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Article

Research on the Influence of Competition between *Festuca arundinacea* Schreb. and *Trifolium pratense* L., Grown in Simple Mixtures, on the Quality of the Fodder Obtained

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Abstract: The aim of the research carried out between 2021 and 2023 was to analyse the influence of competitiveness between *Festuca arundinacea* Schreb. and *Trifolium pratense* L., cultivated in simple mixtures, on the quality of the fodder obtained, under the conditions of the northern Romanian forest steppe. In the experiment organized at the Ezăreni Student Research and Practice Station of Iasi University for Life Sciences, two factors were studied, namely the cultivation system used, with five graduations: a₁ - *Festuca arundinacea* Schreb. (100 % - control); a₂ - *Festuca arundinacea* Schreb. (75 %) and *Trifolium pratense* L. (25 %); a₃ - *Festuca arundinacea* Schreb. (50 %) and *Trifolium pratense* L. (50 %); a₄ - *Festuca arundinacea* Schreb. (25 %) and *Trifolium pratense* L. (75 %); a₅ - *Trifolium pratense* L. (100 %) and mineral fertilization with 5 graduations, respectively: b₁ - unfertilized (control), b₂ - N₅₀P₅₀; b₃ - N₇₅P₇₅; b₄ - N₁₀₀P₁₀₀; b₅ - N₁₅₀P₁₅₀. The obtained results showed that the process of interspecific competitiveness was greatly influenced by the percentage of participation in the sowing norm of the species in the mixture, the biological peculiarities of the species under study, as well as the climatic conditions specific to each agricultural year.

Keywords: crude protein production; RYT index (relative yield total); CR index (competition rate); NDF (neutral detergent fibre); ADF (acid detergent fibre); relative forage quality (RFQ)

1. Introduction

Temporary grassland is grassland established for a fixed period of time and is located in the place of degraded permanent grassland or on areas of arable land intended for the fodder base, using a mixture of perennial forage species adapted to the area and the established use. The contribution of these grasslands to the balance of the forage base is very important, as higher and better-quality forage yields are obtained compared to permanent grassland, thus explaining the current worldwide trend towards the expansion of temporary grassland.

An important technological part in the establishment of a sown grassland is the establishment of the mixture structure, which influences the density and uniformity of the crop, the achievement of an optimal ratio between the component species (especially between grasses and legumes), the choice of the mode of utilization (grassland, animal grazing, mixed use), the duration of use, but also the energy-protein ratio and the quality of the fodder.

The competitive ability of species is also taken into account when determining the relationship between species, as they behave differently at the establishment of the vegetal canopy and during the exploitation of the grassland. There is a multitude of intraspecific and interspecific relationships between species in a grassland's vegetal canopy, permanent competition for vegetation factors, which are important elements in the evolution of the structure of the grassland. From an agronomic point of view, the highest degree of competition is for natural resources, namely water, light, space and nutrients. The combination of forage perennial grasses and perennial forage legumes has a positive

effect on the consumability and quality of forage, as well as on the accessibility of certain soil nutrients for the two groups of species, compared to growing them separately [1–5].

Complex mixtures may be better adapted than simple mixtures to variable climatic conditions, with important effects on dry matter (DM) production and its distribution over the growing season, thus increasing the sustainability of forage production [6,7].

Adaptation to climate change includes an increase in the range of plant species used in sown grasslands, the creation of well-adapted cultivars, and the correct choice of these in mixtures is an important management tool to control the stability and productivity of temporary grasslands under specific climatic conditions [7–10].

Festuca arundinacea Schreb. and *Trifolium sp.* are two species that interact strongly with each other, influencing each other's growth, development and success in the shared habitat [5].

Each species competes for essential resources, such as light, water, nutrients and space, and specialized research has shown how these resources are distributed between the two species and how this distribution influences their performance and physiological response. Competition between tall fescue and clover can influence the composition and structure of plant communities in a given habitat [5].

Worldwide, *Festuca arundinacea* Schreb. is one of the most common perennial meadow grass species in the floristic composition of temporary grasslands used for grazing or mowing [11–13]. This species is almost absent in meadow mixtures used in Switzerland, France, the Netherlands and other European countries, as well as in the USA.

The overlapping of ecological niches of species from different botanical families is attributed to the sharing of one or more ecological resources. This leads to the establishment of different interspecific trophic relationships between species in an ecosystem: predator-prey, parasitism, commensalism, amensalism, protocoeperation, mutualism, but also competition, with effects on productivity and quality of the achieved biomass [14,15]. Interspecific competition determines which species and how many species can coexist within the same community, affects population dynamics, alters species and community structure [16–19].

Maintaining the productive potential of temporary grasslands at the highest possible level is achieved by using valuable species when they are established, by applying fertilizers according to their needs and by sustainable management [20–23].

In the technology of growing mixtures of perennial forage grasses and legumes, with a high legume share, the application of different mineral fertilizer systems, compared to grass-only crops, does not always lead to significant yield increases. This is more evident in the case of nitrogen-based mineral fertilizers, which can negatively influence the atmospheric nitrogen fixation process.

The quality of any forage is determined by the analysis of internationally standardized quality indicators relevant to the characterization of a forage, being influenced by the component species, their proportion, the time of use by mowing or grazing, the level of fertilization, but also by the growing conditions [24–37].

Laboratory analysis measures the total nitrogen content (T_N) of the feed and calculates the crude protein. Relative feed quality (RFQ) is an indicator based on the summative energy equation to estimate the digestibility of nutrients contributing to energy and dry matter intake as a function of the NDF and ADF content of the feed [25–29]. RFQ has a similar range of values as relative feed value (RFV) (80 to 200 points), but in contrast to this, the quality classes of grass forages are better.

Studies on the influence of competition between *Festuca arundinacea* Schreb. and *Trifolium pratense* L. on the quality of the fodder obtained are particularly important for agriculture and animal husbandry, as fodder quality is essential for the nutrition of farm animals and can directly influence their health and performance [5,7,13].

A number of studies have been conducted to assess how competition between crop species, growing conditions affect forage digestibility, taste, palatability, as well as the content of crude fibre, protein, essential amino acids and other important nutrient components [4,5,9,14,20].

A balanced ratio between grasses and perennial legumes on grassland gives the fodder obtained an optimal quality and content of mineral elements, which have positive effects on animals [17,19,23].

Our research aimed to analyse the effect of competition between *Festuca arundinacea* Schreb. and *Trifolium pratense* L., cultivated in simple mixtures, on the quality of forage obtained under the conditions of the northern Romanian forest-steppe.

2. Materials and Methods

The research conducted between 2021-2023 aimed to analyse the influence of the competition between *Festuca arundinacea* Schreb. and *Trifolium pratense* L., cultivated in simple mixtures, on the quality of the fodder obtained, manifested in the content (CP) and the quantity of crude protein achieved (Q_{CP}) and the fodder content in NDF and ADF.

In order to achieve the proposed objectives, an experiment was organized in the experimental field at the Ezăreni Student Research and Practice Station of Iasi University of Life Sciences, in the spring of 2021, following the two-factor subdivided plots method, 5×5, in 3 repetitions, with the size of a 4 m × 3 m plot (12 m²).

The studied factors were: factor A – The species or mixture of perennial grasses and legumes, with 5 graduations: a₁ - *Festuca arundinacea* Schreb. (100%); a₂ - *Festuca arundinacea* Schreb. (75%) + *Trifolium pratense* L. (25%); a₃ - *Festuca arundinacea* Schreb. (50%) + *Trifolium pratense* L. (50%); a₄ - *Festuca arundinacea* Schreb. (25%) + *Trifolium pratense* L. (75%); a₅ - *Trifolium pratense* L. (100%), and factor B - fertilization with mineral fertilizers, with 5 graduations: b₁ - unfertilized; b₂ - N₅₀P₅₀; b₃ - N₇₅P₇₅; b₄ - N₁₀₀P₁₀₀; b₅ - N₁₅₀P₁₅₀. For the productivity indicators, the control was represented by the a₁b₁ (i.e. *Festuca arundinacea* Schreb. (100%), unfertilized), and for the competitiveness indicators, the control was represented by the experience average.

The biological materials used were the species *Festuca arundinacea* Schreb. (tall fescue) – Vio Jucu variety, approved in 2012, developed at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania, and *Trifolium pratense* L. (red clover) – David Liv variety, approved in 2015, developed at the Livada Agricultural Research and Development Station in Romania.

The crude protein content (CP) was calculated using Equation 1.

$$CP = T_N \times 6.25 (\%), \text{ where:} \quad (1)$$

T_N - total nitrogen content (%).

The total nitrogen content (T_N) was determined using the Kjeldahl method.

The total quantity of crude protein (Q_{CP}) was calculated using Equation 2.

$$Q_{CP} = Q_{DM} \times CP (\text{kg-ha}^{-1}), \text{ where:} \quad (2)$$

Q_{DM} - amount of dry matter (kg-ha⁻¹).

The quantity of dry matter (Q_{DM}) was calculated using Equation 3.

$$Q_{DM} = Q_{GM} \times DM (\text{kg-ha}^{-1}), \text{ where:} \quad (3)$$

Q_{GM} - quantity of green mass obtained (kg-ha⁻¹); DM - dry matter content of green mass (%).

The quantity of green mass obtained (Q_{GM}) was determined by weighing the green mass production obtained on the harvestable area of 6 m² of each experimental plot in the three replications, and then determined in proportion to the hectare.

The dry matter content of the green mass (DM) was determined by oven-drying the average green mass samples for 3 hours at 105 °C.

The neutral detergent fibre (NDF) content of the plant was determined by the Van Soest method, the extracted fractions being hemicellulose, cellulose and lignin [24].

The acid detergent fibre (ADF) content of the plant was determined by the Van Soest method, the extracted fractions being cellulose and lignin [24].

Calculation of Relative Forage Quality (RFQ) was performed according to Equation 4 [25–29]:

$$RFQ = (DMI \times TDN) \times 1.23^{-1}, \text{ where:} \quad (4)$$

DMI - dry matter intake (%); TDN - total digestible nutrients (%)

The dry matter intake (DMI) was calculated using Equation 5.

$$\text{DMI} = 120 \times \text{NDF}^{-1} (\%) \quad (5)$$

Total digestible nutrient content (TDN) was calculated using Equation 6.

$$\text{TDN} = 4.898 + 89.796 \times \text{NEL} (\%), \text{ where:} \quad (6)$$

NEL - net energy for lactation (Mcal·kg⁻¹).

Net energy for lactation (NEL) was calculated using Equation 7.

$$\text{NEL} = 1.085 - 0.0124 \times \text{ADF (Mcal/kg)}. \quad (7)$$

The calculation of the Relative Yield Total (RYT) index characterizes the species used in mixtures as regards the ecological resources used, one in relation to the other, and was done using Equation 8 [38]. Depending on the result obtained, the following three situations can be encountered: RYT > 1, the species occupy different eco-niches; RYT = 1, the species use common resources; RYT < 1, the species are in antagonistic relationship.

$$\text{RYT} = Y_{AB} \times Y_{AA}^{-1} + Y_{BA} \times Y_{BB}^{-1}, \text{ where:}$$

$$Y_{AB} = Q_{CP} \text{ for species A, mixed culture; } Y_{AA} = Q_{CP} \text{ for species A, in pure culture; } Y_{BA} = \frac{Q_{CP}}{Q_{CP}} \text{ for species B, mixed culture; } Y_{BB} = Q_{CP} \text{ for species B, in pure culture.} \quad (8)$$

The calculation of the Competition Rate (CR) index characterizes the species used in mixtures as regards their mutual competitiveness and was done using Equation 9 [38]. Depending on the result obtained, the following three situations can be encountered: CR > 1, species A is more competitive than species B; CR = 1, the species are equally competitive; CR < 1, species A is less competitive than species B.

$$\text{CR} = (Y_{AB} \times Y_{AA}^{-1} \times Z_{AB}) \times (Y_{BA} \times Y_{BB}^{-1} \times Z_{BA})^{-1}, \text{ where:}$$

$$Y_{AB} = Q_{CP} \text{ for species A, mixed culture; } Y_{AA} = Q_{CP} \text{ for species A, in pure culture; } Z_{AB} = \frac{\text{proportion of species A and B in mixture}}{Q_{CP}} \text{ for species B, mixed culture; } Y_{BB} = Q_{CP} \text{ for species B, in pure culture; } Z_{BA} = \frac{\text{proportion of species B and A in mixture}}{Q_{CP}}. \quad (9)$$

The obtained data were statistically interpreted by analysis of variance (ANOVA test) and calculation of limited-slip differential (LSD).

In the area where the research was carried out, in terms of climatic conditions, the agricultural period 2021-2023 may be characterized as less favourable for the exploitation of temporary grassland consisting of mixtures of perennial forage grass and legume species. The total amount of precipitation was lower than the multiannual average and it was unevenly distributed, with periods of water stress in October 2022 and March 2023 and from May to June 2023, as shown in Figure 1.

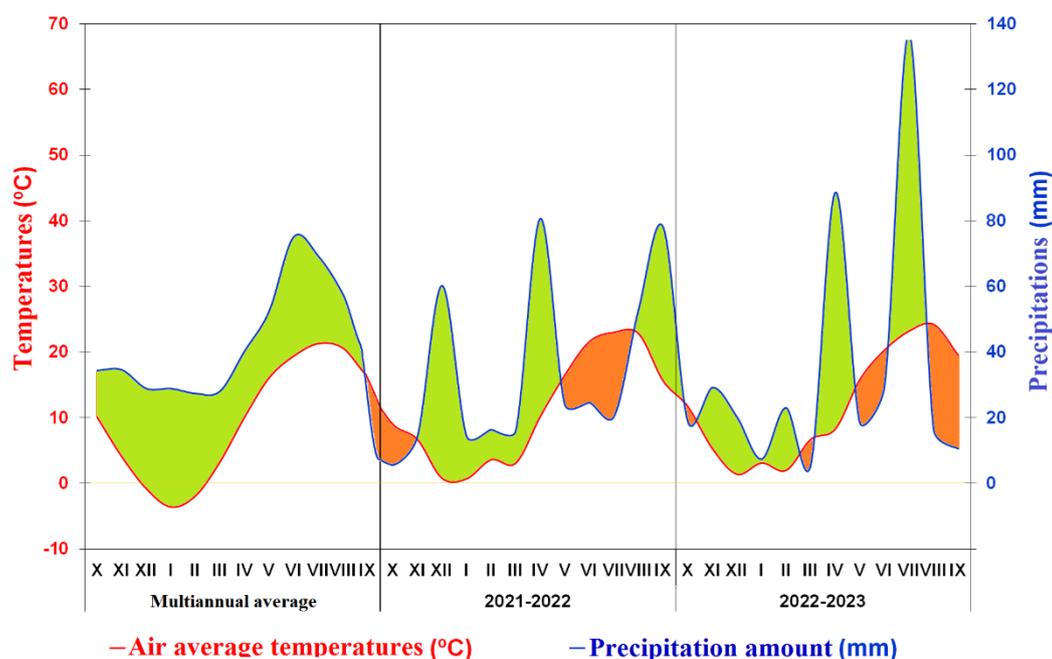


Figure 1. Climadiagram for the agricultural period 2021-2023.

3. Results

3.1. Crude Protein - Content (CP) and Quantity (Q_{CP})

3.1.1. Forage Content in CP in the Second Year of Vegetation

At the Ezăreni Research Station in Iași County, in the agricultural year 2021-2022 (year II of meadow vegetation), in the species *Festuca arundinacea* Schreb. and *Trifolium pratense* L. grown singly or in simple mixtures formed between them, under different fertilization conditions with nitrogen and phosphorus-based mineral fertilizers, the crude protein (CP) content of the obtained forage was analysed. The results obtained, as regards the influence of the interaction between the species or mixture of perennial grasses and legumes and fertilization with mineral fertilizers, showed that the values obtained ranged from 11.74% for variant a₁b₁, *Festuca arundinacea* Schreb. (100%), non-fertilized (control), to 17.01% for variant a₅b₅, *Trifolium pratense* L. (100%), fertilized with N₁₅₀P₁₅₀.

Depending on the species grown alone or the mixture of the two species, in different percentages, the highest values of crude protein content were obtained when *Trifolium pratense* L. was present. Irrespective of the species or mixture of perennial grasses and legumes grown, fertilization with nitrogen and phosphorus mineral fertilizers resulted in higher protein content in the feed.

With the exception of variant a₁b₂, for this parameter, the positive differences obtained in comparison with the control variant were statistically assured, being highly significant for the variants in which *Trifolium pratense* L. was present, regardless of whether or not it was fertilized with mineral fertilizers.

3.1.2. Forage Content in CP in the Third Year of Vegetation

In the agricultural year 2022-2023 (the third year of grassland vegetation), the results obtained, as regards the influence of the interaction between the species or mixture of perennial grasses and legumes and fertilization with mineral fertilizers, showed that the values obtained ranged from 11.83% for variant a₁b₁, *Festuca arundinacea* Schreb. (100%), unfertilized (control variant) and 17.83% for variant a₅b₄, *Trifolium pratense* L. (100%), fertilized with N₁₀₀P₁₀₀ (Table 1).

Table 1. Influence of the interaction between the cropping system used and NP fertilization on the crude protein content and the amount of crude protein achieved.

Variant	CP content (%)		Q _{CP} (Kg-ha) ⁻¹			
	II nd year	III rd year	II nd year	III rd year	Average	
a1 - <i>F.a.</i> (100%)	b ₁ - unfertilized	11.74 ^C	11.83 ^C	212.3 ^{Mt}	333.2 ^{Mt}	272.7 ^{Mt}
	b ₂ - N ₅₀ P ₅₀	12.41	12.29	331.9*	351.3	341.6
	b ₃ - N ₇₅ P ₇₅	12.51*	12.32	623.3***	370.7	497.0**
	b ₄ - N ₁₀₀ P ₁₀₀	12.51*	12.38	674.5***	391.1	532.8**
	b ₅ - N ₁₅₀ P ₁₅₀	12.94**	13.01*	710.4***	447.7	579.0***
a2 - <i>F.a.</i> (75%) + <i>T.p.</i> (25%)	b ₁ - unfertilized	13.57***	13.56***	560.5***	491.8	526.1**
	b ₂ - N ₅₀ P ₅₀	13.71***	13.99***	607.2***	511.0	559.1***
	b ₃ - N ₇₅ P ₇₅	13.83***	13.83***	764.0***	536.8**	650.4***
	b ₄ - N ₁₀₀ P ₁₀₀	14.11***	13.87***	774.1***	594.9**	684.5***
	b ₅ - N ₁₅₀ P ₁₅₀	14.23***	14.14***	868.0***	647.4**	757.7***
a3 - <i>F.a.</i> (50%) + <i>T.p.</i> (50%)	b ₁ - unfertilized	13.99***	14.42***	626.0***	612.6**	619.3***
	b ₂ - N ₅₀ P ₅₀	14.14***	14.39***	688.7***	671.2***	680.0***
	b ₃ - N ₇₅ P ₇₅	14.41***	14.70***	753.4***	661.6***	707.5***
	b ₄ - N ₁₀₀ P ₁₀₀	14.85***	15.02***	901.3***	779.9***	840.6***
	b ₅ - N ₁₅₀ P ₁₅₀	15.09***	15.10***	980.0***	833.9***	906.9***
a4 - <i>F.a.</i> (25%) + <i>T.p.</i> (75%)	b ₁ - unfertilized	14.71***	16.28***	731.0***	762.9***	746.9***
	b ₂ - N ₅₀ P ₅₀	15.02***	16.81***	792.9***	819.8***	806.3***
	b ₃ - N ₇₅ P ₇₅	15.08***	16.76***	917.6***	866.8***	892.2***
	b ₄ - N ₁₀₀ P ₁₀₀	15.19***	16.77***	1007.4***	988.2***	997.8***
	b ₅ - N ₁₅₀ P ₁₅₀	15.43***	16.89***	1100.2***	1022.6***	1061.4***
a5 - <i>T.p.</i> (100%)	b ₁ - unfertilized	15.72***	17.00***	1184.3***	558.7**	871.5***
	b ₂ - N ₅₀ P ₅₀	16.06***	17.43***	1189.6***	585.5**	887.5***
	b ₃ - N ₇₅ P ₇₅	16.69***	17.42***	1345.3***	641.0**	993.1***
	b ₄ - N ₁₀₀ P ₁₀₀	16.95***	17.83***	1283.5***	770.3***	1026.9***
	b ₅ - N ₁₅₀ P ₁₅₀	17.01***	17.82***	1252.2***	816.0***	1034.1***
<i>LSD 0.5</i>	0.76	0.83	82.9	106.6	94.7	
<i>LSD 0.1</i>	1.02	1.10	147.4	189.4	168.4	
<i>LSD 0.01</i>	1.33	1.44	249.9	321.3	285.6	

Depending on the species grown alone or the mixture of the two species, in different percentages, the highest values of crude protein content, as in the previous year, were obtained when *Trifolium pratense* L. was present in the crop.

Irrespective of the species or mix of perennial grasses and legumes grown, fertilization with nitrogen and phosphorus mineral fertilizers resulted in a higher crude protein content in the forage.

With the exception of the variants where only *Festuca arundinacea* Schreb. was present, for this parameter, the positive differences obtained compared to the control variant were highly significant for the variants where *Trifolium pratense* L. was present, regardless of whether or not mineral fertilizers were applied.

3.1.3. Amount of Crude Protein Achieved (Q)_{CP}

Analyses of the influence of the interaction between the species or mixture used and fertilization with mineral fertilizers based on nitrogen and phosphorus on the amount of crude protein achieved (Q_{CP}) showed that the average values obtained in the period 2021-2023 ranged between 272.7 kg-ha⁻¹ for the control variant a₁b₁, *Festuca arundinacea* Schreb. (100%), unfertilized, and 1061.4 kg-ha⁻¹ for variant a₄b₅, *Festuca arundinacea* Schreb. (25%) and *Trifolium pratense* L. (75%) mixture, fertilized with N₁₅₀P₁₅₀ (Table 1).

Depending on the species grown alone or the mixture of the two species, in different percentages, the highest values of crude protein were obtained when *Trifolium pratense* L. was present.

In the second year of vegetation, when *Trifolium pratense* L. was 100% present in the structure of the vegetal canopy, the values obtained were 5.5-6.3 times higher than in the control.

In the third year of vegetation, the highest values were obtained in the mixture *Festuca arundinacea* Schreb. (25%) and *Trifolium pratense* L. (75%), being 2.3-3.0 times higher than in the control.

Irrespective of the species or mix of perennial grasses and legumes grown, fertilization with nitrogen and phosphorus-based mineral fertilizers resulted in higher amounts of crude protein.

With the exception of a₁b₂, for this parameter, the positive differences obtained compared to the control were distinct and highly significant for all other variants, regardless of whether or not mineral fertilizer was applied.

3.2. Competition between *Festuca arundinacea* Schreb. and *Trifolium pratense* L.

3.2.1. Species Competitiveness in the Second Vegetation Year (Table 2)

Analysing the influence of the interaction between the mixture used and fertilization with nitrogen and phosphorus mineral fertilizers on the interspecific relationships in the second year of vegetation, it was found that the RYT index recorded values <1 in all the studied mixture variants. This result indicates that *Festuca arundinacea* Schreb. and *Trifolium pratense* L. are in competition and fight for the same resources to reveal their productive potential. In the second year of vegetation the plants of the *Trifolium pratense* L. species are at the maximum of their productive potential, they have a well-developed underground part and explore deeper soil layers, their symbiotic mechanism is well developed, therefore they are more competitive. Under these conditions, the main vegetation factors are valorised differently than the mixture species.

Table 2. Influence of the interaction between the cropping system used and NP fertilization on RYT and CR indicators.

Variant	II nd year			III rd year			
	RYT	CR <i>F.a.</i>	CR <i>T.p.</i>	RYT	CR <i>F.a.</i>	CR <i>T.p.</i>	
Average (Control)	0.74^c	1.26^c	13.30^c	1.53^c	6.55^c	1.54^c	
a ₂ - <i>F.a.</i> (75%) + <i>T.p.</i> (25%)	b ₁ - unfertilized	0.59 ^{oo}	2.78 ^{***}	0.36 ^{oo}	1.10 ^{oo}	16.59 ^{***}	0.06 ^{oo}
	b ₂ - N ₅₀ P ₅₀	0.60 ^{oo}	2.22 ^{**}	0.45 ^{oo}	1.16 ^{oo}	14.70 ^{***}	0.07 ^{oo}
	b ₃ - N ₇₅ P ₇₅	0.63 ^{oo}	2.17 [*]	0.46 ^{oo}	1.16 ^{oo}	17.01 ^{***}	0.06 ^{oo}
	b ₄ - N ₁₀₀ P ₁₀₀	0.69	3.30 ^{***}	0.30 ^{oo}	1.15 ^{oo}	18.45 ^{***}	0.05 ^{oo}
	b ₅ - N ₁₅₀ P ₁₅₀	0.85 ^{**}	6.74 ^{***}	0.15 ^{oo}	1.13 ^{oo}	17.00 ^{***}	0.06 ^{oo}
a ₃ - <i>F.a.</i> (50%) + <i>T.p.</i> (50%)	b ₁ - unfertilized	0.64 ^{oo}	0.26 ^{oo}	3.89 ^{oo}	1.54	2.45 ^{oo}	0.41 ^o
	b ₂ - N ₅₀ P ₅₀	0.68	0.25 ^{oo}	4.06 ^{oo}	1.62	2.66 ^{oo}	0.38 ^o
	b ₃ - N ₇₅ P ₇₅	0.63 ^{oo}	0.27 ^{oo}	3.70 ^{oo}	1.48	2.49 ^{oo}	0.40 ^o
	b ₄ - N ₁₀₀ P ₁₀₀	0.79	0.30 ^{oo}	3.36 ^{oo}	1.59	2.87 ^{oo}	0.35 ^o
	b ₅ - N ₁₅₀ P ₁₅₀	0.92 ^{***}	0.51 ^o	1.96 ^{oo}	1.53	2.80 ^{oo}	0.36 ^o
a ₄ - <i>F.a.</i> (25%) + <i>T.p.</i> (75%)	b ₁ - unfertilized	0.79	0.04 ^{oo}	25.04 ^{***}	1.87 ^{**}	0.22 ^{oo}	4.52 ^{***}

b ₂ - N ₅₀ P ₅₀	0.77	0.03 ^{oo}	37.58***	1.90**	0.21 ^{ooo}	4.65***
b ₃ - N ₇₅ P ₇₅	0.76	0.03 ^{oo}	39.68***	1.88**	0.23 ^{ooo}	4.43***
b ₄ - N ₁₀₀ P ₁₀₀	0.87**	0.03 ^{oo}	36.20***	1.97**	0.27 ^{ooo}	3.72***
b ₅ - N ₁₅₀ P ₁₅₀	0.95***	0.02 ^{oo}	42.28***	1.85**	0.28 ^{ooo}	3.62***
LSD 0.5	3.23	0.24	1.59	0.99	3.23	0.24
LSD 0.1	4.37	0.32	2.15	1.34	4.37	0.32
LSD 0.01	5.87	0.43	2.92	1.79	5.87	0.43

In the second vegetation year, the CR index for *Festuca arundinacea* Schreb. was higher than for *Trifolium pratense* L. only in 75% rate of participation in the mixture, regardless of the fertilization variant, *Festuca arundinacea* Schreb. being more competitive in this case. The variants in which *Festuca arundinacea* Schreb. had low and very low values of the CR index were those in which the percentage of participation was 50% and 25%. In these cases, *Festuca arundinacea* Schreb. was considered as poorly competitive.

As observed, the values of the CR index for *Trifolium pratense* L. were lower only in the variants with 25% participation, which shows weaker competitiveness compared to *Festuca arundinacea* Schreb., because of the reduced number of individuals. The variants in which *Trifolium pratense* L. showed high and very high values of the CR index were those in which the percentage of participation was 50% and 75%. In these mixtures, *Trifolium pratense* L. was considered highly competitive.

It is observed that when the two species had equal percentages in the mixture (50%+50%), the RC index values showed higher competitiveness of *Trifolium pratense* L., which benefited from high vigour starting the second year of vegetation.

3.2.2. Species Competitiveness in the Third Vegetation Year (Table 2)

The analysis of the influence of the interaction between the mixture used and fertilization with nitrogen and phosphorus mineral fertilizers on the interspecific relationships in the third year of vegetation revealed that the RYT index values recorded were >1, totally different from the previous year. This result indicates that *Festuca arundinacea* Schreb. and *Trifolium pratense* L. occupy different ecological niches, have sufficient resources through low clover production and do not compete fiercely for vegetation factors. It should also be considered that in *Trifolium pratense* L. the number of shoots (and total DM production) is declining, due to the genetic potential of the species, i.e. its low species vivacity and the strong influence of climatic stress on red clover. This is also in contrast to the fact that *Festuca arundinacea* Schreb. plants are better developed, with high vitality and tolerance to climatic stress factors.

In the third vegetation year, the CR index for *Festuca arundinacea* Schreb. was higher than for *Trifolium pratense* L. at a percentage of participation in the mixture of at least 50%, regardless of the fertilization variant, in which case the species was more competitive. The variants in which *Festuca arundinacea* Schreb. recorded low values of the CR index were only when the percentage of participation was 25%, which was different from the previous year. In this case the species was considered poorly competitive.

The CR index values for *Trifolium pratense* L. were lower in the variants where it had a 25 - 50% participation, which indicates lower competitiveness compared to *Festuca arundinacea* Schreb. The variants where *Trifolium pratense* L. had higher CR index values were only in the cases of 75% participation rate, in which case the species was considered more competitive, due to the higher number of individuals.

3.3. NDF and ADF Content and Relative Quality (RFQ) of the Obtained Forage.

3.3.1. NDF and ADF Content and Relative Quality of the Forage Obtained in the Second Year of Vegetation (Table 3)

In the second year of vegetation, the NDF content in the forage obtained ranged from 40.37% in the unfertilized *Trifolium pratense* L. (100%) to 68.02% in the *Festuca arundinacea* Schreb. (100%) fertilized with N₁₅₀P₁₅₀.

Table 3. Influence of the interaction between the species or mixture of perennial grasses and legumes and mineral fertilization on forage NDF, ADF content and RFQ value.

Variant		NDF content (%)		ADF content (%)		RFQ	
		II nd year	III rd year	II nd year	III rd year	II nd year	III rd year
a ₁ - <i>F.a.</i> (100%)	b ₁ - unfertilized	64.53 ^c	56.21 ^c	39.73 ^c	36.12 ^c	87.8 ^c	107.8 ^c
	b ₂ - N ₅₀ P ₅₀	64.96	57.68 [*]	39.70	39.07 ^{***}	87.3	99.5 ^{oo}
	b ₃ - N ₇₅ P ₇₅	64.57	58.81 ^{***}	40.32 [*]	39.94 ^{***}	86.8	96.0 ^{ooo}
	b ₄ - N ₁₀₀ P ₁₀₀	66.95 ^{***}	59.59 ^{***}	43.15 ^{***}	40.57 ^{***}	79.1 ^{oo}	93.6 ^{ooo}
	b ₅ - N ₁₅₀ P ₁₅₀	68.02 ^{***}	59.82 ^{***}	44.60 ^{***}	41.68 ^{***}	75.5 ^{ooo}	91.2 ^{ooo}
a ₂ - <i>F.a.</i> (75%) + <i>T.p.</i> (25%)	b ₁ - unfertilized	57.92 ^{ooo}	55.95	31.56 ^{ooo}	33.45 ^{ooo}	113.2 ^{***}	113.5 [*]
	b ₂ - N ₅₀ P ₅₀	58.94 ^{ooo}	58.32 ^{**}	33.00 ^{ooo}	34.43 ^{ooo}	108.5 ^{***}	107.0
	b ₃ - N ₇₅ P ₇₅	60.39 ^{ooo}	59.42 ^{***}	32.77 ^{ooo}	34.55 ^{ooo}	106.4 ^{***}	104.8
	b ₄ - N ₁₀₀ P ₁₀₀	60.89 ^{ooo}	60.84 ^{***}	35.83 ^{ooo}	36.41	100.0 ^{***}	99.1 ^{oo}
	b ₅ - N ₁₅₀ P ₁₅₀	60.18 ^{ooo}	61.30 ^{***}	34.56 ^{ooo}	38.10 ^{***}	103.5 ^{***}	95.3 ^{ooo}
a ₃ - <i>F.a.</i> (50%) + <i>T.p.</i> (50%)	b ₁ - unfertilized	52.02 ^{ooo}	58.45 ^{***}	32.72 ^{ooo}	31.11 ^{ooo}	123.6 ^{***}	113.0 [*]
	b ₂ - N ₅₀ P ₅₀	52.70 ^{ooo}	58.66 ^{***}	32.29 ^{ooo}	31.68 ^{ooo}	122.9 ^{***}	111.5
	b ₃ - N ₇₅ P ₇₅	51.84 ^{ooo}	59.36 ^{***}	31.92 ^{ooo}	33.64 ^{ooo}	125.7 ^{***}	106.6
	b ₄ - N ₁₀₀ P ₁₀₀	54.51 ^{ooo}	60.24 ^{***}	34.86 ^{ooo}	35.72	113.7 ^{***}	101.3 ^o
	b ₅ - N ₁₅₀ P ₁₅₀	54.60 ^{ooo}	60.82 ^{***}	35.65 ^{ooo}	39.45 ^{***}	111.9 ^{***}	93.7 ^{ooo}
a ₄ - <i>F.a.</i> (25%) + <i>T.p.</i> (75%)	b ₁ - unfertilized	48.94 ^{ooo}	54.55 ^o	30.07 ^{ooo}	34.80 ^{oo}	137.2 ^{***}	113.7 [*]
	b ₂ - N ₅₀ P ₅₀	51.05 ^{ooo}	56.14	31.20 ^{ooo}	34.81 ^{oo}	129.2 ^{***}	110.5
	b ₃ - N ₇₅ P ₇₅	55.04 ^{ooo}	56.20	36.80 ^{ooo}	36.26	108.8 ^{***}	107.5
	b ₄ - N ₁₀₀ P ₁₀₀	55.69 ^{ooo}	57.29	36.62 ^{ooo}	37.01 [*]	107.8 ^{***}	104.1
	b ₅ - N ₁₅₀ P ₁₅₀	54.60 ^{ooo}	58.29 ^{**}	35.88 ^{ooo}	38.69 ^{***}	111.4 ^{***}	99.2 ^{oo}
a ₅ - <i>T.p.</i> (100%)	b ₁ - unfertilized	40.37 ^{ooo}	55.04	32.97 ^{ooo}	34.49 ^{ooo}	158.6 ^{***}	113.3 [*]
	b ₂ - N ₅₀ P ₅₀	41.94 ^{ooo}	55.86	33.43 ^{ooo}	35.08 ^o	151.5 ^{***}	110.5
	b ₃ - N ₇₅ P ₇₅	43.27 ^{ooo}	57.93 ^{**}	35.12 ^{ooo}	35.11 ^o	142.6 ^{***}	106.5
	b ₄ - N ₁₀₀ P ₁₀₀	49.57 ^{ooo}	59.38 ^{***}	38.66 ^{ooo}	36.44	116.7 ^{***}	101.5 ^o
	b ₅ - N ₁₅₀ P ₁₅₀	47.74 ^{ooo}	60.60 ^{***}	38.58 ^{ooo}	37.15 [*]	121.3 ^{***}	98.1 ^{ooo}
	LSD 0.5	0.77	1.26	0.58	0.84	5.2	5.1
	LSD 0.1	1.03	1.68	0.78	1.12	7.0	6.8
	LSD 0.01	1.34	2.18	1.02	1.46	9.1	8.9

In the same year, the ADF content of the obtained forage ranged from 30.07% in the unfertilized *Festuca arundinacea* Schreb. (50%) + *Trifolium pratense* L. (50%) variant to 44.60% in the *Festuca arundinacea* Schreb. variant (100%) fertilized with N₁₅₀P₁₅₀.

The lowest values of cell walls in the obtained forage, represented by NDF and ADF, were obtained in the variants where *Trifolium pratense* L. was present in higher percentage, positively influencing the quality of the forage.

Festuca arundinacea Schreb. and *Trifolium pratense* L. grown singly or in simple mixtures formed between them, under different nitrogen and phosphorus mineral fertilizer conditions, the relative forage quality (RFQ) was determined on the basis of NDF and ADF values.

Thus, as regards the influence of the interaction between the species or mixture of perennial grasses and legumes and fertilization with NP-based mineral fertilizers, the values obtained ranged from 75.5 for the variant a₁b₅ (low quality forage), *Festuca arundinacea* Schreb. (100%), fertilized with N₁₅₀P₁₅₀ and 158.6 for the variant a₅b₁, *Trifolium pratense* L. (100%) unfertilized (excellent forage quality).

In terms of the influence of the species grown alone or the mixture of the two species, in different percentages, on the RFQ value, the highest values were determined when *Trifolium pratense* L. was present, as the positive effect of red clover on forage quality is well known, due to the lower accumulation of cell walls.

Irrespective of the species or mixture of perennial grasses and legumes grown, fertilization with nitrogen and phosphorus-based complex mineral fertilizers resulted in forage of lower relative quality as the dose of nitrogen and phosphorus fertilizer applied increased, due to the elongation of plant stem internodes and thus the accumulation of more cell walls.

For this parameter, except for the variants a₁b₂ and a₁b₃, *Festuca arundinacea* Schreb. (100%), fertilized with N₅₀P₅₀ or N₇₅P₇₅, for all the other fertilized variants the differences obtained compared to the control variant were very significant, but negative for variants a₁b₄ and a₁b₅, *Festuca arundinacea* Schreb. (100%), fertilized with N₁₀₀P₁₀₀ or N₁₅₀P₁₅₀, and positive for all other variants, in which *Trifolium pratense* L. was present, irrespective of the percentage of participation in the vegetal canopy or fertilization variant.

3.3.2. NDF and ADF Content and Relative Quality of Forage Obtained in the Third Year of Vegetation (Table 3)

In the third year of vegetation, the NDF content of the forage obtained ranged between 54.55% for the unfertilized mixture of *Festuca arundinacea* Schreb. (25%) + *Trifolium pratense* L. (75%) and 61.30% for the mixture of *Festuca arundinacea* Schreb. (75%) + *Trifolium pratense* L. (25%), fertilized with N₁₅₀P₁₅₀, and the ADF content of the obtained forage varied between 31.11% in the unfertilized mixture of *Festuca arundinacea* Schreb. (50%) + *Trifolium pratense* L. (50%) and 41.68% in the variant *Festuca arundinacea* Schreb. (100%), fertilized with N₁₅₀P₁₅₀.

Analysing the influence of the interaction between the species or mixture of perennial grasses and legumes and fertilization with mineral fertilizers, the RFQ values obtained ranged from 91.2 for the variant a₁b₅, *Festuca arundinacea* Schreb. (100%), fertilized with N₁₅₀P₁₅₀ (medium quality forage) and 113.7 for the variant a₄b₁, the unfertilized mixture of *Festuca arundinacea* Schreb. (25%) + *Trifolium pratense* L. (75%) (good quality forage).

And in the third year of vegetation, in terms of the influence of the species cultivated alone or the mixture of the two species, in different percentages, on the RFQ value, the highest values were recorded when *Trifolium pratense* L. was present.

Irrespective of the species or mixture of perennial grasses and legumes grown, fertilization with nitrogen and phosphorus-based complex mineral fertilizers resulted in a forage of lower relative quality as the dose of nitrogen and phosphorus fertilizer applied increased, due to the elongation of stem internodes and increased cell wall share.

For this parameter, for each of the variants in which *Festuca arundinacea* Schreb. was 100% sown, regardless of the fertilizer dose applied, the differences obtained compared to the control variant were negative, distinct and highly significant.

In almost all variants in which *Trifolium pratense* L. was present, irrespective of the percentage of participation in the vegetal canopy, and fertilized with N₁₀₀P₁₀₀ or N₁₅₀P₁₅₀, the differences obtained compared to the control variant were also negative, distinct and highly significant.

The only positive, significant differences were recorded in the variants with *Trifolium pratense* L. under non-fertilization conditions.

4. Discussions

4.1. Discussion on Crude Protein Content and Quantity

Crude protein indicates the amount of nitrogen in the feed. The crude protein content of forages usually varies depending on the plant species composing the forage, the stage of development of the plants at the time of harvest and the fertilization applied [11,20,23,34,36]. The crude protein content of legumes ranges on average from 15 to 19%, while the average crude protein content of grasses ranges from 8 to 14%.

The amount of nitrogen used for fertilization can be reduced by using a balanced ratio of grass and legume species in the mixtures, which achieve very high yields [16,36].

Our study showed that, in general, the crude protein content of the plants was influenced by mineral fertilization, especially in the case of 100% sown *Festuca arundinacea* Schreb.

4.2. Discussion on the Competition between *Festuca arundinacea* Schreb. and *Trifolium pratense* L. Species

The composition of the mixtures of perennial grass and legume species for forage is conditioned by the biological properties of the species, according to the manner of use and period of use of the established temporary grassland [37,39].

The competitive ability or competitiveness between species will also be taken into account when composing mixtures. Introducing aggressive species into mixtures alongside species with low competitive ability will eventually lead to the elimination of the latter from the vegetal canopy. Competitive ability is a species-specific trait, but it is strongly influenced by environmental conditions and management [2,9,40,41].

Balanced fertilization of temporary pastures influences their productive capacity, resulting in higher quality forage [42,43]. There is also a change in vegetation structure, with grass species usually benefiting from nitrogen fertilization. In addition, there is a change in soil properties, soil microorganism activity and carbon dynamics [44].

In the case of the study conducted in the period 2021-2023, at Ezăreni Student Research and Practice Station of Iasi University of Life Sciences, the interspecific competitiveness was influenced by the percentage of participation in the sowing norm of the species in the mixture, the nitrogen and phosphorus mineral fertilizers administered, the biological characteristics of the species under study, as well as the specific climatic conditions of each agricultural year.

4.3. Discussion on NDF and ADF Content and Relative Feed Quality (RFQ)

Feed quality refers to a set of chemical, organoleptic, nutritional and wholesomeness properties that express the degree to which feed meets the nutritional requirements of the animal organism, depending on the biological background, farming technology and feeding technology. The composition and nutritive value of forages highly vary from one type to another, as well as for the same type of forage, depending on a wide range of factors [45-52]. Balanced fertilization contributes to the improvement of forage chemical composition and floristic structure [43,53,54]. The quality of a forage depends on the 'nutritive value' (nutrient supply potential, digestibility and nutrient levels), the amount of forage intake and the presence of quality-degrading elements [46,55-57].

The chemical composition of plants is conditioned by the genetic potential of the species, by vegetation factors and then by competition between individuals of the same species and individuals of different species.

The basic component of the plants that make up forage is the cell. The plant cell consists of the primary cell wall (ADF, made of cellulose and lignin), the secondary cell wall (NDF, made of hemicellulose, cellulose and lignin), the cytoplasm and the vacuole. As cell wall content increases, feed quality decreases. The lowest values of NDF and ADF were determined in the forage where *Trifolium pratense* L. was present and low doses of mineral fertilizers were used.

Following the study conducted from 2021 to 2023, the RFQ values obtained ranged between 75.5 for variant a1b5, *Festuca arundinacea* Schreb. (100%), fertilized with N₁₅₀P₁₅₀ and 158.6 for variant a5b1,

Trifolium pratense L. (100%), unfertilized, in the second year of vegetation, and in the third year of vegetation ranged from 91.2 in variant a1b5, *Festuca arundinacea* Schreb. (100%), fertilized with N₁₅₀P₁₅₀ to 113.7 in variant a4b1, mixture of *Festuca arundinacea* Schreb. (25%) + *Trifolium pratense* L. (75%), unfertilized.

Irrespective of the fertilization variant, the highest RFQ values were determined when *Trifolium pratense* L. was present.

5. Conclusions

In the second year of vegetation, the CP content in the forage obtained ranged from 11.74% in the control variant, represented by *Festuca arundinacea* Schreb. (100%), unfertilized, to 17.01% in the variant represented by *Trifolium pratense* L. (100%), fertilized with N₁₅₀P₁₅₀.

In the third year of vegetation, the CP content values in the forage obtained ranged from 1.83% in the control variant, *Festuca arundinacea* Schreb. (100%), unfertilized, to 17.83% in the variant *Trifolium pratense* L. (100%), fertilized with N₁₀₀P₁₀₀.

The highest values of crude protein content were obtained when *Trifolium pratense* L. was present, and fertilization with nitrogen and phosphorus mineral fertilizers resulted in higher protein content in the forage.

On average, in the period 2021-2023, the total amount of crude protein achieved ranged from 272.7 kg-ha⁻¹ in the control variant, *Festuca arundinacea* Schreb. (100%), unfertilized, to 1061.4 kg-ha⁻¹ in the variant consisting of *Festuca arundinacea* Schreb. (25%) and *Trifolium pratense* L. (75%), fertilized with N₁₅₀P₁₅₀.

Interspecific competitiveness was influenced by the percentage participation in the sowing norm of the species in the mixture, the nitrogen and phosphorus mineral fertilizers applied, the biological characteristics of the species under study, and the climatic conditions specific to each crop year.

In the second year of vegetation, the RYT index registered values <1, showing that *Festuca arundinacea* Schreb. and *Trifolium pratense* L. are in antagonistic relationships, fighting for the same resources to manifest their productive potential, *Festuca arundinacea* Schreb. being more competitive than *Trifolium pratense* L. only in case of 75% participation rate in the mixture, regardless of the fertilization variant. The variants in which *Festuca arundinacea* Schreb. recorded low and very low values of the CR index were those in which the participation percentage was 25% and 50%, the species being considered as weakly competitive.

In the third year of vegetation, the RYT index recorded values >1, indicating that *Festuca arundinacea* Schreb. and *Trifolium pratense* L. occupy different ecological niches, the resources being sufficient for the productive potential shown, and the CR index for *Festuca arundinacea* Schreb. was higher than for *Trifolium pratense* L. in cases of 50% and 75% rate of participation in mixtures, regardless of the fertilization variant, in which case the species was more competitive. The variants in which *Festuca arundinacea* Schreb. had low CR index values were only at 25% participation rate, being considered as poorly competitive. In the case of *Trifolium pratense* L., the CR index was lower in the variants with 25% and 50% participation, indicating a weaker competitiveness compared to *Festuca arundinacea* Schreb.

In the second year of vegetation, the RFQ values obtained ranged from 75.5 for *Festuca arundinacea* Schreb. (100%), fertilized with N₁₅₀P₁₅₀ (poor quality forage) to 158.6 for *Trifolium pratense* L. (100%), unfertilized (forage of excellent quality), and in the third year of vegetation they ranged from 91.2 for *Festuca arundinacea* Schreb. (100%), fertilized with N₁₅₀P₁₅₀ (forage of average quality) to 113.7 for *Festuca arundinacea* Schreb. (25%) + *Trifolium pratense* L. (75%), unfertilized (forage of good quality).

Irrespective of the fertilization variant, the highest RFQ values were determined when *Trifolium pratense* L. was present.

Irrespective of the species or mixture of perennial grasses and legumes grown, fertilization with nitrogen and phosphorus-based complex mineral fertilizers resulted in a lower relative forage quality as the amount of nitrogen and phosphorus fertilizer applied increased, due to the elongation of stem internodes and increased cell wall share.

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