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Evaluation of soil nitrogen availability by growing tufts of nitrophilic species in an intensively grazed biodiverse legume-rich pasture

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Abstract

Biodiverse legume-rich pastures (BLRP) have been recommended for extensive animal production since they can improve productivity and pasture quality. However, the consequences for the N balance within the agro-system, due to the increase in biological N_2 fixation, must be monitored. A field trial was carried out to evaluate the soil N availability in a BLRP in comparison with an adjacent unsown pasture. The field experiment consisted of growing tufts of nitrophilic species (turnip, *Brassica campestris* and rye, *Secale cereale*) in the pastures rounded by PVC rings. Soil inorganic-N levels were monitored during a period of one year. The potentially available soil N was determined by growing ryegrass (*Lolium multiflorum*) in a pot experiment and carrying out several chemical extraction methods. The mean values of N recovered by field-grown turnip and rye were, respectively, 30.6 and 31.1 kg ha^{-1} in BLRP, not statistically higher than that recovered in the unsown pasture. This is consistent with the very low levels of soil inorganic-N observed both in BLRP and the unsown pasture. Nitrogen recovered by ryegrass grown in pots was significantly higher in the soil collected from the BLRP than in soil from the unsown pasture. In this study, plant-available inorganic-N appeared as a strong limiting factor for the growth of the non-legume component. The BLRP seems to be currently environmentally sound, since the risk of N loss is practically non-existent. However, the potentially mineralisable organic N is increasing, which requires a further monitoring of the soil N dynamic as the pasture ages.

Additional key-words: chemical tests; multispecies pastures; potential N losses; soil available N indicators; soil inorganic-N.

Resumen

Evaluación de la disponibilidad de N en suelo en pastos biodiversos ricos en leguminosas mediante el cultivo de plantas nitrófilas dentro de la dehesa

Los pastos biodiversos ricos en leguminosas (PBRL) están siendo recomendados para la ganadería extensiva, ya que pueden mejorar la calidad de los pastos. Sin embargo, las implicaciones en el balance de nitrógeno (N) en suelo, debido a la fijación biológica de N, deben ser monitorizadas. Este estudio se estableció para evaluar la disponibilidad de N en suelo en PBRL en comparación con pastizales naturales. El ensayo consistió en el establecimiento de especies nitrófilas (nabo y centeno) en matas rodeadas por anillos de PVC. Durante un año se monitorizaron también los niveles de N mineral en suelo. El N potencialmente disponible se determinó mediante el cultivo de raigrás en macetas y mediante varios análisis químicos de laboratorio. En los PBRL los valores medios de N recuperado por el nabo y el centeno fueron 30,6 y 31,1 kg ha^{-1}, respectivamente, no existiendo diferencias significativas con los valores registrados en los pastos naturales. Durante todo el año se registraron niveles bajos de N inorgánico en suelo. El N recuperado por el raigrás fue significativamente más elevado en los PBRL que en el pasto natural. Los resultados mostraron que el N fue un factor ecológico muy limitante para el crecimiento de las especies no leguminosas. Por otro lado, en los PBRL el riesgo de pérdida de N para el medio ambiente fue prácticamente nulo. Sin embargo, el N orgánico potencialmente mineralizable aumenta en el suelo, lo cual justificaría la necesidad de realizar nuevos análisis de dinámica del N en el suelo en un futuro.

Palabras clave adicionales: análisis químicos; indicadores de disponibilidad de nitrógeno en el suelo; nitrógeno inorgánico en el suelo; pastos biodiversos.

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Introduction

The fertility of soils in most of the Portuguese territory is low, firstly limited by soil shallowness. The abundance of steep slopes in the landscape encourages soil erosion which counteracts pedogenesis, the process of soil formation. Extensive animal production is one of the most interesting possibilities of land use. Since native pastures are poor in terms of productivity and pasture quality, some farmers have increased the productivity of their lands by sowing subterranean clover-based pastures. However, in the last few years, biodiverse legume-rich pastures (BLRP) have been recommended to the farmers by Fertiprado, the biggest seed company in the country. It is expected that these multispecies and multi-varietal pastures will show high environmental plasticity, with each species exploiting different ecological micro-niches, increasing the productivity, the inter-annual production stability and also the longevity of the pasture.

The greatest benefit of the improved pastures arises mainly from nitrogen (N) fixation by the legume component. The N fixation capacity of legumes can vary greatly according to plant species, management practices and environmental conditions. Values of N fixation exceeding 200 kg N ha^{-1} yr^{-1} can be obtained in temperate/boreal areas, as reported by Elgersma and Hassink (1997) for white clover grown for forage. In a study involving 158 pastures either based in annual legume species (annual medics, clovers and vetch) or alfalfa, over a 1250 km north-south transect of eastern Australia, Peoples *et al*. (2001) reported an average amount of 30 to 160 kg shoot N fixed ha^{-1} yr^{-1} for annual species and 37 to 128 kg N ha^{-1} yr^{-1} for alfalfa. Much lower amounts of fixed N_2, ranging from 11 to 18 kg N ha^{-1} yr^{-1}, were reported by Riffkin *et al*. (1999) for grazed dairy pastures of white clover in south-western Victoria. The differences found in N_2 fixation between legume species and agro-systems are mainly due to the fact that N_2 fixation is primarily regulated by legume dry matter yield. Peoples *et al*. (2001) reported N_2 fixation amounts for pasture and crop legumes varying between 20 and 25 kg shoot N for every tonne of shoot dry matter produced, which is not very dissimilar to values reported by Carlsson and Huss-Danell (2003), Chen *et al*. (2004) and Goh and Bruce (2005).

The benefit of the legume component in the swards comes also from the transfer of N to the companion grasses. This transfer may involve the decomposition of litter and roots of the legume (Viera-Vargas *et al*., 1995; Chen *et al*., 2004) and also root exudates (Snoeck *et al*., 2000). A study in mezotrons by using ^{15}N labelling showed that the rhizodeposited N compounds by red clover and white clover growing in mixtures with ryegrass, constituted more than 80% of the total plant-derived N in the soil (Høgh-Jensen and Schjoerring, 2001). It is also important to note that most of the ingested N is excreted by grazing animals. Nitrogen in meat and wool, for instance, is insignificant in respect to N_2 fixation in improved pastures. Ledgard (1991) estimated that below-ground transfer of fixed N in white clover to grasses was 70 kg N ha^{-1} yr^{-1}, whereas above-ground transfer, via cow excreta, was 60 kg N ha^{-1} yr^{-1}.

In spite of the many positive aspects of biological N_2 fixation, the substantial input of N to the soil may have great consequences for the ecosystem N balance. Thus, the intensively grazed legume-based pastures may pose significant environmental concerns, such as denitrification and nitrous oxide emissions (Luo *et al*., 2000; Pu *et al*., 2001; Macadam *et al*., 2003; Zaman *et al*., 2009), ammonia volatilization (Fillery, 2001; Zaman *et al*., 2009) and nitrate leaching (Eriksen *et al*., 2004; Ridley *et al*., 2004; Di and Cameron, 2005).

The Portuguese agriculture department is currently subsidizing the seeding of BLRP, thereby causing an increase of this kind of pastures in the country over the next years. Previously reported data have shown that BLRP increased productivity and nutritional quality in comparison to natural pastures (Carneiro *et al*., 2008). However, studies on the N dynamic associated to BLRP have not yet been done. Thus, the main objectives of this study were: i) to evaluate the risk of environmental impact of BLRP, due to the potential losses of NO_3-N by leaching and/or denitrification; ii) to test the suitability of a technique which consists of the growing of nitrophilic species (turnip and rye) in micro-plots sown in the pasture as a means of monitoring soil N availability and the transfer of N from legume to non-legume species; and iii) to evaluate the potentially mineralisable N in a BLRP determined from N recovered by ryegrass grown in a pot experiment and from chemical analyses of soil N availability.

Material and methods

Site description

The field experiment was carried out in a farm named «Quinta de França» located near the city of

Covilhã (40° 16' N; 7° 30' W) in Portugal. In a biodiverse (mixture of up to 20 different species of grasses and legumes) legume-rich pasture sown seven years ago, three locations were selected across the pasture's landscape (top slope, middle slope and bottom slope). The slope of the pasture is around 2-3%. A fourth location was selected in an adjoining native unsown pasture grown in similar soil and climatic conditions. The BLRP is currently dominated by *Trifolium subterraneum* subsp. s*ubterraneum* and subsp. *yanninicum*, with a mean ground cover above 80% in middle spring (unpublished). Other common species in the BLRP are: *Trifolium cernuun* (abundant in wet years), *T. glomeratum*, *T. dubium*, *Agrostis salmantica* (only in wet years), *Vulpia myurus, Plantago coronopus*, *Leontodon longirostris* and *Chamaemelum mixtum*. In the BLRP two functional groups are particularly important among the indigenous plant species: plants adapted to compacted soils, due to sheep walking (*e.g. Plantago coronopus* and *Spergularia purpurea*); and oligotrophic annual species, such as *Aira caryophyllea* and *Tolpis barbata*. Plants of temporally wet soils (*e.g. T. cernuun* and *A. salmantica*) and nitrophilous species (*e.g. Chamaemelum mixtum*) are also relevant. The legume component of the native pasture is very poor: punctually some *Trifolium glomeratum* and *T. campestre*. This pasture is dominated by annual species with a short growing cycle and low productivity like *A. salmantica* (only in wet years), *Bromus hordeaceus*, *Molineriella laevis*, *V. myurus*, *Plantago coronopus* and *Spergularia purpurea.* The production differences between the two pastures types in the production cycle of 2007-2008 were overwhelming: 656 g m^{-2} and 106 g m^{-2}, respectively in the BLRP and in the native pasture (unpublished).

The region benefits from a Mediterranean type climate. Mean annual temperature and accumulated precipitation in the period 1930-1960 were 13.9°C and 1,034 mm (Mendes and Bettencourt, 1980). The soil is a Leptosol derived from granites, with an effective depth of about 20 cm. Selected physical and chemical properties of the soil in the four different locations, selected as treatments in the experimental design, are shown in Table 1. The BLRP received 1,500 kg lime ha^{-1} before sowing in October 2001. Superphosphate 18% was also biennially applied in the BLRP at a rate of 150 kg ha^{-1}.

Both the BLRP and the native pastures have been managed in an intensive grazing system that includes a herd of cattle and a flock of sheep. Grazing is stopped in spring to allow for enough seed production for pasture self-reseeding.

Inorganic-N in soil

Soil inorganic-N was determined through monthly soil coring during the year. Soil was sampled at 0-20 cm depth (15 replicates for each composite sample), sieved (2-mm mesh) in the field and immediately transported to the laboratory and frozen until analysis to restrict microbial activity. Soil extracts were prepared from 20 g of soil and 40 mL 2 M KCl. The suspension was shaken for 1 hour and filtered in Watmann #42 filter paper. Nitrate and ammonium concentrations in the extracts were analyzed in an UV-VIS spectrophotometer Varian Cary 50. The moisture content of the soil samples was also determined by drying the samples at 40°C to allow expression of the results on a dry soil basis.

Table 1. Selected properties of soils in October 2007 according to the location in the pasture

Parameters	Top slope	Middle slope	Bottom slope	Unsown pasture
Texture (USDA)	Loamy sand	Loamy sand	Loamy sand	Loamy sand
pH (soil:water, 1:2.5)	5.1	5.3	5.3	5.1
Organic C (Walkley-Black) (g kg^{-1})	14.3	13.9	15.0	14.5
Extractable P (Egner-Rhiem) (mg kg^{-1})[a]	17.8	26.6	17.3	23.3
Extractable K (Egner-Rhiem) (mg kg^{-1})[a]	150.9	105.9	112.3	106.4
Exchangeable bases (ammonium acetate, pH 7)				
Ca (cmol$_c$ kg^{-1})	3.2	4.4	6.0	2.0
Mg (cmol$_c$ k^{-1})	2.2	3.7	3.9	0.7
K (cmol$_c$ kg^{-1})	0.5	0.7	0.4	0.6
Na (cmol$_c$ kg^{-1})	0.7	1.0	1.7	0.2

[a] Extracted by ammonium lactate plus acetic acid, buffered at pH 3.7.

Micro-plots of nitrophilic species

In circular micro-plots of 154 mm diameter two nitrophilic species, turnip (*Brassica campestris* L.) and rye (*Secale cereale* L.), were sown in the autumn of 2007. Turnip has a high growth rate and N demand in autumn and early winter months, giving information about soil N availability in the autumn/winter period. Rye shows a higher growth rate in spring, giving information about soil N availability mainly after the end of March. Within the micro-plots the soil was hoed to allow the seeding of turnip and rye. Twenty and ten seeds of turnip and rye were respectively sown per micro-plot. The micro-plots were rounded by a PVC ring with 2 cm height to avoid the invasion of the inside space by the legume species, but at the same time allowing for rizosphere interactions. In each location 16 micro-plots were prepared, eight for each individual species, in groups of four, two of each species. The four micro-plots in each group were placed in close proximity to permit its protection by pasture exclosure cages (Fig. 1). During the experimental period the legume plants that emerged in the micro-plots were weeded out. The turnip and rye plants were mowed on 23 February and 19 April, respectively. Above-ground plant biomass was oven-dried at 70°C and ground. Dry matter yields were recorded and tissue N concentrations determined in a Kjeltec auto 1030 analyzer.

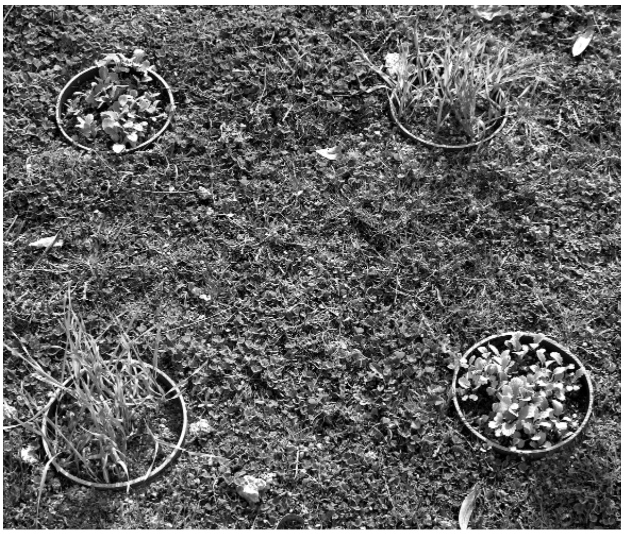

Figure 1. Turnip and rye growing in circular micro-plots on 16 November 2007, after being sown on 8 October 2007. The arrangement of the micro-plots allows them to be covered with protective stock cages in groups of four.

Pot experiment

A pot experiment with Italian ryegrass (*Lolium multiflorum* L.) was carried out from soil samples collected in October 2008 in the four locations of the experimental design. Ryegrass was selected for the pot experiment taking into account its continuous and extended period of growth and the ability of the grass species to take up N. The soil samples were separated into two depths, 0-10 cm and 10-20 cm. The samples were air dried and sieved (2-mm mesh). Five replicates (5 pots) per location and depth were filled with 1 kg dry soil. After sowing the ryegrass the pots were watered with 150 mL of distilled water and placed in a greenhouse. The growing plants were irrigated 1 to 3 times per week, depending on the temperature in the greenhouse and the developing level of the sward. To avoid denitrification due to the excess of water the volumes used in each irrigation event were low ($\approx$ 75 mL pot^{-1}). The experiment was maintained for nine months until the growth of ryegrass decreased due to the exhaustion of soil available N. During the growing period two intermediate harvests were made by cutting the plants 3.0 cm above the soil surface. In the final harvest the plants were cut close to the soil surface. Plant material was oven-dried at 70°C, weighed and ground. Total N content in dry material was determined in a Kjeltec 1030 auto-analyzer. Nitrogen recovered was estimated from above-ground dry matter yield and N concentration in dry matter.

Soil N analyses

From the same soil samples prepared for the pot experiment several laboratory analyses were performed to assess the potentially available soil N. The methods followed were essentially the same as reported by Sharifi *et al*. (2007) and Soon *et al*. (2007). Extractable NH_4-N and NO_3-N were determined from 10 g of soil and 40 mL 2 M KCl shaken for 1 hour in 150-mL centrifuge tubes. The suspension was thereafter filtered through a Whatman #42 filter paper. The concentrations of NH_4-N and NO_3-N in the extracts were determined by UV-vis. spectrophotometry. Hot KCl extractable NH_4-N was determined by heating (100°C) 5 g of soil and 20 mL of 2 M KCl in 150-mL digestion tubes for 4 h in a digestion block. Thereafter the tubes were removed and allowed to cool and the extract filtered through a Whatman #42. Hydrolysable NH_4-N was obtained by subtracting initial NH_4-N (cold KCl extraction) from hot KCl extractable NH_4-N. The ultraviolet absorbance of $NaHCO_3$ extracts were prepared by shaking 2.5 g soil with 0.01 mol L^{-1} $NaHCO_3$ for 15 min in 125-mL Erlenmeyer flasks. The suspension was filtered through a Whatman #42 filter paper. The ultraviolet absorbance of the extracts was measured at 205 and 260 nm. UV absorbance at 205 nm reflects both organic and mineral forms of N, whereas the UV absorbance at 260 nm is only related to organic forms (Sharifi *et al.*, 2007). Extractable NH_3-N was determined by adding 5 g of soil to 20 mL NaOH (12.5 mol L^{-1}). The suspension was directly distilled in Kjeltec 1030 autoanalyzer. The C:N ratio of soil samples were estimated from total soil carbon and Kjeldahl N determinations. Total soil carbon was determined by dry combustion which consisted of the mass difference of 1 g oven-dried sample at 100°C and heated in a furnace at 400°C for 6 h.

Data analysis

Comparisons among locations were provided by ANOVA. Means with significant differences were separated by Tukey-Kramer HSD test ($\alpha = 0.05$). Comparisons between two different soil depths were made by the t test for paired samples ($\alpha = 0.05$).

Results

Soil inorganic nitrogen

Soil NH_4-N concentration was very low from 8 Oct. 2007 to 23 Jan. 2008. In spring, soil NH_4-N levels progressively increased, reaching the higher levels in the summer months (Fig. 2). The variation pattern was similar among locations and the mean values were often not statistically different. Soil NH_4-N levels were close to 0.3 mg kg^{-1} on 8 Oct. 2007 and reached 6.5 mg kg^{-1} on 4 Jun. 2008. The experimental variability also increased in the summer months.

Soil NO_3-N concentrations were low during the entire year and for all the locations of the pastures. The mean values of soil NO_3-N concentration ranged from 0.8 to 3.1 mg kg^{-1} (Fig. 3). The differences among the mean values of the different locations were usually not statistically significant.

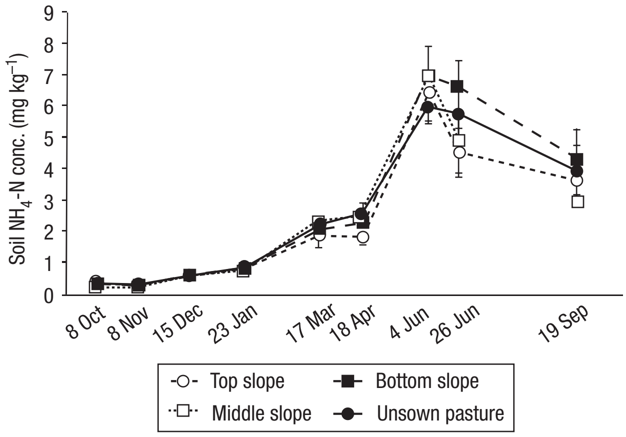

Figure 2. Soil NH_4-N concentration in soil during the growing season (8 Oct. 2007 to 19 Sep. 2008). Vertical bars are the mean standard deviations.

Turnip and rye dry matter yield and plant N recovery

The dry matter yields of the turnip plants cultivated in the PVC rings were very low considering the area of the rings and extrapolated to the hectare. The values were lower than 2.5 Mg ha^{-1} and did not differ significantly with the locations, including the unsown pasture (Table 2). The mean values of plant N concentration varied from 14.22 to 15.48 g kg^{-1} and the differences among treatments were not statistically significant. Nor were there any statistically significant differences in plant N recovery. The experimental variability was

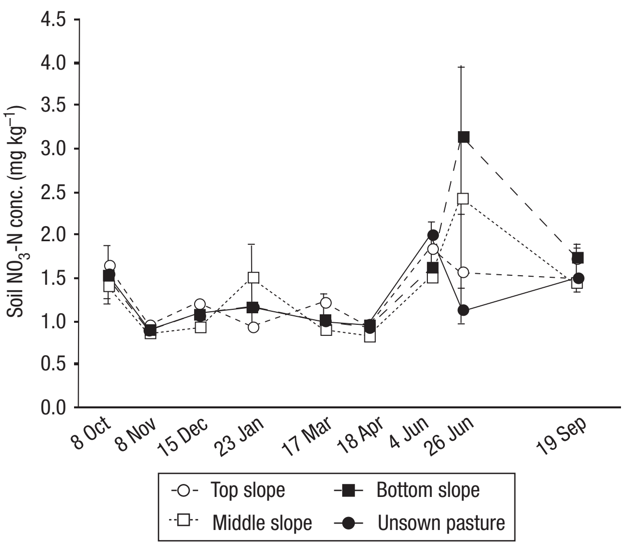

Figure 3. Soil NO_3-N concentration in soil during the growing season (8 Oct. 2007 to 19 Sep. 2008). Vertical bars are the mean standard deviations.

Table 2. Dry matter (DM) yield, plant N concentration (PNC) and plant N recovered (PNR) of turnip and rye plants grown in circles of 154 mm diameter in the pasture rounded by PVC rings of 2 cm height. Means with the same letter in the columns for each depth are not statistically different by Tukey-Kramer HSD test ($\alpha = 0.05$)

Landscape position	DM yield (Mg ha^{-1})	PNC (g kg^{-1})	PNR (kg ha^{-1})
Turnip			
Top slope	2.41^{a}	14.22^{a}	34.27^{a}
Middle slope	2.02^{a}	14.53^{a}	29.29^{a}
Bottom slope	1.82^{a}	15.48^{a}	28.17^{a}
Unsown pasture	2.30^{a}	15.44^{a}	33.21^{a}
Rye			
Top slope	2.25^{a}	11.79^{a}	26.60^{a}
Middle slope	3.11^{a}	11.20^{a}	34.84^{a}
Bottom slope	2.77^{a}	10.90^{a}	31.91^{a}
Unsown pasture	3.06^{a}	9.42^{a}	28.81^{a}

high, with great differences in DM yields and plant N recoveries among replicates of the same treatment, contributing to the lack of statistical differences among the four locations.

The growth of the winter rye was also poor, and the mean vales of DM yield, plant N concentration and plant N recovery were not statistically different among treatments (Table 2). The mean DM yields varied from 2.25 to 3.11 Mg ha^{-1}, plant N concentration from 9.42 to 11.79 g kg^{-1}, and plant N recovery varied from 26.60 to 34.84 kg ha^{-1}.

Potentially available soil nitrogen

Ryegrass DM yield in the pot experiment was significantly higher in the soils from the BLRP in comparison with the unsown pasture for both the 0-10 cm and 10-20 cm soil layers (Table 3). In the 0-10 cm soil samples the mean values ranged from 3.17 to 3.46 g pot^{-1} for the BLRP, whereas for the unsown pasture the mean DM yield was 2.46 g pot^{-1}. In the 10-20 cm soil layer, mean DM yields ranged from 1.66 and 1.74 g pot^{-1} for the BLRP and reached the value 0.86 g pot^{-1} for the native pasture. Plant N concentrations were not statistically different among treatments for both the 0-10 cm and 10-20 cm soil depths. Plant N recovery was usually significantly higher in the soils from the BLRP than from the soils of the native pasture for both the soil depths, reflecting the influence of the DM yield

Table3. Dry matter (DM) yield, plant N concentration (PNC) and plant N recovered (PNR) in ryegrass grown in a pot experiment in a greenhouse using soil provided from two depths (0-10 and 10-20 cm). Means with the same letter in the columns for each depth are not statistically different by Tukey-Kramer HSD test ($\alpha = 0.05$)

Landscape position[1]	DM yield (g pot^{-1})	PNC (g kg^{-1})	PNR (mg pot^{-1})
0-10 cm soil layer			
Top slope	3.33^{a}	16.98^{a}	56.68^{a}
Middle slope	3.17^{a}	15.88^{a}	50.59^{ab}
Bottom slope	3.46^{a}	16.73^{a}	57.79^{a}
Unsown pasture	2.46^{b}	16.98^{a}	41.37^{b}
10-20 cm soil layer			
Top slope	1.68^{a}	14.69^{a}	24.64^{a}
Middle slope	1.74^{a}	13.83^{a}	24.12^{a}
Bottom slope	1.66^{a}	14.02^{a}	23.17^{a}
Unsown pasture	0.86^{b}	14.74^{a}	12.63^{b}

[1] Means of DM yield, PNC and PNR are statistically different between the two soil depths by the t test ($\alpha = 0.05$) for paired samples.

in the estimation of plant N recovery. The mean values of plant N recovery in the 0-10 cm soil layer ranged from 50.59 to 57.79 mg N pot^{-1} for the BLRP and was 41.37 mg N pot^{-1} for the unsown pasture. In the deeper layer the mean plant N recovery ranged from 23.17 to 24.64 mg N pot^{-1} for BLRP and was 12.63 mg N pot^{-1} for the unsown pasture.

The C:N ratio of soil was significantly lower in BLRP in comparison to unsown pasture in the 10-20 cm soil layer (Table 4). In the 0-10 cm layer the means were not statistically different ($p = 0.14$), but the mean values were slightly higher in the unsown pasture, which may indicate that significant differences would be expected

Table 4. C:N ratio of the soil samples from the four locations and the two depths

Landscape position	Soil layer	
	0-10 cm	10-20 cm
Top slope	9.8^{a1}	13.9^{b}
Middle slope	9.4^{a}	13.7^{b}
Bottom slope	10.2^{a}	15.0^{ab}
Unsown pasture	10.7^{a}	16.3^{a}
Average	10.0^{B2}	14.7^{A}

[1] Means with the same letter in columns are not statistically different by Tukey-Kramer HSD test ($\alpha = 0.05$). [2] The means of the two soil depths in the bottom line are statistically different by the t test ($\alpha = 0.05$) for paired samples.

in the next few years. The upper soil layer showed significantly lower C:N ratio (10.0) than the deeper layer (14.7) probably as a result of the presence of more debris from the above-ground biomass and the higher density of the rooting system.

Most of the laboratory chemical methods tested to assess the easily mineralisable soil N showed a significant linear relationship with N recovered by ryegrass grown in the pot experiment. The six laboratory indices showing the highest coefficients of determination (R^2) are shown in Figure 4. The highest R^2 was provided by the soil C:N ratio (0.84), which was followed by hot KCl NH_4-N extraction (0.74), cold KCl inorganic-N extraction (0.69), NH_3-N extracted by NaOH (0.60), UV absorbance at 205 nm of $NaHCO_3$ extracts (0.56) and UV absorbance at 260 nm of $NaHCO_3$ extracts (0.51).

Discussion

In both the BLRP and the native pasture the NH_4-N soil levels were very low particularly during the winter

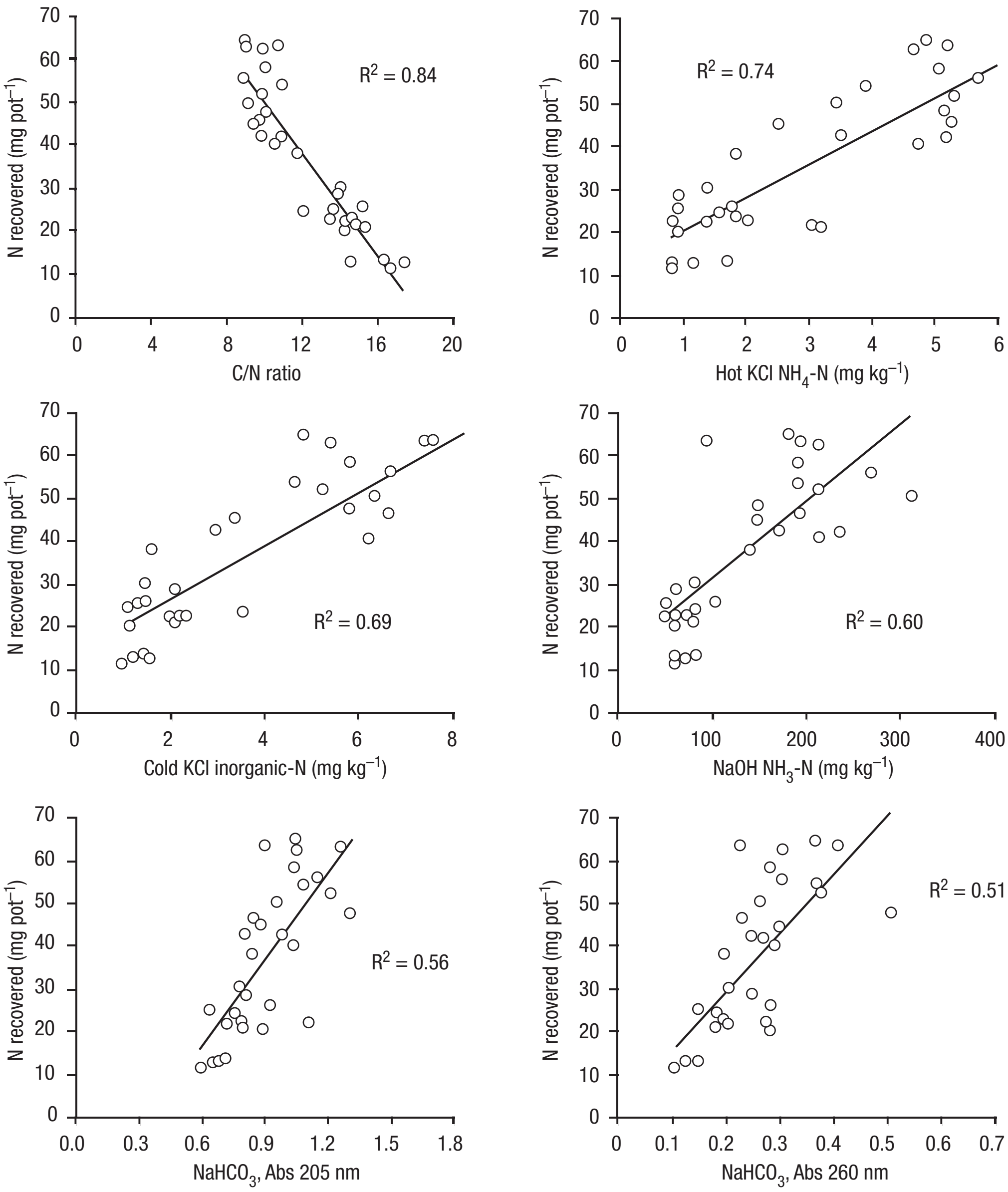

Figure 4. Relationship between chemical laboratory indices of potentially available soil N and N recovered by ryegrass grown in the pot experiment.

and early spring. In summer, as the soil moisture decreased NH_4-N levels increased. Maximum values reached 6.5 mg kg^{-1} on 4 June 2008. It seems that the activity of the nitrifying bacteria stopped first as the soil moisture decreased, rather than the activity of the microorganisms responsible for the ammonification of the organic substrates, allowing for a slight accumulation of NH_4^+ in soil. The increase of NH_4^+ in the soil as the soil moisture decreases is a well documented phenomenon as previously reported by Harris (1988). The accumulation of NH_4^+ in the soil was possible since no alive vegetation was present in the pasture at that time. However, the accumulation of NH_4^+ in this soil would not be an environmental concern regarding NH_3 volatilization due to the low soil pH (Table 1). According to Schmidt (1982) the amount of N in non-ionized forms (NH_3) is insignificant at pH below 7.

Soil nitrate levels were very low during all the experimental period, in comparison to the values that had been recorded in agricultural fields (Rodrigues *et al.*, 2002; Rodrigues, 2004) or even in other pastures (Sigua *et al.*, 2010). The low soil nitrate levels found in the pastures indicates that N availability is an important limiting factor for the growth of the non-leguminous component and may also justify the good persistence of the legume species in the BLRP. Furthermore, the risk of nitrate leaching would be insignificant, since one of the most important prerequisites is not met, *i.e.,* the presence of high soil NO_3-N levels (Stevenson and Cole, 1999). Available soil nitrate is also the primary factor limiting biological denitrification (Luo *et al.*, 2000; Pu *et al.*, 2001), allowing the exclusion of denitrification as a current environmental concern.

The DM yields of turnip and rye grown in the micro-plots in the field were very low in comparison to the values reported for these crops grown in well established stands (Jung *et al.*, 1986; Jedel and Salmon, 1995; Moreira, 2002). The low turnip and rye DM yields agreed with the low inorganic-N levels measured directly by soil coring. The growing plants showed marked symptoms of N deficiency, such as small leaves (turnip), poor tillering (rye) and chlorosis (both crops). Both crops showed a very poor N nutritional status, if plant N concentrations were compared to the sufficiency ranges published for these species. The sufficiency range of N for turnip is between 3.5-5.0% in the middle of growing season, analyzing the most recent fully developed leaves (Mills and Jones, 1996). In this study, plant N concentration was close to 1.5%, a very low value even taking into account that the standard for tissue selection was not rigorously followed. The sufficiency range for rye is 4-5% N at panicle initiation, analyzing whole tops (Mills and Jones, 1996). For rye, the standard for tissue selection was followed and the plant N concentrations were in the range of 0.9-1.1%. Plant N recovery was also very low since it was estimated from DM yield and plant N concentration. The lack of significant differences in DM yields, plant N concentrations and plant N recoveries between the three locations of the BLRP and the unsown pasture is the definitive evidence of the low soil available N even in the BLRP. This also means that the transfer of N from legume to the non-legume species would be very low, in line with previous findings that the release of N from alfalfa and subsequent uptake by ryegrass was lower than 10 kg N ha^{-1} during two years in mixed swards (Hardarson *et al.*, 1988). The very low abundance of non-legume plants in the BLRP also supports the thesis that the transfer of N from the legume to the non-legume component was low. However, the present study was not designed to provide a definitive statement about the N transfer from legume to non-legume species in BLRP. In other studies it has been demonstrated that legumes are capable of transferring significant amounts of fixed N to grasses (Gebhart *et al.*, 1993; Viera-Vargas *et al.*, 1995).

The experimental variability associated with the DM yield among individual turnip micro-plots was particularly high. This was probably due to urine patches that increased soil N availability in the proximity of the rooting zone of those particular plots. The phenomenon was less evident in rye, probably due to the lower soil moisture and higher temperature in spring which would promote the rapid evaporation of urine, limiting the inorganic-N to reach the rooting zone. However, this would not represent necessarily an environmental problem since the soil inorganic-N levels were generally low and the extra NH_4-N and NO_3-N would be quickly absorbed by the growing plants. In addition, the soil acidity may prevent NH_3 volatilization.

The DM yield in the pot experiment was significantly higher in the soil samples collected in the BLRP in comparison to the native pasture. In addition, the C:N ratio of soil samples was significantly lower in BLRP. In the pot experiments, several environmental variables regulating the mineralization pattern of organic substrates (aeration, temperature, soil moisture...) were quite different to those found in the field. The better aeration and soil moisture and a high temperature usually increase soil microbial activity. Taking into account that the lower C:N ratio favors net N mineralization (Stevenson and Cole, 1999), it was possible, from the pot experiment, to obtain significant differences in N release from organic residues when the soil samples of the BLRP and the unsown pasture were compared. The higher amount of N mineralized from BLRP soil samples in the pot experiments was probably due to the higher amount of debris and roots of the legume component.

Most of the chemical laboratory indices showed positive linear relationships with N recovered by ryegrass, in particular the C:N ratio. Since these methods have been successfully used to predict N release from soils (Gianello and Bremner, 1986; Jalil *et al*., 1996; Soon *et al*., 2007; Sharifi *et al*., 2008), the result strengthens the thesis that the potentially available soil N has increased in the BLRP.

In summary, it can be concluded that the soil inorganic-N levels were very low in both BLRP and unsown pasture, indicating that N was an important limiting resource for the non-leguminous plant species' growth. No signs of significant risks of N losses (nitrate leaching, denitrification or NH_3 volatilization) were observed in these pastures. Seven years after the BLRP had been sown it appears to be a very environmentally sound agro-system. The C:N ratio of soil is decreasing and the potentially available soil N is increasing in BLRP, probably due to the increase in the debris and roots of the legume species, which underlines the need for further analysis of the soil N dynamics at larger time intervals after establishment of the BLRP. The nitrophilic plants sown in the pasture seemed to be a promising tool for monitoring N dynamic in the field, in spite of its apparent sensitivity to the urine patches.

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