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Dynamics of nitrogen concentration on intercropped ryegrass

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ABSTRACT. The dynamics of nitrogen (N) in ryegrass intercropped with different forage species with regard to dry matter (DM) accumulation within an agroecological transition system was evaluated. Annual ryegrass was *intercropped* with black oats (RY + BO), white clover (RY + WC) and forage peanut (RY + FP). The experimental design was completely randomized, with three replications. The study applied the N dilution model for plant tissue which comparatively uses modifiable factors of the environment at optimal levels. The model shows decline in N concentration due to the DM accumulation of temperate grass pastures. Data were compared to model by the root mean square deviation analysis. Dilution coefficient (β), used in all pasture samples, was lower than -0.60. Highest N concentration decline in plant tissues, due to DM accumulation, occurred in RY + WC pastures (β = -0.94), followed by RY + FP (β = - 0.86) and RY + BO (β = - 0.67), respectively. Ryegrass intercropped with white clover showed the highest N content in plant tissue, with a nitrogen nutrition index close to the proposed model.

Keywords: dilution coefficient, forage peanut, legumes, nutrients, oat, white clover.

Dinâmica da concentração de nitrogênio em azevém consorciado

RESUMO. O trabalho objetivou avaliar a dinâmica do nitrogênio (N) no azevém consorciado com diferentes espécies forrageiras, em função do acúmulo de matéria seca (MS), em um sistema de transição agroecológica. Foram avaliados os consórcios de azevém anual cv. Comum, com aveia preta (AZ + AV), trevo branco (AZ + TB) e amendoim forrageiro (AZ + AF). O delineamento experimental foi o inteiramente casualizado, com três repetições. Utilizou-se o modelo de diluição do N no tecido vegetal, o qual utiliza comparativamente os fatores modificáveis do meio ambiente em um nível ótimo. O modelo descreve o declínio da concentração do N em função do acúmulo de forragem de pastagens temperadas. Os dados de campo foram confrontados ao modelo utilizando-se a análise do desvio da raiz do quadrado médio. O valor do coeficiente de diluição (β) em todas as pastagens foi inferior a -0,60. O declínio mais intenso da concentração de N no tecido vegetal em função do acúmulo de MS ocorreu na pastagem de AZ + TB (β = -0,94), seguido das pastagens de AZ + AF (β = -0,86) e AZ + AV (β = -0,67), respectivamente. O azevém consorciado com trevo branco apresentou maior conteúdo de N no tecido vegetal, com índice de nutrição nitrogenada mais próxima ao modelo estudado.

Palavras-chave: coeficiente de diluição, amendoim forrageiro, leguminosas, nutrientes, aveia, trevo branco.

Introduction

If environmental limiting factors did not exist, biomass production capacity of pasture systems would be unlimited owing to high solar energy supply. Water, nutritional and temperature limitations, frequent in pastoral ecosystems, impair the adequate development of the leaf area of the vegetal canopy. Essential nutrients, such as carbon, nitrogen, phosphorus and others, integrate biochemical processes and the plants' metabolic pathways which affect energy retrieval and flow within the system (MARENCO; LOPES, 2009).

Nitrogen (N), one of the most absorbed nutrients, is of prime importance to plant growth. According to Quadros et al. (2003), biomass amount produced by vegetation in forage plant communities is often limited by N availability. According to Plénet and Cruz (1997), N availability in soil is mostly insufficient for culture demands up to the start of flowering period. Consequently, the proper management of nitrogen fertilization in forage cultures should provide N in sufficient quantities during the vegetation development so that they could achieve their growth potential in the wake of intercepted light amounts.

According to Lemaire et al. (1991), N requirements decreased in proportion to development of plants due to higher contents of structural material and increasing shading by older foliage. Nitrogen remobilization for younger leaves occurs. Further, mineralization rates of soil N vary throughout the year according to response to temperature and humidity, in the main. Intercropping between leguminous plants and grasses may increase the availability of N in the soil and the sustainability of agricultural and cattle-raising systems by N biological fixation or by the recycling of the nutrient. According to Assmann et al. (2007), soil cultivated with winter leguminous plants has higher rates of N-nitrate on the surface when compared to soil cultivated only with oats.

The dynamics of N concentration in ryegrass culture intercropped with leguminous plants was evaluated by an N dilution model which comparatively employs the modifiable factors of the environment at optimum levels and estimates the plant's N status at different development phases.

Material and methods

The experiment was undertaken on a property which belongs to the Department of Animal Science of the Federal University of Santa Maria (UFSM), Santa Maria, Rio Grande do Sul State, Brazil, between 4th June and 31st October 2005. The area lies in the physiographical region of the Central Depression of the state of Rio Grande do Sul, Brazil, 95m above sea level, at 29° 43' S and 53° 42' W. Soil, classified as Dystrophic Red Arenite Argisoil, belongs to São Pedro map unit (EMBRAPA, 2006) and the region's climate is humid subtropical climate according to Köppen classification (MORENO, 1961), featuring mean yearly rainfall 1769 mm; mean yearly temperature 19.2°C; mean minimum temperature 9.3°C in July and mean maximum temperature 24.7°C in January; yearly sunny period 2212 hours; relative air humidity 82%.

Pasture was established within an agricultural-ecological transition system, with low manure, after two soil preparations, in 100 m^2 enclosures. Common cultivar ryegrass (*Lolium multiflorum* Lam.) culture intercropped with black oats (*Avena strigosa* Schreb.), Comum cultivar (RY + BO); white clover (*Trifollium repens* L.), cultivar Yi (RY + WC); and forage peanut (*Arachis pintoi* Krapov & Gregory), cultivar Amarillo (RY + FP) was evaluated. Treatments consisted of the following seeding densities: RY + BO, 40 kg ha^{-1} of ryegrass and 80 kg ha^{-1} of oats; RY + WC, 40 kg ha^{-1} of ryegrass and 4 kg ha^{-1} of clover; RY + FP, 40 kg ha^{-1} of ryegrass and 12 kg ha^{-1} of forager peanut. Forager peanut was sown the previous year, in December 2004. Experimental design was totally randomized, with three repetitions (enclosures) per treatment.

Basic manure consisted of 40 kg ha^{-1} phosphorus and potassium, based on soil analysis and according to recommendations of the Committee of Soil fertility RS/SC (COMISSÃO DE FERTILIDADE DO SOLO RS/SC, 2004). Covering manure consisted of 20 kg ha^{-1} of N in one application, after the first pasturing. Soil had the following features: texture = 4; pH-H_2O = 5.0; SMP index = 5.8; P_2O_5 = 31.85 mg dm^{-3}; K_2O = 98.2 mg dm^{-3}; organic matter (MO) = 3.2%; Al saturation = 3%; saturation per base = 58%.

Areas were rotation pastured by milking Holstein cows, mean weight 530 + 30.5 kg and average daily milk production amounting to 17 + 2.31 kg day^{-1}, fed on supplement with concentrate and maize silage. Animals were admitted to pasture when the latter reached a height of 20 cm. Forage mass, calculated by double sample technique with 5 cuts and 20 visual estimates, was assessed prior to the admittance of animals. Forage provision between 8 and 10 DM 100 kg^{-1} of live weight, based on double sampling-estimated forage mass, was maintained to determine animal load. Whereas animal load was variable so that animals could remain one day in each pastureland enclosure, the animals were kept in the experimental areas from 9h a.m. to 4h p.m. and from 6h p.m to 7h a.m, with shade, water and mineral salt.

Approximately one tenth of each enclosure (10 m^2) was isolated after the second pasture. Three cuttings at a height of 5 cm from the soil were undertaken weekly in each isolated area making use of a 0.3 m^2 frame when forage provided more than a ton of dry matter per hectare. According to Plénet and Lemaire (1999), contents of the whole plant's critical N remains constant at early growth stages and biomass stays at less than 1.0 t ha^{-1}. Each harvest was weighed and approximately 300 g of pasture sample was retrieved to separate ryegrass and its components (stem and leaf blade), the intercropped species, dead matter and other non-identified species. Fresh samples with only ryegrass (leaves and stem) were then weighed, conditioned in paper bags and taken to a forced air circulation buffer at 55°C for approximately 72 hours. The partially dry matter was weighed to calculate culture production. Samples with only ryegrass were ground in a 1 mm mesh sieve Wiley mill.

Dry matter, processed in a buffer at 105°C during 16 hours, and total ryegrass N were calculated by micro Kjeldahl method (AOAC, 1995). N dilution in vegetal texture method was employed to estimate the plant's N concentration. The method compares the environment's modifying factors at optimized levels:

$$N\ (\%) = 4.8\ (MS)^{-0.32}$$

Where: N (%) is the concentration of N as a percentage of produced mass and DM is the amount of dry matter produced by the aerial part of the pasture in t ha^{-1}. Coefficient $\alpha = 4.8$ represents the concentration of N in the first ton of the plants' aerial part. Coefficient $\beta = -0.32$ is the decrease in N contents in the plant for each DM accumulated unit. Rather, the behavior of N concentration in the plant's aerial part is characterized proportionately to accumulation of dry matter. Above model, suggested by Lemaire et al. (1984) and validated by Greenwood et al. (1991), describes concentration decrease of optimized N according to forage accumulation of temperate pastures.

The nitrogen nutrition index (NNI) was calculated for the pastures, obtained by the quotient between the culture's N content (sample) and the N critical concentration (model). According to the model, every N concentration below optimized N may limit the culture's productivity. Data were checked to the model through an analysis of the root mean square deviant (RMSD) proposed by Kobayashi and Salam (2000).

Results and discussion

Increase of accumulated DM in pastures was followed by decrease in their N rates (Figure 1). This is due to the fact that as plants grow and accumulate DM they also obtain increasing storing structural components with low N even though sufficient supplement of N and of other components already exists (GREENWOOD et al., 1991; LEMAIRE, et al., 1984). Controls on soil fertility and acidity, the existence of invaders and water, and solar radiation and environmental temperature on pastures were not undertaken in current analysis. In fact, the curve rates from field conditions and the model's curve proposed by Lemaire et al. (1984) showed considerable distancing (Figure 1).

Figure 1 shows that the N curve of RY + WC pasture was closer to the curve proposed by Lemaire et al. (1984) during the whole period, with RMSD 1.26 against 1.55 and 1.81 respectively for pastures RY + BO and RY + FP. The above evidences that ryegrass intercropped with white clover provides a lower deviant when compared to model. Consequently, intercropping provided better conditions for the development of ryegrass. Deviants demonstrate differences with regard to culture and management conditions between the two analyses. Data and curves do not diverge from the model; rather they show the solid basis and preciseness of the model, which characterizes deficits on the culture and management of pastures to the extent that data do not position themselves on the optimized curve (Figure 1).

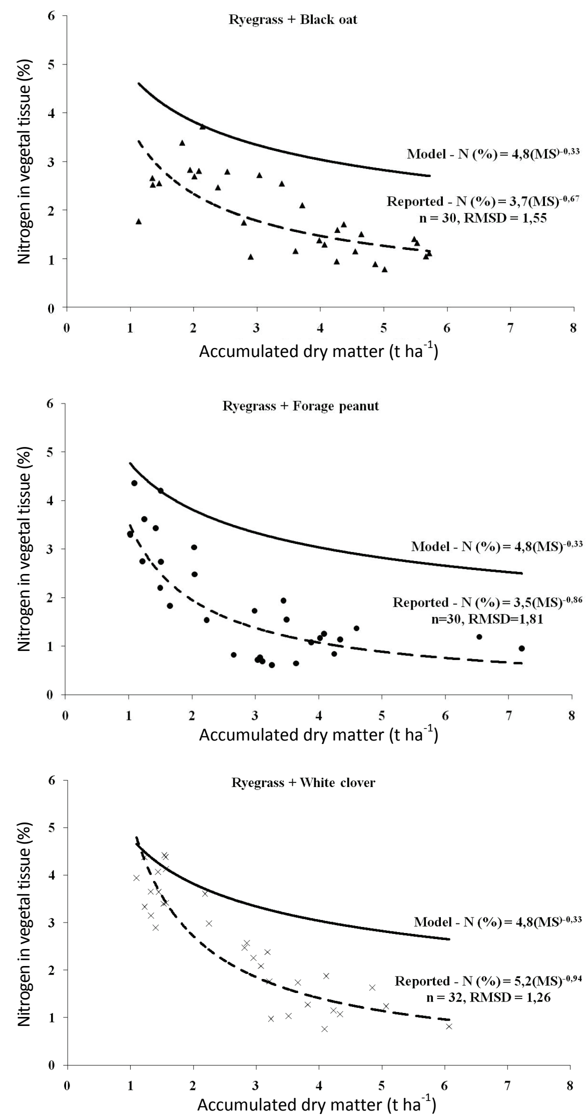

Figure 1. Nitrogen dilution curve in annual ryegrass plant intercropped with black oats, forage peanut and white clover.

When all modifiable factors of the environment are optimized, potential productivity is achieved. Only environmental variables which cannot be controlled in field conditions, such as radiation and temperature, limit the plants' productive potential. When a modifiable factor (water, nutrients, health and others) is kept within limiting levels, actual productivity is obtained and is less than the potential.

According to Lemaire et al. (1984), the environment's influence is great even in ideal conditions of fertilization and humidity. The model was consequently developed to give stable rates for coefficients α and β so that they could be employed in different edaphological conditions, years and cultivars for pastures that receive sufficient N rates so that it could be guaranteed that growth would not be restricted to N availability in the soil. However, the above coefficients cannot be applied unrestrictedly but must be adjusted to each species, cultivar, culture conditions, forage systems and other factors.

According to Hernandez and Bolagños Aguilar (2007), nitrogen dilution curves have different parameters among the species and even within the same species and these differences exhibit the nitrogen needs of each culture to achieve the best growth rate (α) and pinpoint which genotypes have the greatest reduction in N contents with regard to DM accumulation (β). The above authors show that differences in coefficient rates (α and β) exist among different yearly evaluations with important environmental effects on the coefficients even in non-limiting fertility and humidity conditions.

Ryegrass's N curve in RY + FP pasture showed the highest deviant when compared to the model, featuring a pronounced decrease in N contents proportionally to DM accumulation (β = -0.86) and with the lowest N contents (α = 3.5) in the vegetal tissue (Figure 1). Results show that intercropping with forage peanut failed to provide the best conditions for the development of ryegrass and are similar to RY + BO pastures. This is due to the fact that intercropped leguminous plants concentrate their production during the summer, or rather, they do not have a vegetative development during the winter and present mean 2.8% of total biomass of the pasture during this study. Providing ryegrass culture with N during this period is not efficacious.

According to Miranda et al. (2003), different accesses of forage peanut may fix between 36 and 90% of their N nutritional requirements in the soil. Although forage peanut fixed N in the soil during the summer period, it was not received by the ryegrass during the winter since, according to Magdoff (1991), mineral N in the soil's arable layer has insignificant electrostatic adsorption which favor its lixiviation on the surface for unexplored depths by the roots. Assmann et al. (2003) reported that plantation of white clover in the winter did not produce any residual N for the subsequent culture (maize, for instance) in summer.

Coefficients (α) in pastures with RY + FP and RY + BO were respectively 3.5 and 3.7. Although rates are lower when compared to the model adopted in this study, they are similar to the coefficient for tropical plants, N (%) = 3.6 $(MS)^{-0.32}$ and show nitrogen deficiency in pastures. Difference between coefficient rates for temperate and tropical species, respectively 4.8 and 3.6, represents the differences in the carbohydrate assimilation pathways. Within the non-limiting conditions for N supplementation, the above difference shows that plants C_4 need only 75% of N required by plants C_3 to produce the same amount of biomass.

Highest coefficient α rate for pasture with RY + WC (5.2), which is even higher than that of the proposed model (4.8), shows that ryegrass has higher N contents in the vegetal tissue when intercropped with white clover. Further, sometime in its development N absorption by plant was higher than its requirements due to a higher availability of the mineral in the soil (Figure 1). White clover's greater participation, featuring 24.75% of the biomass of pasture after 35 days exclusion, may have cooperated towards N fixation in the soil and its reception in the pasture during the initial evaluation stage. Higher N concentration in the vegetal tissue during this period and intense reduction of N rates in ryegrass due to DM accumulation (β = -0.94) underpins the above hypothesis (Figure 1).

Biological fixation of N and nutrient recycling attributed to the use of leguminous plants solely or mixed with grasses may be applied as a strategy to increase N availability in the soil and in the agriculture and pasture system. However, the determination of the moment in which release occurs is problematic. According to Whitehead (1995), white clover in the mixture is a weak competitor for inorganic nitrogen. Thus, the ryegrass intercropped with white clover intercropped recovered greater quantities of N from the soil.

Cruz and Lemaire (1986) showed that experiments undertaken in France with dactylis (*Dactylis glomerata*) intercropped with alfalfa (*Medicago sativa*) failed to demonstrate a direct pathway of N from the leguminous plant towards the other through biological fixation; moreover, alfalfa competes with the grass for inorganic N in the soil. According to Collins et al. (1991), there is only a slight N transference during the first months after planting an intercrop pasture with grass and leguminous plants. Moreover, the greatest transference pathway, in pasture and in cutting, consists of the decomposition of the leguminous plant's dead matter, such as roots, leaves and stems.

All dilution coefficient rates (β) are lower than -0.6, or rather, high decrease in N rates in the vegetal tissue in proportion of DM accumulation. Progressive vegetation stage of the pasture and the absence of pasturing reduce light intensity on white clover culture due to the erect-growing ryegrass shading. Consequently, within the pasture stand after 35 days of exclusion, the white clover contributes decreasingly from 24.75 to 1.78% after 78 days of exclusion. Pronounced decrease of white clover percentage in pasture during the period coincides with an intense decline in N rates in intercropped ryegrass (β = -0.94) as from the moment the contribution of ryegrass increases in the pasture.

N dilution curves are based on N concentration in the vegetal tissue and are used to diagnose the cultures' nutritional status during the different phases of the growth and development cycle. In fact, they indicate N status when they pinpoint deficiencies and excesses so that a fast diagnosis is provided for the correction of any stages in plant growth. Nitrogen nutrition index (NNI) is obtained by dilution curves and is the ratio between N contents of a plant at a certain instance and the N critical concentration. If rate is higher than 1, there is an excess of N in the culture and, consequently, great intake of available nutrient (from soil or from the fertilizers). If rate is equal to 1, an adequate nutrition is available with regard to nitrogen. On the other hand, if the rate is less than 1, there is N deficit and therefore maximum rate of culture growth will not be achieved.

The determination of NNI rate for growth stages of a culture guarantees safe decisions with regard to the application of an extra N dose. All pastures studied were N deficient at different stages of growth (Figure 2). When compared to others, pasture with RY + WC was closest to the model since NNI was higher than estimated and reached the 102% mark after 41 days of exclusion. This fact may have occurred because of the biological fixture of N in the soil and its reception by the pasture due to the leguminous plant's high contribution. In fact, its peak occurred on the 35th day of pasture exclusion when it became senescent due to luminosity competition with ryegrass. The above data indicate a type of management for winter intercropped pastures since fallow periods over 41 days bring liabilities for maintenance of white clover stands or of low leguminous plants. It would impair the adequate N sustenance for ryegrass or for the winter intercropped upright grass.

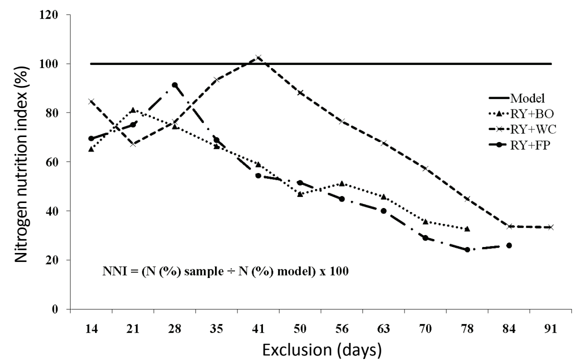

Figure 2. Nitrogen nutrition index (NNI) in annual ryegrass plant intercropped with black oats (RY+BO), white clover (RY+WC) and forage peanut (RY+FP).

Conclusion

No leguminous plant, neither white clover nor forage peanut, is able to fix and provide N in sufficient amounts to supply the demand of intercropped ryegrass culture. The white clover has the greatest capacity to fix and transfer N to the ryegrass and thus contributes towards an increase in N rates in the culture. Forage peanut is not efficacious in fixing and providing N during the winter period due to slight participation in the pasture's biomass.

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