

Article

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

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Abstract: The aim of this research carried out in the period 2021–2023 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, at an altitude of 117 m, with coordinates 47°07'27" N latitude and 27°30'25" E longitude, on a cambic chernozem soil, with 2.40% humus, 0.178% Nt, 26 ppm P_{AL} and 242 ppm K in the 0–30 cm layer, 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%); and a₅—*Trifolium pratense* L. (100%), and mineral fertilization, with five graduations, respectively: b₁—unfertilized (control), b₂—N₅₀P₅₀; b₃—N₇₅P₇₅; b₄—N₁₀₀P₁₀₀; and 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 and the biological peculiarities of the species under study, as well as the climatic conditions specific to each agricultural year, with consequences for the quality of the feed. The crude protein quantity (Q_{CP}) obtained in the mixture of *Festuca arundinacea* Schreb. (25%) and *Trifolium pratense* L. (75%), fertilized with N₁₅₀P₁₅₀, was higher by 788.7 kg·ha^{−1} than that in the control variant, represented by the *Festuca arundinacea* Schreb. species (100%), unfertilized. The lowest values of NDF (neutral detergent fiber) and ADF (acid detergent fiber) were recorded in the variants where the *Trifolium pratense* L. species was present and low doses of mineral fertilizers were used, determining a better quality of the forage. In the third year of vegetation, relative forage quality (RFQ) had values of 113.7 in the variant represented by the mixture of *Festuca arundinacea* Schreb. (25%) + *Trifolium pratense* L. (75%), unfertilized, and only 91.2 in the variant represented by the *Festuca arundinacea* Schreb. species (100%), fertilized with N₁₅₀P₁₅₀.



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Keywords: crude protein quantity (Q_{CP}); 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 established for a fixed period of time and is located in the place of a degraded permanent meadow 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. Their contribution 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 meadows [1–3].

An important technological part of the establishment of a sown grassland is the mixture structure, which not only 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) and 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 are a multitude of intraspecific and interspecific relationships between species in the vegetal canopy, 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 perennial forage grasses and 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 and 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, proto cooperation, mutualism and also competition, with effects on the productivity and quality of the achieved biomass [5,14]. Interspecific competition determines which species and how many species can coexist within the same community, affects population dynamics, and alters the species and community structure [15–18].

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 [9,19–21].

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 and also by the growing conditions [22–33].

Laboratory analysis measures the total nitrogen content (T_N) of the feed and calculates the crude protein (CP). Relative forage 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 neutral detergent fibre (NDF) and acid detergent fibre (ADF) content of the feed [23–27]. RFQ has a similar range of values to 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 and growing conditions affect forage digestibility, taste and palatability, as well as the content of crude fibre, protein, essential amino acids and other important nutrient components [4,5,9].

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 [16,18,21].

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. *Festuca arundinacea* Schreb. and *Trifolium pratense* L. are two important forage species for Romania, which are undergoing a breeding process and are cultivated in various mixtures or pure crops.

2. Materials and Methods

The research conducted in the period 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 crude protein content and the quantity of crude protein achieved and the fodder content in NDF and ADF.

In order to achieve the proposed objectives, an experiment was organized in the Ezăreni Student Research and Practice Station of Iasi University of Life Sciences, on the 26 March 2021, at an altitude of 117 m, with coordinates 47°07'27" N latitude and 27°30'25" E longitude, on a cambic chernozem soil, with 2.40% humus, 0.178% Nt, 26 ppm P_{AL} and 242 ppm K in the 0–30 cm layer, 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* (F.a.) 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%); and a₅—*Trifolium pratense* (T.p.) L. (100%), and factor B—annual fertilization with mineral fertilizers, with 5 graduations: b₁—unfertilized; b₂—N₅₀P₅₀; b₃—N₇₅P₇₅; b₄—N₁₀₀P₁₀₀; and b₅—N₁₅₀P₁₅₀ (Table 1). For fertilization, a complex fertilizer was used, type NPK 20-20-0, with nitric nitrogen (NO₂) at 8.7% and ammoniacal nitrogen (NH₄) at 13.3%.

For the productivity indicators, the control was represented by 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 cultivar, tuft grass, approved in 2012, developed at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania, and *Trifolium pratense* L. (red clover), David Liv cultivar, approved in 2015, developed at the Livada Agricultural Research and Development Station in Romania.

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

$$CP = T_N \times 6.25 (\%), \quad (1)$$

where:

T_N—total nitrogen content (%).

Table 1. The experimental design.

Factor A	Factor B
a ₁ — <i>Festuca arundinacea</i> (F.a.) Schreb. (100%—control)	b ₁ —N ₀ P ₀ (control)
	b ₂ —N ₅₀ P ₅₀
	b ₃ —N ₇₅ P ₇₅
	b ₄ —N ₁₀₀ P ₁₀₀
	b ₅ —N ₁₅₀ P ₁₅₀
a ₂ — <i>Festuca arundinacea</i> Schreb. (75%) și <i>Trifolium pratense</i> L. (25%)	b ₁ —N ₀ P ₀
	b ₂ —N ₅₀ P ₅₀
	b ₃ —N ₇₅ P ₇₅
	b ₄ —N ₁₀₀ P ₁₀₀
	b ₅ —N ₁₅₀ P ₁₅₀
a ₃ — <i>Festuca arundinacea</i> Schreb. (50%) și <i>Trifolium pratense</i> L. (50%)	b ₁ —N ₀ P ₀
	b ₂ —N ₅₀ P ₅₀
	b ₃ —N ₇₅ P ₇₅
	b ₄ —N ₁₀₀ P ₁₀₀
	b ₅ —N ₁₅₀ P ₁₅₀
a ₄ — <i>Festuca arundinacea</i> Schreb. (25%) și <i>Trifolium pratense</i> L. (75%)	b ₁ —N ₀ P ₀
	b ₂ —N ₅₀ P ₅₀
	b ₃ —N ₇₅ P ₇₅
	b ₄ —N ₁₀₀ P ₁₀₀
	b ₅ —N ₁₅₀ P ₁₅₀
a ₅ — <i>Trifolium pratense</i> (T.p.) L. (100%)	b ₁ —N ₀ P ₀
	b ₂ —N ₅₀ P ₅₀
	b ₃ —N ₇₅ P ₇₅
	b ₄ —N ₁₀₀ P ₁₀₀
	b ₅ —N ₁₅₀ P ₁₅₀

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).

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

where:

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

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

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

where:

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 h 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 [22].

The acid detergent fibre (ADF) content of the plant was determined by the Van Soest method, the extracted fractions being cellulose and lignin [22].

Calculation of the relative forage quality (RFQ) was performed according to Equation (4) [23–27]:

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

where:

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).

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

where:

NEL—net energy for lactation (Mcal·kg^{−1}).

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

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

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 performed using Equation (8) [34]. 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; and RYT < 1, the species are in an antagonistic relationship.

$$\text{RYT} = Y_{AB} \times Y_{AA}^{-1} + Y_{BA} \times Y_{BB}^{-1}, \quad (8)$$

where:

Y_{AB} = Q_{CP} for species A, mixed culture;

Y_{AA} = Q_{CP} for species A, in pure culture;

Y_{BA} = Q_{CP} for species B, mixed culture;

Y_{BB} = Q_{CP} for species B, in pure culture.

The calculation of the Competition Rate (CR) index characterizes the species used in mixtures as regards their mutual competitiveness and was performed using Equation (9) [34]. 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; and CR < 1, species A is less competitive than species B.

$$\text{CR} = (Y_{AB} \times Y_{AA}^{-1} \times Z_{AB}) \times (Y_{BA} \times Y_{BB}^{-1} \times Z_{BA})^{-1}, \quad (9)$$

where:

Y_{AB} = Q_{CP} for species A, mixed culture;

Y_{AA} = Q_{CP} for species A, in pure culture;

Z_{AB} = proportion of species A and B in mixture;

Y_{BA} = Q_{CP} for species B, mixed culture;

Y_{BB} = Q_{CP} for species B, in pure culture;

Z_{BA} = proportion of species B and A in mixture.

The obtained data were statistically interpreted by analysis of variance (ANOVA test) and the calculation of Least Significant Difference (LSD), using SPSS version 29 Academic.

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. In the area where the research was conducted, the multiannual average of precipitation is 517.8 mm, while in the agricultural years 2021–2022 and 2022–2023, the sum of precipitation was 407.4 mm and 404.6 mm, respectively, unevenly distributed over months and decades.

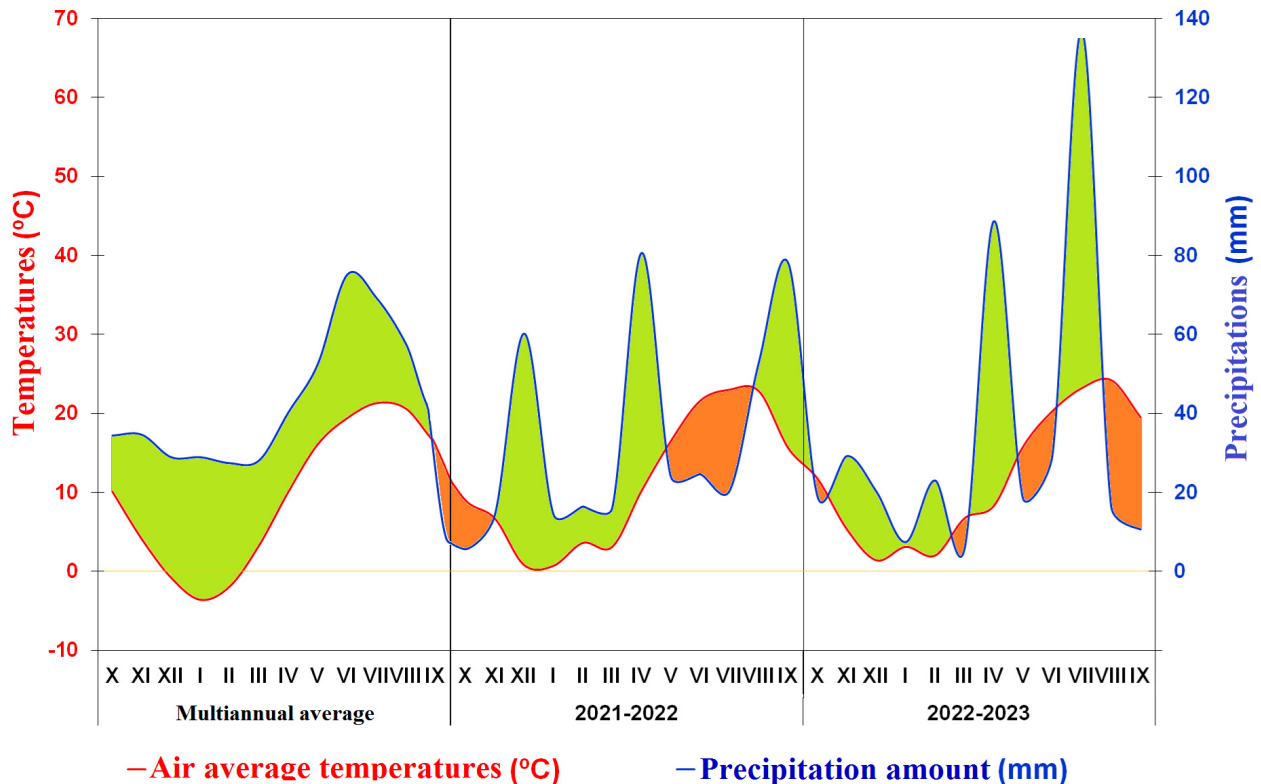


Figure 1. Climadiagram for the agricultural period 2021–2023. Note: Green color: the precipitation is above the evapotranspiration level; red color: the periods of water stress for plants.

3. Results and Discussions

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 ranged from 11.74% for variant a_1b_1 , *Festuca arundinacea* Schreb. (100%), non-fertilized (control), to 17.01% for variant a_5b_5 , *Trifolium pratense* L. (100%), fertilized with $N_{150}P_{150}$.

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 the case of a_1b_2 , for this parameter, the positive differences obtained in comparison with the control 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 feed content in CP was higher than in the previous year in all variants where *Trifolium pratense* L. had a share equal to or greater than 50%. The values obtained ranged from 11.83% in variant a₁b₁, *Festuca arundinacea* Schreb. species (100%), unfertilized (control), to 17.83% in the case of a₅b₄, *Trifolium pratense* L. species (100%), fertilized with N₁₀₀P₁₀₀ (Table 2).

Table 2. 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		
		2nd Year	3rd Year	2nd Year	3rd Year	Average
a ₁ — <i>F.a.</i> (100%)	b ₁ —unfertilized	11.74 ^C	11.83 ^C	212.3 ^C	333.2 ^C	272.7 ^C
	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 ***
a ₂ — <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 ***
a ₃ — <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 ***
a ₄ — <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 ***
a ₅ — <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 ***
LSD 0.05		0.76	0.83	82.9	106.6	94.7
LSD 0.01		1.02	1.10	147.4	189.4	168.4
LSD 0.001		1.33	1.44	249.9	321.3	285.6

Note: LSD 0.05 = Least Significant Difference 5%; LSD 0.01 = Least Significant Difference 1%; LSD 0.001 = Least Significant Difference 0.1%; * = significant positive differences, ** = distinct significant positive differences, *** = highly significant positive differences; ^C = control.

Depending on the species grown alone or the mixture studied, 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 (a₁b₁) 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 nitrogen and phosphorus on the amount of crude protein achieved (Q_{CP}) showed that the average values obtained in the period 2021–2023 for variant a₄b₅, *Festuca arundinacea* Schreb. (25%) and *Trifolium pratense* L. (75%) mixture, fertilized with N₁₅₀P₁₅₀,

are higher by 788.7 kg-ha^{-1} than those in the control variant a_1b_1 , *Festuca arundinacea* Schreb. (100%), unfertilized (Table 1).

Depending on the species grown alone or the mixture studied, 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 resulted in higher amounts of crude protein. With the exception of a_1b_2 , 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.

Crude protein indicates the amount of nitrogen in the feed. The crude protein content 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 [9,11,21,32,35]. 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 [15,36].

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.

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

3.2.1. Species Competitiveness in the Second Vegetation Year

Analysing the influence of the interaction between the factors studied (mixture $\times$ fertilization) 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 and fertilization variants (Table 3).

This result indicates that for *Festuca arundinacea* Schreb. and *Trifolium pratense* L., although they occupy different ecological niches, the resources were insufficient to express the high 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, and their symbiotic mechanism is well developed; therefore, they are more competitive.

In the second vegetation year, the CR index for *Festuca arundinacea* Schreb. was higher than for *Trifolium pratense* L. only for the 75% rate of participation in the mixture, regardless of the fertilization variant, with *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 to be 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. for all the fertilization variants, which benefited from high vigour starting the second year of vegetation.

Table 3. Influence of the interaction between the cropping system used and NP fertilization on RYT and CR indicators.

Variant		RYT	2nd Year CR <i>F.a.</i>	CR <i>T.p.</i>	RYT	3rd Year 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 —N ₀ P ₀	0.59 ⁰⁰	2.78 ***	0.36 ⁰⁰⁰	1.10 ⁰⁰⁰	16.59 ***	0.06 ⁰⁰
	b ₂ —N ₅₀ P ₅₀	0.60 ⁰⁰	2.22 **	0.45 ⁰⁰	1.16 ⁰⁰	14.70 ***	0.07 ⁰⁰
	b ₃ —N ₇₅ P ₇₅	0.63 ⁰⁰	2.17 *	0.46 ⁰⁰⁰	1.16 ⁰⁰	17.01 ***	0.06 ⁰⁰
	b ₄ —N ₁₀₀ P ₁₀₀	0.69	3.30 ***	0.30 ⁰⁰⁰	1.15 ⁰⁰	18.45 ***	0.05 ⁰⁰
	b ₅ —N ₁₅₀ P ₁₅₀	0.85 **	6.74 ***	0.15 ⁰⁰⁰	1.13 ⁰⁰	17.00 ***	0.06 ⁰⁰
a ₃ — <i>F.a.</i> (50%) + <i>T.p.</i> (50%)	b ₁ —N ₀ P ₀	0.64 ⁰⁰	0.26 ⁰⁰	3.89 ⁰⁰⁰	1.54	2.45 ⁰⁰⁰	0.41 ⁰
	b ₂ —N ₅₀ P ₅₀	0.68	0.25 ⁰⁰	4.06 ⁰⁰⁰	1.62	2.66 ⁰⁰⁰	0.38 ⁰
	b ₃ —N ₇₅ P ₇₅	0.63 ⁰⁰	0.27 ⁰⁰	3.70 ⁰⁰⁰	1.48	2.49 ⁰⁰⁰	0.40 ⁰
	b ₄ —N ₁₀₀ P ₁₀₀	0.79	0.30 ⁰⁰	3.36 ⁰⁰⁰	1.59	2.87 ⁰⁰⁰	0.35 ⁰
	b ₅ —N ₁₅₀ P ₁₅₀	0.92 ***	0.51 ⁰	1.96 ⁰⁰⁰	1.53	2.80 ⁰⁰⁰	0.36 ⁰
a ₄ — <i>F.a.</i> (25%) + <i>T.p.</i> (75%)	b ₁ —N ₀ P ₀	0.79	0.04 ⁰⁰	25.04 ***	1.87 **	0.22 ⁰⁰⁰	4.52 ***
	b ₂ —N ₅₀ P ₅₀	0.77	0.03 ⁰⁰	37.58 ***	1.90 **	0.21 ⁰⁰⁰	4.65 ***
	b ₃ —N ₇₅ P ₇₅	0.76	0.03 ⁰⁰	39.68 ***	1.88 **	0.23 ⁰⁰⁰	4.43 ***
	b ₄ —N ₁₀₀ P ₁₀₀	0.87 **	0.03 ⁰⁰	36.20 ***	1.97 **	0.27 ⁰⁰⁰	3.72 ***
	b ₅ —N ₁₅₀ P ₁₅₀	0.95 ***	0.02 ⁰⁰	42.28 ***	1.85 **	0.28 ⁰⁰⁰	3.62 ***
LSD 0.05		3.23	0.24	1.59	0.99	3.23	0.24
LSD 0.01		4.37	0.32	2.15	1.34	4.37	0.32
LSD 0.001		5.87	0.43	2.92	1.79	5.87	0.43

Note: LSD 0.05 = Least Significant Difference 5%; LSD 0.01 = Least Significant Difference 1%; LSD 0.001 = Least Significant Difference 0.1%; * = significant positive differences, ** = distinct significant positive differences, *** = highly significant positive differences, ⁰ = significant negative differences, ⁰⁰ = distinct significant negative differences, ⁰⁰⁰ = highly significant negative differences; ^C = control.

3.2.2. Species Competitiveness in the Third Vegetation Year

The analysis of the influence of the interaction between the mixture used and fertilization with nitrogen and phosphorus 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 (Table 2). This result indicates that *Festuca arundinacea* Schreb. and *Trifolium pratense* L. occupy different ecological niches, with sufficient resources, through low clover production. 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 the 75% participation rate for all the fertilization variants, in which case, the species was considered more competitive due to the higher number of individuals.

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 [36–39].

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 [40,41]. 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 [42].

In the case of this 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 and the biological characteristics of the species under study, as well as the specific climatic conditions of each agricultural year.

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

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 4).

Table 4. 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	
		2nd Year	3rd Year	2nd Year	3rd Year	2nd Year	3rd Year
a ₁ — <i>F.a.</i> (100%)	b ₁ —N ₀ P ₀	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 ⁰⁰
	b ₃ —N ₇₅ P ₇₅	64.57	58.81 ^{***}	40.32 [*]	39.94 ^{***}	86.8	96.0 ⁰⁰⁰
	b ₄ —N ₁₀₀ P ₁₀₀	66.95 ^{***}	59.59 ^{***}	43.15 ^{***}	40.57 ^{***}	79.1 ⁰⁰	93.6 ⁰⁰⁰
	b ₅ —N ₁₅₀ P ₁₅₀	68.02 ^{***}	59.82 ^{***}	44.60 ^{***}	41.68 ^{***}	75.5 ⁰⁰⁰	91.2 ⁰⁰⁰
a ₂ — <i>F.a.</i> (75%) + <i>T.p.</i> (25%)	b ₁ —N ₀ P ₀	57.92 ⁰⁰⁰	55.95	31.56 ⁰⁰⁰	33.45 ⁰⁰⁰	113.2 ^{***}	113.5 [*]
	b ₂ —N ₅₀ P ₅₀	58.94 ⁰⁰⁰	58.32 ^{**}	33.00 ⁰⁰⁰	34.43 ⁰⁰⁰	108.5 ^{***}	107.0
	b ₃ —N ₇₅ P ₇₅	60.39 ⁰⁰⁰	59.42 ^{***}	32.77 ⁰⁰⁰	34.55 ⁰⁰⁰	106.4 ^{***}	104.8
	b ₄ —N ₁₀₀ P ₁₀₀	60.89 ⁰⁰⁰	60.84 ^{***}	35.83 ⁰⁰⁰	36.41	100.0 ^{***}	99.1 ⁰⁰
	b ₅ —N ₁₅₀ P ₁₅₀	60.18 ⁰⁰⁰	61.30 ^{***}	34.56 ⁰⁰⁰	38.10 ^{***}	103.5 ^{***}	95.3 ⁰⁰⁰
a ₃ — <i>F.a.</i> (50%) + <i>T.p.</i> (50%)	b ₁ —N ₀ P ₀	52.02 ⁰⁰⁰	58.45 ^{***}	32.72 ⁰⁰⁰	31.11 ⁰⁰⁰	123.6 ^{***}	113.0 [*]
	b ₂ —N ₅₀ P ₅₀	52.70 ⁰⁰⁰	58.66 ^{***}	32.29 ⁰⁰⁰	31.68 ⁰⁰⁰	122.9 ^{***}	111.5
	b ₃ —N ₇₅ P ₇₅	51.84 ⁰⁰⁰	59.36 ^{***}	31.92 ⁰⁰⁰	33.64 ⁰⁰⁰	125.7 ^{***}	106.6
	b ₄ —N ₁₀₀ P ₁₀₀	54.51 ⁰⁰⁰	60.24 ^{***}	34.86 ⁰⁰⁰	35.72	113.7 ^{***}	101.3 ⁰
	b ₅ —N ₁₅₀ P ₁₅₀	54.60 ⁰⁰⁰	60.82 ^{***}	35.65 ⁰⁰⁰	39.45 ^{***}	111.9 ^{***}	93.7 ⁰⁰⁰
a ₄ — <i>F.a.</i> (25%) + <i>T.p.</i> (75%)	b ₁ —N ₀ P ₀	48.94 ⁰⁰⁰	54.55 ⁰	30.07 ⁰⁰⁰	34.80 ⁰⁰	137.2 ^{***}	113.7 [*]
	b ₂ —N ₅₀ P ₅₀	51.05 ⁰⁰⁰	56.14	31.20 ⁰⁰⁰	34.81 ⁰⁰	129.2 ^{***}	110.5
	b ₃ —N ₇₅ P ₇₅	55.04 ⁰⁰⁰	56.20	36.80 ⁰⁰⁰	36.26	108.8 ^{***}	107.5
	b ₄ —N ₁₀₀ P ₁₀₀	55.69 ⁰⁰⁰	57.29	36.62 ⁰⁰⁰	37.01 [*]	107.8 ^{***}	104.1
	b ₅ —N ₁₅₀ P ₁₅₀	54.60 ⁰⁰⁰	58.29 ^{**}	35.88 ⁰⁰⁰	38.69 ^{***}	111.4 ^{***}	99.2 ⁰⁰
a ₅ — <i>T.p.</i> (100%)	b ₁ —N ₀ P ₀	40.37 ⁰⁰⁰	55.04	32.97 ⁰⁰⁰	34.49 ⁰⁰⁰	158.6 ^{***}	113.3 [*]
	b ₂ —N ₅₀ P ₅₀	41.94 ⁰⁰⁰	55.86	33.43 ⁰⁰⁰	35.08 ⁰	151.5 ^{***}	110.5
	b ₃ —N ₇₅ P ₇₅	43.27 ⁰⁰⁰	57.93 ^{**}	35.12 ⁰⁰⁰	35.11 ⁰	142.6 ^{***}	106.5
	b ₄ —N ₁₀₀ P ₁₀₀	49.57 ⁰⁰⁰	59.38 ^{***}	38.66 ⁰⁰⁰	36.44	116.7 ^{***}	101.5 ⁰
	b ₅ —N ₁₅₀ P ₁₅₀	47.74 ⁰⁰⁰	60.60 ^{***}	38.58 ⁰⁰⁰	37.15 [*]	121.3 ^{***}	98.1 ⁰⁰⁰
LSD 0.05		0.77	1.26	0.58	0.84	5.2	5.1
LSD 0.01		1.03	1.68	0.78	1.12	7.0	6.8
LSD 0.001		1.34	2.18	1.02	1.46	9.1	8.9

Note: LSD 0.05 = Least Significant Difference 5%; LSD 0.01 = Least Significant Difference 1%; LSD 0.001 = Least Significant Difference 0.1%; * = significant positive differences, ** = distinct significant positive differences, *** = highly significant positive differences, ⁰ = significant negative differences, ⁰⁰ = distinct significant negative differences, ⁰⁰⁰ = highly significant negative differences; ^C = control.

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 registered in the variants where *Trifolium pratense* L. was present in a higher percentage, positively influencing the quality of the forage.

On the basis of NDF and ADF values, the relative forage quality was determined. Thus, as regards the influence of the interaction between the species or mixture of perennial grasses and legumes and fertilization with NP, the RFQ values obtained ranged from 75.5 for the variant a₁b₅ (low quality forage), *Festuca arundinacea* Schreb. (100%), fertilized with N₁₅₀P₁₅₀, to 158.6 for the case of 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 (NDF and ADF).

Irrespective of the species or mixture of perennial grasses and legumes grown, fertilization with nitrogen and phosphorus resulted in forage of lower relative quality as the dose of nitrogen and phosphorus applied increased due to the accumulation of more cell walls. For this parameter (RFQ), except for the cases of a₁b₂ and a₁b₃, *Festuca arundinacea* Schreb. (100%), with N₅₀P₅₀ or N₇₅P₇₅, for all the other fertilized variants in which *Trifolium pratense* L. was present, the differences obtained compared to the control were positive and highly significant. For the cases of a₁b₄ and a₁b₅, *Festuca arundinacea* Schreb. (100%), with N₁₀₀P₁₀₀ or N₁₅₀P₁₅₀, the differences were negative, distinct and highly significant.

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 NP, the RFQ values obtained ranged from 91.2 for the variant a₁b₅, *Festuca arundinacea* Schreb. (100%), with N₁₅₀P₁₅₀ (medium quality forage), to 113.7 for the case of a₄b₁, the unfertilized mixture of *Festuca arundinacea* Schreb. (25%) + *Trifolium pratense* L. (75%) (good quality forage).

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

For the RFQ parameter, at 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 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 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.

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 vary highly from one type to

another, as well as for the same type of forage, depending on a wide range of factors [43–49]. Balanced fertilization contributes to the improvement in the forage chemical composition and floristic structure [41,50,51]. The quality of a forage depends on the ‘nutritive value’ (nutrient supply potential, digestibility and nutrient levels), the amount of forage intake, being influenced by the content in ADF and NDF [44,52–54].

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 the 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.

4. Conclusions

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.

The largest amount of crude protein was obtained in the variant consisting of *Festuca arundinacea* Schreb. (25%) and *Trifolium pratense* L. (75%) fertilized with N₁₅₀P₁₅₀, being greater by 788.7 kg·ha^{−1} than that in the control variant a₁b₁, *Festuca arundinacea* Schreb. (100%), unfertilized.

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 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

In the third year of vegetation, the CR index for *Festuca arundinacea* Schreb. was higher than for *Trifolium pratense* L. in cases of a 50% and 75% rate of participation in mixtures, regardless of the fertilization variant, in which case, the *Festuca arundinacea* Schreb. species was more competitive than *Trifolium pratense* L.

The highest RFQ values were determined when *Trifolium pratense* L. was present in the crops. Increasing the dose of NP fertilizer applied causes a decrease in RFQ due to the higher NDF and ADF contents of the feed.

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