

Fertilization response and nitrogen nutrition diagnosis of a natural grassland in Southern Brazil

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Abstract: Natural grasslands found in Campos Biome in Southern Brazil are the main forage source for production approximately 13 million cattle and 5 million sheep. The forage yield is often limited by the soil fertility, which is naturally low. This study aimed to test the response of natural grassland to fertilization and to assess the level of N nutrition index (NNI) achieved with fertilization by using a dilution curve model. The experimental design was a factorial arrangement in sub-divided plots, where the landscape was considered the main plot and fertilization the subplots, with three replicates. In the fertilized treatment were applied 310 kg ha⁻¹ of N, 160 kg ha⁻¹ of P₂O₅, 160 kg ha⁻¹ of K₂O, and 3 Mg ha⁻¹ of dolomitic lime. No fertilization was applied in the non-fertilized treatment (natural soil fertility). Herbage mass was evaluated over 224 days of growth by harvesting all the aboveground plant biomass in a 0.25 m² quadrat. Herbage mass was higher in the fertilized treatment from 180 to 224 days of growth. At 180 days of growth, herbage mass was 7.8 Mg ha⁻¹ in the fertilized treatment and 3.0 Mg ha⁻¹ in the non-fertilized treatment. Generally, herbage mass accumulation from 83 to 180 days of growth was 3.2 times as high in the fertilized (62 kg ha⁻¹ day⁻¹) than in the non-fertilized treatment (20 kg ha⁻¹ day⁻¹). The N dilution curve model was successfully tested under high and low soil fertility conditions. At 83, 109, and 136 days of growth in the fertilized treatment, the nitrogen nutrition index (NNI) was 95, 103 and 92, respectively; while in the non-fertilized treatment, the NNI was always lower than 52. Therefore, the level of nitrogen nutrition index (NNI) estimated by the N dilution curve model was a good indicator of N availability to the natural grassland, and can be used for assessing the N nutrition status during the growth of natural grassland in Southern Brazil.

Keywords: Campos Biome, herbage mass production, N dilution curve.

Resposta à fertilização e diagnose de nutrição com nitrogênio em uma pastagem natural no Sul do Brasil

Resumo: As pastagens naturais encontradas no Bioma Campos do Sul do Brasil são a principal fonte de forragem para produção de aproximadamente 13 milhões de bovinos e 5 milhões de ovinos. O rendimento forrageiro dessas pastagens é muitas vezes limitado

pela fertilidade do solo, que é naturalmente baixa. Este estudo teve como objetivo avaliar a resposta de uma pastagem natural à fertilização e avaliar o nível de nutrição de N alcançado com a fertilização utilizando um modelo de curva matemático de diluição N. O desenho experimental foi um arranjo fatorial em parcelas subdivididas, onde a paisagem foi considerada a parcela principal e fertilização das sub-parcelas, com três repetições. No tratamento fertilizado foram aplicados 310 kg ha⁻¹ de N, 160 kg ha⁻¹ de P₂O₅, 160 kg ha⁻¹ de K₂O e 3 Mg ha⁻¹ de calcário dolomítico, enquanto nenhuma fertilização foi aplicada no tratamento não-fertilizado. A massa de forragem foi avaliada em 224 dias de crescimento colhendo toda a biomassa da planta acima do solo contido em uma área de 0,25 m². A massa de forragem foi maior no tratamento fertilizado dos 180 aos 224 dias de crescimento. Aos 180 dias de crescimento, a massa de forragem foi de 7,8 Mg ha⁻¹ no tratamento fertilizado e 3,0 Mg ha⁻¹ no tratamento não-fertilizado. A acumulação de massa de forragem de 83 até os 180 dias de crescimento foi cerca de 3,2 vezes maior no tratamento fertilizado (62 kg ha⁻¹ dia⁻¹) do que no tratamento não-fertilizado (20 kg ha⁻¹ dia⁻¹). O modelo de curva de diluição de N foi testado com sucesso em condições de alta e baixa fertilidade do solo. Aos 83, 109 e 136 dias de crescimento no tratamento fertilizado, o índice de nutrição de nitrogênio (INN) foi 95, 103 e 92, respectivamente, enquanto que no tratamento não-fertilizado a INN foi sempre menor que 52. Portanto, o índice de nutrição nitrogenada (NNI) estimado pelo modelo de curva de diluição de N foi um bom indicador da disponibilidade de N para as pastagens naturais e pode ser utilizado para avaliar o estado nutricional de N durante o crescimento de pastagens naturais no Sul do Brasil.

Palavras-chave: Bioma Campos, produção de massa de forragem, curva de diluição de N.

Introduction

In Southern Brazil, Campos grasslands cover 176,496 km² of the national territory and has more than 3,000 plant species, of which 450 grasses species, and they are the main forage source for production approximately 13 million cattle and 5 million sheep (CARVALHO and BATELLO, 2009). Nonetheless, this natural forage resource has poor natural soil fertility and, therefore, low production potential is attributed to this system, which resulted in the conversion of approximately 58% of natural grasslands into cropfields and forest plantations (HASENACK et al., 2010).

Fertilizing these grasslands increases production of herbage mass (HM). Nitrogen is one of the nutrients that most limits the forage production of natural grasslands and due to its high mobility in the soil, especially in the form of NO₃⁻, it is important that the amount of

N applied does not exceed the requirement for plant growth in order to reduce production costs and to avoid environmental contamination. Therefore, it is important to generate diagnostic tools to evaluate the nutritional state of these grasslands.

The amount of available N in the soil depends largely on organic matter and vegetal residues mineralization, which changes according to the seasonal environmental conditions (temperature, humidity), hampering the assessment of the N availability precisely throughout the growing period. To overcome this problem, diagnostic tools based on plant N deficiency were developed in France by Lemaire and Salette (1984). They demonstrated that for grasses, decrease in plant N concentration was related to plant dry matter accumulation, regardless of weather conditions of the year, species or genotype.

This decrease in N concentration was described by a negative power function called the “dilution curve”, defined as a critical N concentration representing the minimum N required for maximum growth (BÉLANGER and GASTAL, 2000). This approach was tested for ryegrass in Argentina (MARINO *et al.*, 2004), maize in China (LIET *et al.*, 2012), and for C4 species (LEMAIRE *et al.*, 2008). Duru and Ducroq (1996) also tested this approach in permanent pasture with C3 species. However, to our knowledge, the critical N dilution curve has never been tested for natural grasslands dominated by C4 species, and a validation is required for the characteristics of natural grasslands in Southern Brazil.

Therefore, the objectives of this study were (i) to test the fertilization-response of natural grasslands and (ii) to assess the level of N nutrition achieved with N fertilization by using the model of the N dilution curve for grasslands in Southern Brazil.

Material and methods

2.1. Site description

The experiment was carried out from August 2006 to March 2007 on a natural grassland of Restinga Seca, in Rio Grande do Sul State, Southern Brazil (29°48'46" S and 53°22'30" W). Climate is subtropical humid Cfa according to the Köppen classification. The site is located 95 m above sea level (a.s.l.), with a mean annual rainfall of 1,769 mm. Mean monthly air temperature in the summer (December–February) is 24.2°C, and 14.5 °C in the winter (June–August), with few frosts between May and August (Figure 1). The soils were classified as “Argissolo Vermelho Distrófico arênico” in well-drained slope positions and as “Argissolo Bruno-Acinzentado Alítico úmbrico” in poorly-drained lower areas in the landscape, according to the Brazilian System of Soil Classification (EMBRAPA, 2013), and as sandy Acrisols according to the World Reference Base for Soil Resources (WRB) (IUSS WORKING GROUP WRB, 2006).

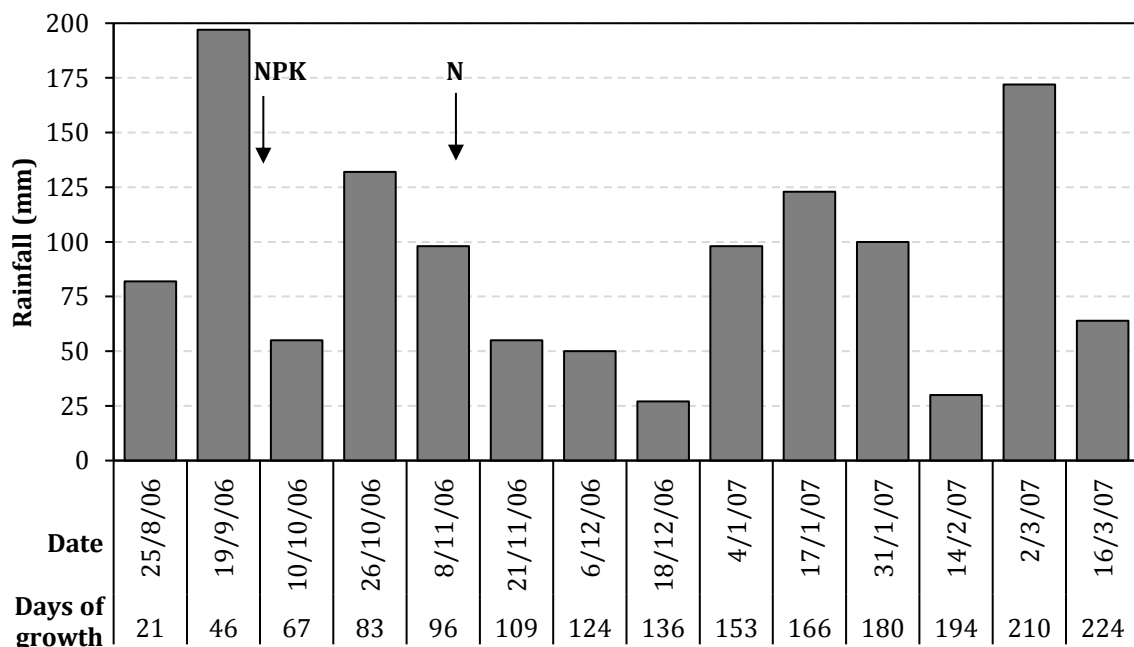


Figure 1. Accumulated precipitation for each sampling period. Arrows indicate fertilizer application dates.

The grasslands in the region have been used for more than 50 years for livestock production in an extensive system, receiving no fertilization or liming, resulting in low forage allowance during the winter and lush growth during the summer. Mainly grass species (65%), senescent material (28%), legumes (1%), and others (6%) composed the aboveground plant biomass of the experimental area. The C4 grass species with greatest contribution to the grassland were *Paspalum notatum* and *Andropogon lateralis* on slopes and *Axonopus affinis*, *Paspalum pumilum*, *Setaria glauca*, *Paspalum urvillei*, and *Paspalum pauciciliatum* in the lower parts of the landscape.

2.2. Experimental design

The experimental design was a factorial arrangement in sub-divided plots, where the landscape was considered the main plot and fertilization the subplots. The landscape positions chosen were a *slope area* at 88 a.s.l. (29°48'23" S; 53°24'6" W) and a *lowland area* at 64 a.s.l. (29°47'40" S; 53°24'3" W). In each landscape position, each fertilization treatment was performed over an area of 40 m² in three replicates. The fertilization treatments were one with fertilization (*fertilized*) and another without fertilization (*non-fertilized*).

The non-fertilized treatment received neither fertilizers nor lime. On 2 December 2005, the fertilized treatment received 30 kg ha⁻¹ of N, 120 kg ha⁻¹ of P₂O₅, 120 kg ha⁻¹ of K₂O, and 3 Mg ha⁻¹ dolomitic lime. Grazing continued on experimental plots until 4 August 2006, when the plots were fenced off to exclude cattle. On 10 October 2006 (67 days of growth), the fertilized treatment received 145 kg ha⁻¹ of N, 40 kg ha⁻¹ of P₂O₅, and 40 kg ha⁻¹ of K₂O. On 14 November 2006 (102 days of growth), the fertilized treatment received 135 kg ha⁻¹ of N.

Therefore, total amount of fertilizers was 310 kg ha⁻¹ of N, 160 kg ha⁻¹ of P₂O₅, and 160 kg ha⁻¹ of K₂O. The application of P, K, and liming in the fertilized treatment was performed to obtain the maximum response capacity of the natural pasture to the nitrogen fertilization, simulating an optimal soil fertility condition.

2.3. Soil sampling and analysis

Soil samples were taken in November 2006 and March 2007 in the 0–10 cm soil layer. Soil samples were air dried, sieved through 2 mm mesh, and stored for further analysis. Soil pH was determined in water suspension (1:1, v/v). Exchangeable Al³⁺, Ca²⁺, and Mg²⁺ were extracted by 1.0 mol L⁻¹ KCl (1:10, v/v). Exchangeable Al³⁺ was determined by titration with standardized 0.0125 mol L⁻¹ NaOH solution and Ca and Mg by atomic absorption spectroscopy. Available P and K were extracted by Mehlich-1 (1:10, v/v), and then K was measured by flame photometer and P was colorimetrically (MURPHY and RILEY, 1962). The potential acidity (H⁺ + Al³⁺) was calculated based on SMP index as proposed by CQFS-RS/SC, (2016). Effective cation exchange capacity (CEC_{ef}) was calculated as the sum of Al³⁺ + Ca²⁺ + Mg²⁺ + K⁺. Cation exchange capacity at pH 7.0 (CEC_{pH7}) was calculated as the sum of (H⁺ + Al³⁺) + Ca²⁺ + Mg²⁺ + K⁺. Aluminum saturation (m) was calculated as follow: $m (\%) = Al / (CTC_{ef}) \times 100$. Base saturation (V) was calculated as follow: $V (\%) = (Ca + Mg + K) / (CTC_{pH7}) \times 100$.

2.4. Herbage mass assessment

Growth evaluation began on 4 August 2006 (when the plots were excluded from grazing). Herbage mass (HM) was measured at 21, 46, 67, 83, 96, 109, 124, 136, 153, 166, 180, 194, 210, and 224 days of growth, respectively (Figure 1). Evaluation of HM was performed by harvesting the

aboveground shoots of all plants in 0.25 m². The harvested area was then staked to avoid resampling the same area. Each subsample was weighed and separated into green and senescent material (SM). The harvested herbage was dried at 60°C in a forced-air oven for 72 h. HM accumulation (HMA) was calculated as the HM available at the end of each period subtracted from the HM available at the end of the previous period divided by the number of days between periods.

2.5. N dilution model and N nutrition index

N concentrations in the shoots were determined by the micro Kjeldahl method following protocol described by Tedesco *et al.* (1995). N dilution in shoot biomass of the natural grassland was predicted with the following model:

$$N(\%) = b_0 \times (HM^{-b})$$

where b_0 is the initial N concentration in shoot biomass when HM of the grassland reaches 1 Mg ha⁻¹, and $-b$ is an N-dilution coefficient. To calculate the N nutrition index (NNI), we used the evaluations after 83 growth days, when HM exceeded 1 Mg ha⁻¹. The final evaluations (210 and 224 days of growth) were not used because the pasture was composed mainly by senescent material. The critical N content (N_c) was estimated by applying the N dilution curve developed by Cruz and Lemaire (1996) for C4 plants:

$$N_c(\%) = 3.6 \times (HM^{-0.34})$$

From this model, NNI was calculated as follow:

$$NNI = \frac{N(\%)}{N_c(\%)} \times 100$$

where $\%N$ is the percentage of N in shoot biomass and $\%N_c$ is the percentage of N predicted by the critical curve for identical biomass (DURU and DUCROQ, 1996).

2.6. Statistical analysis

For analysis of variance (ANOVA) of the soil parameters, HM and SM data in each growth period, the following statistical model was used:

$$Y_{ijk} = \mu + \alpha_i + \mathcal{R}_j(\alpha_i) + \beta_k + (\alpha\beta)_{ik} + \varepsilon_{ijk}$$

where μ = the overall experimental average, α_i = effect of landscape position factor ($i = 1, 2$), $\mathcal{R}_j(\alpha_i)$ = error a (effect of replication within the landscape position factor), β_k = effect of fertilization factor ($k = 1, 2$), $(\alpha\beta)_{ik}$ = effect of interaction between landscape position and fertilization, ε_{ijk} = residual random error. When effects of treatments were significantly different ($p < 0.05$) according to the F test, differences between treatment means were compared using the Tukey test ($p < 0.05$).

Results and discussion

3.1. Soil properties

There was no effect of the landscape on the soil properties (data not shown). However, some soil chemical properties were modified in the fertilized treatment compared to the non-fertilized (Table 1), mainly those related to soil acidity parameters due to the application of lime. Accordingly, the fertilized treatment resulted in higher soil pH, higher contents of base saturation (4-fold increase), exchangeable Ca and Mg (Table 1). On the other hand, fertilization decreased exchangeable Al, Al saturation (4-fold decrease), and potential acidity (H+Al). Moreover, K availability also increased 1.8-fold due to fertilization.

Table 1. Average of soil chemical characterization of 0–10 cm soil layer for two sampling periods (2006 and 2007) after fertilization of a natural grassland from Southern Brazil.

Soil property	Fertilized	Non-fertilized
Clay (g dm ⁻³)	21 a	21 a
Organic matter (g dm ⁻³)	2.1 a	2.1 a
Soil pH H ₂ O (1:1)	4.8 a	4.3 b
Base saturation (%)	47 a	11 b
Aluminum saturation (%)	18 b	74 a
Al (cmol _c dm ⁻³)	0.5 b	2.8 a
Ca (cmol _c dm ⁻³)	2.6 a	0.8 b
Mg (cmol _c dm ⁻³)	0.7 a	0.2 b
H + Al (cmol _c dm ⁻³)	4.0 b	8.0 a
P (mg dm ⁻³)	2.2 a	3.1 a
K (mg dm ⁻³)	58 a	32 b
CEC _{ef} (cmol _c dm ⁻³)	3.9 a	3.9 a
CEC _{pH7} (cmol _c dm ⁻³)	7.4 a	8.9 a

Means followed by the same letter in the row are not different by Tukey test at $p < 0.05$.

3.2. Herbage mass assessment

There was also no effect of the landscape on HM accumulation (data not shown). From 21–109 days of growth, HM was similar between fertilization treatments (Figure 2a). However, from 124–194 days of growth, HM was generally 2.6 times as high in the fertilized as in the non-fertilized treatment. At 180 days of growth, HM was 7.8 Mg ha⁻¹ in the fertilized treatment and 3.0 Mg ha⁻¹ in the non-fertilized treatment. There was a decrease on HM on the sampling dates after 180 days of growth, and the amount of HM per hectare was similar between the fertilized and non-fertilized treatments on the last two sampling periods. On average, HMA from 83–180 days of growth was 3.2 times as high in the fertilized (62 kg ha⁻¹ day⁻¹) as in the non-fertilized treatment (20 kg ha⁻¹ day⁻¹). From 21–166 days of growth, SM was similar between the treatments (Figure 2b), after that, SM was generally 2.9 times as high in the fertilized as in the non-fertilized treatment.

Usually, annual production of HM without fertilization at experimental sites in Southern Brazil natural grasslands ranges from 3.8 Mg ha⁻¹ year⁻¹ (SOARES *et al.*, 2005) to 9.8 Mg ha⁻¹ year⁻¹ (PELLEGRINI *et al.*, 2010). In our study, the grassland with fertilization reached 8 Mg ha⁻¹ in 180 days, while the non-fertilized grassland produced only 3 Mg ha⁻¹. These results agree with experiments conducted by Siewerdt *et al.* (2001) and Sallis and Siewerdt (2000), who observed yields of 5.3 and 3.9 Mg ha⁻¹ in 166 and 180 days of growth, respectively, without applying fertilizer. When applying 100, 200, and 300 kg ha⁻¹ of N, Siewerdt *et al.* (2001) reported HM yields of 8.2, 9.7, and 10 Mg ha⁻¹, respectively. These results demonstrate the high response of Campos grasslands to fertilization, highlighting that this natural ecosystem is highly productive and can even compete with other agricultural system, ensuring the conservation of natural grasslands in Southern Brazil (TIECHER *et al.*, 2014; OLIVEIRA *et al.*, 2015).

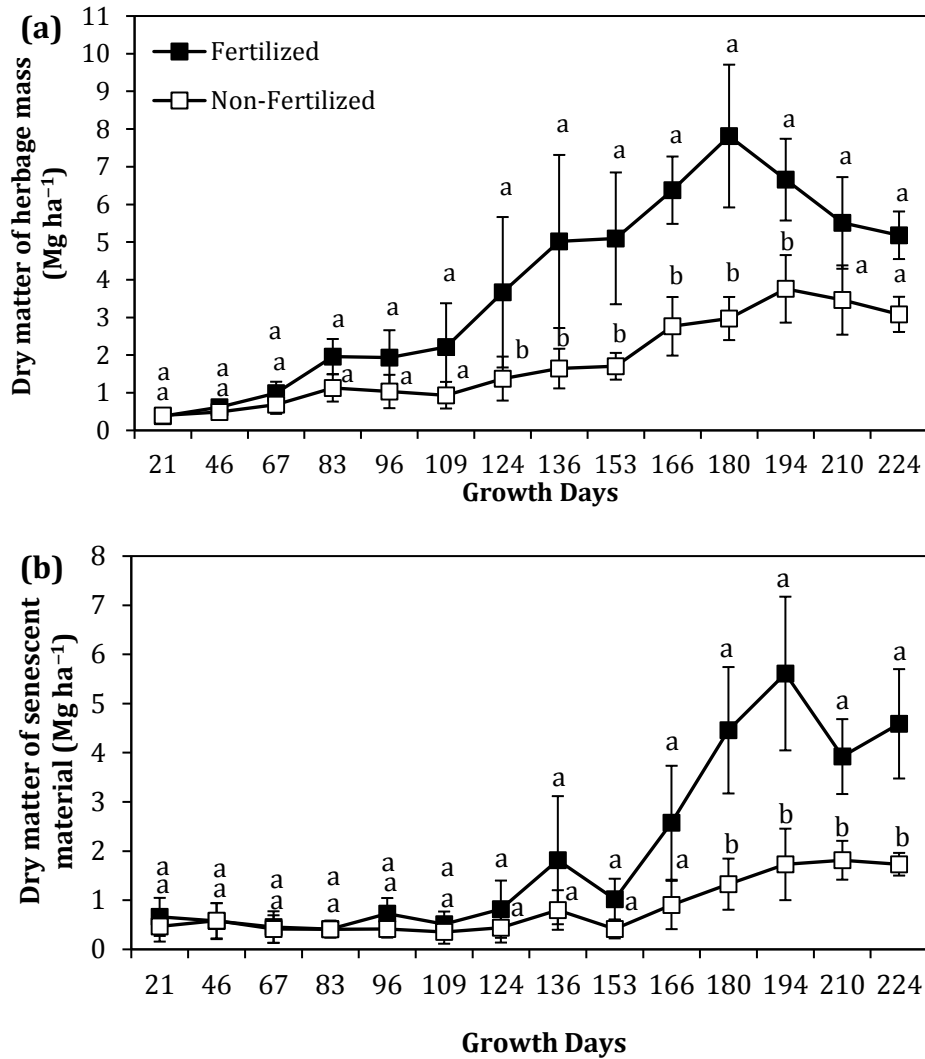


Figure 2. Herbage mass (a) and senescent material mass (b) as a function of growth. Means \pm 1 standard deviation. Means followed by the same letter in the growth period are not different by Tukey test at $p < 0.05$.

3.3. N dilution model and N nutrition index

Dilution model and nitrogen nutrition index (NNI) in shoot biomass of the natural grassland fit well to the general model $N = b_0 \times HM^{-b}$, with adjusted R^2 equal to 0.74 and 0.93 for non-fertilized and fertilized treatments, respectively (Figure 3a). Initial N availability (b_0) in shoot biomass was 2.8 times as high in the fertilized as in the non-fertilized treatment ($p = 0.042$). The shoot biomass N dilution coefficient (b) was 1.7 times higher in the fertilized treatment compared to the non-fertilized

treatment ($p = 0.001$). At 83, 109, and 136 days of growth in the fertilized treatment, the NNI exceeded 80, and it decreased at 153 and 166 days of growth, remaining above 66 (Figure 3b). In the non-fertilized treatment, the NNI never exceeded 52.

Reduction in N concentration with growth and herbage mass accumulation over time has been reported extensively in many studies (DURU and DUCROCQ 1996; LEMAIRE *et al.*, 2008; LI *et al.*, 2012). The decrease in N concentration is mainly due to two reasons. First, self-shading of leaves induces a non-uniform leaf N concentration, with high N

concentrations in upper canopy layers and low N concentrations in shaded layers (GASTAL and LEMAIRE, 2002). Second, the proportions of plant

structural and storage tissues, which have lower N concentrations, increase in total plant biomass.

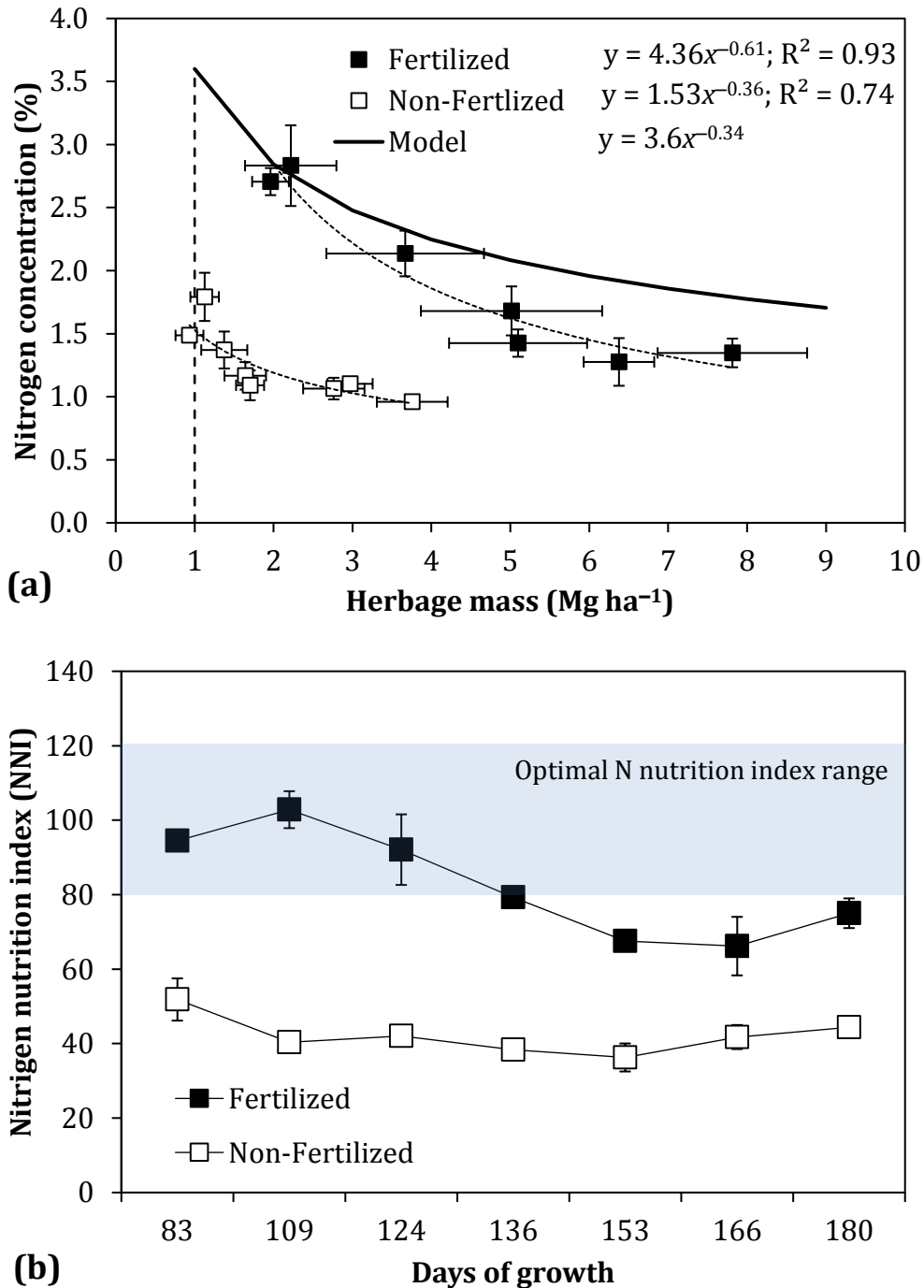


Figure 3. Nitrogen concentration dilution curve (a) as a function of herbage mass (HM) ($N = 3.6 \times HM^{-0.34}$, CRUZ and LEMAIRE, 1996). Nitrogen nutrition index (b) (NNI) as a function of growth days. Means \pm 1 standard deviation. Blue zone indicates optimal N nutrition index range.

Our data indicate that HM higher than approximately 1 Mg ha⁻¹ in the fertilized treatment lay above the critical N dilution curve proposed by Cruz and Lemaire (1996). However, when HM exceeded 4 Mg ha⁻¹, especially in the non-fertilized treatment, it lay below the critical curve (Figure 3a). In the first growing stage (*i.e.* approximately 1 Mg ha⁻¹) in the fertilized treatment, initial N availability (parameter $b_0 = 4.36$) was slightly higher than that considered as critical by the N dilution curve proposed by Cruz and Lemaire (1996), and much higher than that observed in the non-fertilized treatment ($b_0 = 1.53$). However, the coefficient dilution in the fertilized treatment was lower in our model ($b = -0.61$) compared to Cruz and Lemaire (1996) ($b = -0.34$), indicating higher dilution of N with increasing HM.

The model of optimal N dilution describes N availability when N does not limit crop growth, which results in maximum growth rate (BÉLANGER and GASTAL, 2000). In this sense, the NNI estimates N deficiency by the relation between the actual N concentration and the optimal N concentration predicted by the model (LEMAIRE *et al.*, 2008). The NNI for the fertilized treatment indicates that N supply was satisfactory from 83–136 days of growth because NNI exceeded 80, which may be considered as a high level of N nutrition (NNI = 100±20 is optimal) (Figure 3b). This value was not observed for any period in the non-fertilized treatment, demonstrating that the soil naturally has the capacity to supply only 40% of N required by natural grasslands for maximum growth rate. Thus, the non-fertilized grassland grew 20 kg ha⁻¹ day⁻¹, while the fertilized pasture was able to grow approximately 62 kg ha⁻¹ day⁻¹. These results demonstrate that growth rates of 12–15 kg ha⁻¹ day⁻¹ observed in other studies of grasslands from Southern Brazil

(MOOJEN and MARASCHIN, 2002; SOARES *et al.*, 2005; SANTOS *et al.*, 2008; CARASSAI *et al.*, 2008) are below the potential capacity of herbage mass accumulation of Campos grasslands. This was also demonstrated by Berreta *et al.* (1998), Boggiano (2000) and Guma (2005), who reported growth rates up to 48, 54, and 84 kg ha⁻¹ day⁻¹ with N fertilization.

After 109 days of growth, the NNI dropped to values below the optimal, indicating that there was deficiency of N in the fertilized treatment (Figure 3b). In order to achieve higher herbage mass, another application of N could be necessary after 136 days of growth. Another possibility could be splitting the second application (performed at 102 days of growth) into two or three applications to ensure a better absorption efficiency by the grassland. These results highlight that the NNI based on the N dilution curve model could be used as a tool for fertilizing programs of natural grassland in Southern Brazil.

Conclusions

The natural grasslands in Southern Brazil are highly responsive to fertilization. Application of lime, N, P, and K increased three times the growth rate compared to the natural condition. The N dilution curve model was successfully tested under high and low soil fertility conditions. The level of nitrogen nutrition index (NNI) estimated by the N dilution curve model was a good indicator of N availability to the natural grassland, and can be used for assessing the N nutrition status during the growth of natural grassland in Southern Brazil.

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