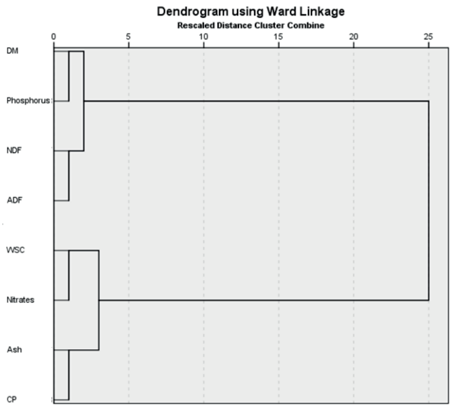


Figure 9. Dendogram of forage quality variables

The affiliation of each parameter to a certain cluster in the first stage of clustering was the forwards. Cluster 1: nitrates and WSC; Cluster 2: DM and phosphorus. In the second stage, ash aggregated to cluster 1 and NDF to cluster 2. CP, respectively ADF were involved in the third stage of clustering led to distinct two clusters.

The analysis of the classification of the indicators of forage quality based on their values determined in all 12 samples shows an optimum number of two classes. These clearly demarcate the forage macronutrients (CP, WSC), the nitrates potential involved in their increasing concentration, and the ash on the one part and dry matter, fibers, and the other compound by interest in fertilization (phosphorus), respectively.

Regarding the potential of tested biological material for riparian grassland renovation, we can conclude that the V1 (Valahia alfalfa cultivar) and V6 (the complex grass mixture with five species) variants performed better in terms of forage yield and overall quality, and thus further experiments should consider screenings of V1-V6 mixtures with various participations of alfalfa and grasses, and optimized levels of fertilization.

CONCLUSIONS

The forages must fulfill increased requirements, primarily regarding the concentration of nutrients and biologically active substances. Increased requirements are addressed not only to the amount of nutrients in forage but also to their quality and accessibility for the animal body. The nutrients' requirements can differ according to the degree of their utilization by various species of animals, and their physiological status.
According to our results, the nutritive value of grass mixtures was higher than legumes pure cultures. The stage of harvesting and the type of cultivar/type of plant mixtures should be carefully analyzed in relationship with the proposed fertilization scheme.
Further research will be conducted to extend the characterization of the plant material by determination of other chemical constituents and the digestibility of nutrients. The next experiments will consider legumes and grass mixtures with various participations based on the significance of the current results. Also, the correlation between the doses of fertilizers applied and the plant metabolism in terms of parameters of interest (i.e., for animal nutrition) will be on focus.

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