Revista Brasileira de Ciência do Solo

Division - Soil Use and Management | Commission - Soil Fertility and Plant Nutrition

Phosphate sources affect P and N nutrition in pluri-specific natural grasslands in the Brazilian Pampa biome

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Received: November 09, 2021

Approved: May 06, 2022

How to cite: Oliveira LB, Tiecher T, Marques ACR, Somavilla A, Ruthes BES, Crestani C, Kaschuk G, Rheinheimer DS, Quadros FLF. Phosphate sources affect P and N nutrition in pluri-specific natural grasslands in the Brazilian Pampa biome. Rev Bras Cienc Solo. 2022;46:e0210145.
https://doi.org/10.36783/18069657rbcs20210145

Editors: José Miguel Reichert and Leandro Souza da Silva.

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ABSTRACT: High acidity and low soil P availability in the soils from the Pampa natural grasslands generally limit the forage growth and economic return. Thus, to increase the economic return and avoid environmental degradation and biodiversity loss due to replacement by intensive cropping systems, it is essential to improve the soil P availability. This study aimed to diagnose the nutritional status of the pluri-specific natural grasslands amended with different history of P sources. Additionally, we also try to indicate the most appropriate range of soil P availability for maximizing forage growth. The experiment started in 1997 in an area of Pampa natural grasslands in the state of Rio Grande do Sul, Brazil. The treatments evaluated the application of Gafsa hyperphosphate (HP) and triple superphosphate (SP) for four years (1997, 1998, 2002, and 2010). Soil and aboveground biomass sampling were performed five times from October 2010 to March 2011 to access aboveground biomass production, botanical composition, soil available P, and N and P nutrition index. Greater P availability in the soil amended with SP produced more forage and resulted in a higher P accumulation than in the soil amended with HP. The ideal range of soil P availability in the soils of natural grasslands ranged from 14 to 20 mg kg^{-1}, varying for different plant species. The species *Paspalum plicatulum* and *Aristida laevis* produce less aboveground biomass and accumulate less P in their tissue than *Dichantelium sabulorum* and *Eustachys uliginosa*, and *P. notatum*. Therefore, areas of natural grasslands dominated by *D. sabulorum*, *E. uliginosa* and *P. notatum* demand higher soil P availability to maintain high forage production. Soil P fertilization of pluri-specific natural grasslands in the Brazilian Pampa biome must consider the dominant forage species in the area and the soil P availability.

Keywords: nitrogen nutrition index, phosphorus nutrition index, sandy Acrisols, triple superphosphate, Gafsa hyperphosphate.

INTRODUCTION

Pampa biome, in southern Brazil, is historically used to raise cattle, but in the last five decades, it lost more than 50 % of its original area to intensive grain crops production and forest plantations, limiting the traditional cattle ranching to less fertile soils and areas where agricultural mechanization is difficult (Roesch et al., 2009; Oliveira et al., 2017). Pampa has soils with high acidity and low nutrient availability, especially phosphorus (P), which limits the native forage growth (Oliveira et al., 2011). Both P and nitrogen (N) are the nutrients that most limit photosynthetic production in aquatic and terrestrial environments (Fay et al., 2015). Thus, to increase the economic return of natural grasslands, to avoid environmental degradation and biodiversity loss, or their replacement by cropping systems, it is essential to improve the nutritional levels of the soil, especially P.

Studies have shown that liming and P fertilization of soils under natural grasslands can be used as an alternative to increase the productivity of forage species and result in greater animal production per hectare (Gatiboni et al., 2000, 2007; Tiecher et al., 2014; Oliveira et al., 2015; Somavilla et al., 2021a). These strategies should contribute to the maintenance of natural grasslands and the biodiversity conservation of this ecosystem. Fertilization guidelines for Pampa natural grasslands are traditionally based on soil chemical analyses (CQFS-RS/SC, 2016). However, the soil analysis may be insufficient to identify the variations in nutritional needs of the different forage species that compose the Pampa vegetation. Plant diversity is often neglected when fertilization is based solely on soil analysis. In addition, the frequent use of phosphate fertilizers can favor groups of species that are more efficient in the use of nutrients and change the dynamics and diversity of the natural grasslands (Blanck et al., 2011; Ceulemans et al., 2013; Harpole et al., 2016; Somavilla et al., 2021b).

Pampa natural grasslands are mostly formed by C_4 megathermal grasses, with the occurrence of few C3 species (Boldrini, 2009). Native grasses may have different adaptive mechanisms for P uptake and use in environments limited by this nutrient (Aerts and Chapin, 1999), resulting in different P content in aboveground biomass. Furthermore, plant growth characteristics that result in differences in leaf anatomy can alter the efficiency of light interception (Marques et al., 2020a) and so, within the same metabolic efficiency group, the dilution of N and P may be different (Lemaire et al., 2019). Thus, the study of the dilution of N and P in dry matter over productive periods in different native species can improve fertilization guidelines for natural grasslands in Southern South America.

This study aimed to diagnose the nutritional P status of the pluri-specific natural grasslands amended with a different history of P sources to verify the most appropriate range of soil P availability for natural grasslands and its relationship with N nutritional status.

MATERIALS AND METHODS

Experimental site

The experiment started in 1997 in an area of natural grassland of the Pampa biome, in the Central Depression of Rio Grande do Sul, Brazil, at coordinates 29° 43’ S and 53° 42’ W, and altitude of 95 m. The vegetation is classified as Mesic Pampa grassland subtype 5b (Andrade et al., 2019). The climate is humid subtropic, Cfa, according to Köppen classification system. The average rainfall and temperature from 1981 to 2011 are shown in table 1. The soil was classified as “Argissolo Vermelho Distrófico arênico” (Santos et al., 2013), which correspond to a sandy Acrisols (Soil Survey Staff, 2014). The soil chemical properties before the establishment of the experiment are presented in table 1.

Table 1. Monthly rainfall and temperature over the study period (2010-2011) and over a 30-years period (1981-2011), and soil physical and chemical properties in the 0.10 m soil depth in natural grassland from Southern Brazil before the establishment of the experimental area

Month	Rainfall		Temperature		Soil property	
	2010-2011	1981-2011	2010-2011	1981-2011		
	Mm		°C			
September	244.9	153.6	16.7	16.6	Clay (g dm^{-3})	170
October	49.3	145.9	18.1	19.1	Soil organic carbon (g dm^{-3})	10
November	71.3	132.2	20.7	21.6	$pH(H_2O)$ (1:1)	4.5
December	157.9	133.5	24.3	24.4	Exchangeable K (mg dm^{-3})	60
January	127.1	145.1	25.6	24.7	Exchangeable Ca ($cmol_c$ dm^{-3})	1.2
February	165.8	130.2	24.6	24.7	Exchangeable Mg ($cmol_c$ dm^{-3})	0.7
March	54.9	181.7	22.5	23.0	Exchangeable Al ($cmol_c$ dm^{-3})	1.3
April	164.9	134.7	19.4	19.7	CEC_{pH7} (1) ($cmol_c$ dm^{-3})	8.0
May	54.9	129.1	15.5	16.9	V (2) (%)	40
					m (3) (%)	22

(1) Potential cation exchange capacity at soil pH 7.0 = sum of H+Al, Ca^{2+}, Mg^{2+} and K^+. (2) Cation saturation = $[(Ca^{2+}+Mg^{2+}+K^+)/(Al^{3+}+Ca^{2+}+Mg^{2+}+K^+)] \times 100$. (3) Aluminum saturation = $[Al^{3+}/(Al^{3+}+Ca^{2+}+Mg^{2+}+K^{1+})] \times 100$.

Experimental design and treatments

The experiment design is a randomized block with three replications and three treatments, which were: i) fertilization with Gafsa hyperphosphate (HP); ii) fertilization with triple superphosphate (SP); and iii) control with no fertilization. The P fertilizers were applied to the soil surface in 1997 (79 kg of P ha^{-1}), 1998 (39 kg of P ha^{-1}), 2002 (44 kg of P ha^{-1}) and 2010 (44 kg of P ha^{-1}), totaling 206 kg of P ha^{-1}. In 1997, all plots were fertilized with 108 kg of K ha^{-1}, using potassium chloride. On August 27, 2010, all plots were fertilized with 30 kg of N ha^{-1}, as urea.

During the winter season of 1997, 1998 and 2002, pastures were mowed, and ryegrass (*Lolium multiflorum*) and arrowleaf clover (*Trifolium vesiculosum*) were sown. After that, pastures were mowed once a year in the winter. The last mowing before sampling was done on August 27, 2010, when the straw debris were removed from all plots to let the pasture sprouts to grow freely.

Forage sampling and analysis

Forage biomass was sampled on 10/16/2010, 11/08/2010, 01/03/2011, 02/03/2011 and 03/01/2011, respectively, which represent 50, 83, 129, 160 and 186 days after the mowing. In each plot, a composite sample was formed with four subsamples collected from four spots measuring 0.25 m^2 each. Subsamples were taken in different spots in each sampling. The composite samples were split into seven subsamples: (i) senescing biomass, (ii) *Paspalum notatum*, (iii) *Paspalum plicatulum*, (iv) *Eustachys uliginosa*, (v) *Dichantelium sabulorum*, (vi) *Aristida laevis* and (vii) other species. Ryegrass was found only in two samples, and therefore, it was included in the “other species” group. The arrowleaf clover was not found.

Forage biomass was dried in the oven with forced air circulation at ±65 °C until reaching constant weight (approximately 72 h), and used to quantify dry matter and the chemical composition of the forage tissue. The accumulated biomass included the biomass of all forage species and the senescing biomass. The net biomass was the sum of all forage species biomasses less the senescing biomass. Net biomass was ground at 1 mm mesh and submitted to analyses of P and Kjeldahl-N (Tedesco, 1995).

Nutritional index calculations

Nutrition indices were calculated according to an equation that measures the distance between the sample and the model for maximum growth rate (Nc). The equation for nitrogen nutrition index (NNI) is equation 1.

$$NNI = \frac{\%N}{\%Nc} \times 100 \qquad \text{Eq. 1}$$

in which: %N is the N concentration (%) in the aboveground biomass of forages; and %Nc is the critical concentration of the model (Equation 2).

$$Nc = 3.6 \times NB^{-0.34} \qquad \text{Eq. 2}$$

in which NB corresponds to Net biomass (Bélanger and Gastal, 2000).

The phosphorus nutrition index was calculated using the equation 3.

$$PNI = \frac{\%P}{\%Pc} \times 100 \qquad \text{Eq. 3}$$

in which: %P is the P concentration (%) in the aboveground biomass of forages; and %Pc is the critical concentration of the model (Equation 4).

$$\%Pc = 1.5 + 0.065 \times \%N \qquad \text{Eq. 4}$$

in which: %N is the N concentration (%) in the aboveground biomass of pastures (Duru and Ducrocq, 1996).

Soil sampling and analyses

Composite soil samples were collected with eight subsamples: four subsamples were taken from two spots where forage biomasses were collected, and the other four were taken randomly within the plot. Soil samples were dried in the oven with forced air circulation at ±45 °C, ground at 2 mm mesh, and submitted to P analyses using an anion exchange resin (AER). First, 0.2 g of each soil sample, 10 mL of distilled water, and one sheet of bicarbonate-saturated AER (AR 103 QDP 434 Ionics Inc.) were put into 15 mL *falcon* tubes. Then, tubes with the suspensions were stirred in an oven-shaker for 16 h. The resin sheets were removed, washed with distilled water and eluted in 10 mL of HCl 0.5 mol L^{-1}. The inorganic P in the HCl extracts was determined according to Murphy and Riley (1962).

Statistical analyses

Data of soil P available extracted by anion exchange resin was submitted to analysis of variance (ANOVA) with the equation 5.

$$Y_{ijkl} = \mu + B_i + S_j + D_l + SD + error\ (i, j,) \qquad \text{Eq. 5}$$

in which: μ is the overall experiment average; B is the blocks (i = 1, 2, 3); S is the phosphate source (j = control, HP, SP); D is the days of growth after mowing; and *error* is the experimental error. When the treatment effects were significant at 5 % probability of error by the F test, the differences were compared by the least significant difference (LSD).

A comparison of biomass of individual native species was based on randomization tests. This approach was chosen because the distribution of the results did not meet the assumptions of normality for implementing ANOVA. The analyses tested the effects of phosphate sources, sampling periods, and the interaction between them using the Euclidean distance for dissimilarity measure.

Statistical analysis of species contribution dynamics in total forage biomass as a function of different phosphate sources and sampling periods was based on an ordination analysis by principal coordinates (PCOA), using Euclidean distance as the dissimilarity measure. The ordination analysis and the randomized tests were performed using MULTIV software (Pillar, 2004).

RESULTS

Available soil P

At 50, 83 and 129 days of growth, the available P in the HP and SP treatments was, on average, 4 and 12 times higher than in control, respectively (Figure 1). Despite the expected decrease in the content of available P over time (after 159 and 183 days), the P availability in the HP and SP treatments was, on average, 5 times greater than the control. It is important to emphasize that only at the beginning of the evaluations, in the SP treatment, the content of soil available P was close to the critical level of 20 mg kg^{-1} proposed by the regional guidelines (CQFS-RS/SC, 2004).

Forage biomass

In SP, HP and Control treatments, the net biomass had a linear growth over time (Figure 2), with constant accumulation rates of 25.0, 14.6 and 15.5 kg ha^{-1} day^{-1}, respectively. At the end of the evaluation period, the forage net biomass of plots fertilized with SP (4.5 Mg ha^{-1}) was 1.2 and 1.4 times higher than that of control (3.7 Mg ha^{-1}) and HP plots (3.3 Mg ha^{-1}), respectively (Figure 2).

Phosphorus nutrition index (PNI) and relationships of P and N in the forage biomass

The PNI of the pasture ranged from 35 to 94 among the treatments (Figure 3a). On average, the PNI of SP treatments were higher than HP, which were higher than control (Figure 3a). The concentration of P in the forage increased with the increase in the concentration of N (Figure 3b) as predicted by the model of Duru and Thelier-Huche

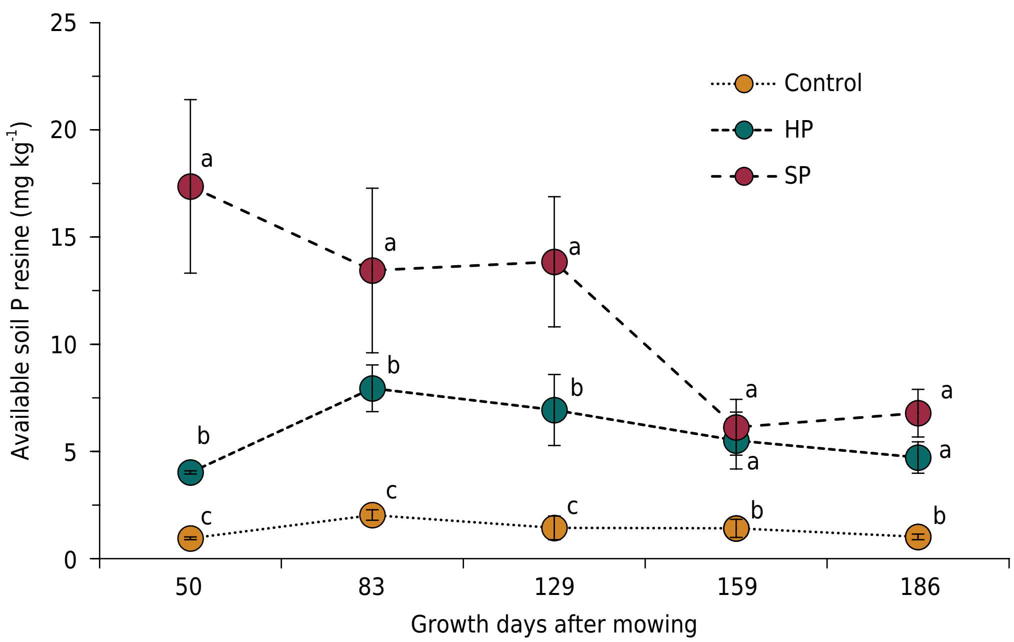

Figure 1. Soil available P over time in a natural grassland, from Southern Brazil, affected by the application of different P sources. Control: without P addition; HP: hyperphosphate; and SP: superphosphate.

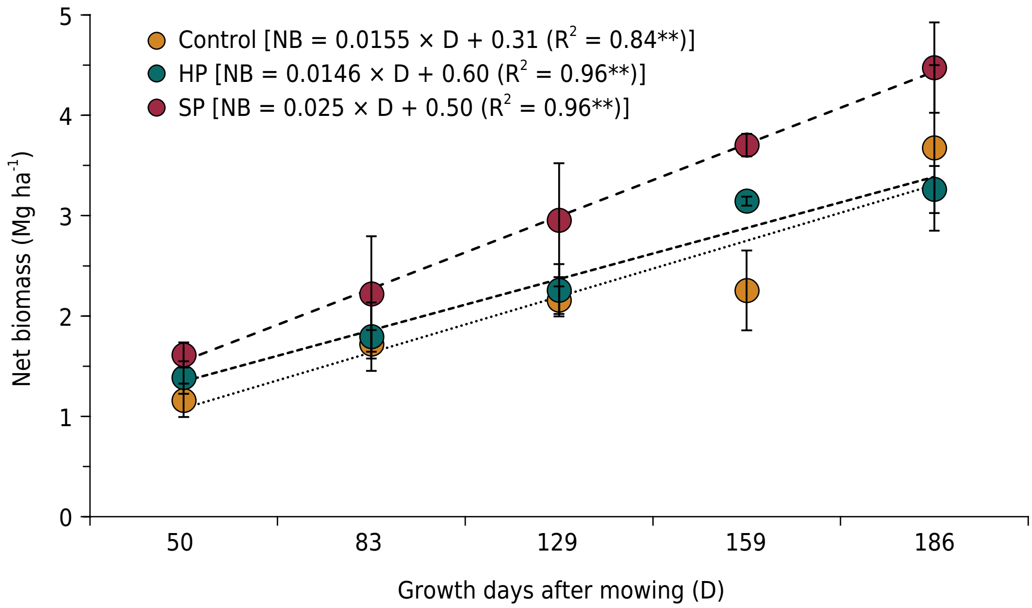

Figure 2. Net biomass (NB) over time in a natural grassland, from Southern Brazil, with the application of different P sources. Control: without P addition; HP: hyperphosphate; SP: superphosphate.

(1997). Moreover, the corresponding content of soil available P to reach 100 PNI was 17.0 mg kg^{-1} (Figure 3c).

Nitrogen nutrition index (NNI)

Nitrogen concentration in the aboveground forage decreased with the increase of the net forage biomass in all treatments, which made possible an adjustment of the data to the potential dilution model (Figure 4a). The initial availability of N (*a*-value) was higher (p<0.05) in the SP (3.2) than in the HP (2.5) and the control (2.2) treatments. However, the initial availability of N in all treatments was lower than the model proposed by Duru and Ducrocq (1996), that is, $N = 3.6 \times HM^{-0.34}$. In addition, the N-dilution coefficients (*b*-value) were similar in all treatments (average of 1.01; p>0.05) and three times bigger than the b-value in the model of Duru and Ducrocq (1996), which was 0.34.

The NNI of the pasture was similar between treatments during the period of evaluation. The maximum value of NNI was 71 in the beginning of the evaluation (Figure 4b), but it decreased over time. This means that there was a limitation of N for forage growth during the period due to the low amount of N in the soil.

Phosphorus accumulated in forage species

Phosphorous accumulated in the five forage species was plotted through a linear relationship with the amount of biomass accumulated by each species (Figure 5). In the non-fertilized plots, all species had similar biomass production responses for each unit of absorbed P, whereas in the plots fertilized with SP and HP, the species showed divergent responses. In the situation with greater soil P availability, the species *D. sabulorum* and *E. uliginosa* accumulated more P per unity of plant biomass than the species *P. plicatulum.* Moreover, *A. laevis* had lower P absorption per unity of plant biomass.

Botanical composition

There was no interaction of P source treatments and the time of samplings for all forage species (p>0.05; Table 2), and all forage species accumulated biomass linearly over time (p<0.01). This means that the species contribution increased with the total dry biomass of the pasture over the growing days and this pattern was shown in the ordering diagram

(Figure 6). The increase in mass of all treatments can be visualized by positioning the variables net biomass and the biomass of senescent material correlated 0.97 and 0.87 with the x-axis.

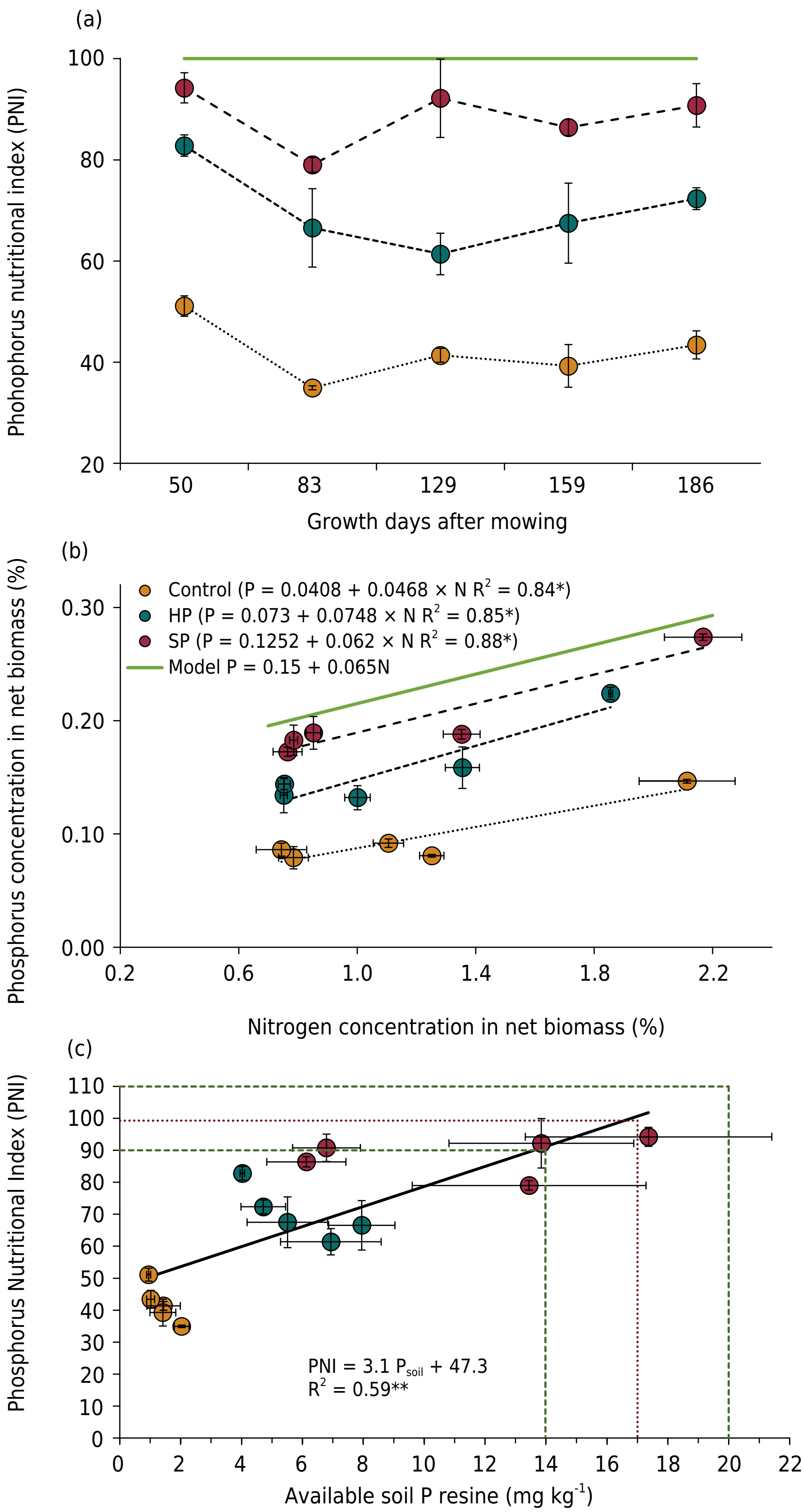

Figure 3. Phosphorus nutrition index (PNI) (a); relationship between Ps and N content in dry matter (b), relationship between available P and PNI (c) in a natural grassland from Southern Brazil with the application of different P sources. Control: without P addition; HP: hyperphosphate; SP: superphosphate. Model: model suggested by Duru and Thelier-Huche (1997).

During the growing days, the species that contribute to the mass variation of the treatments were *E. uliginosa* and “others species” [correlation coefficient (R) with x axis of 1.00 and 0.97, respectively] and the species *P. notatum* (R = 0.99 with the y axis).

Aboveground biomass of the *E. uliginosa* and the “others species” component was higher in SP than the other treatments (Table 2). The aboveground biomass of *D. sabulorum* species was higher in control. The species with the highest biomass in HP was the grass *P. notatum*. The variables NNI, PNI and Soil P (Figure 3) evidenced that SP was the treatment with greater availability of P and, consequently, higher N and P nutrition levels, especially during the beginning of the growing days.

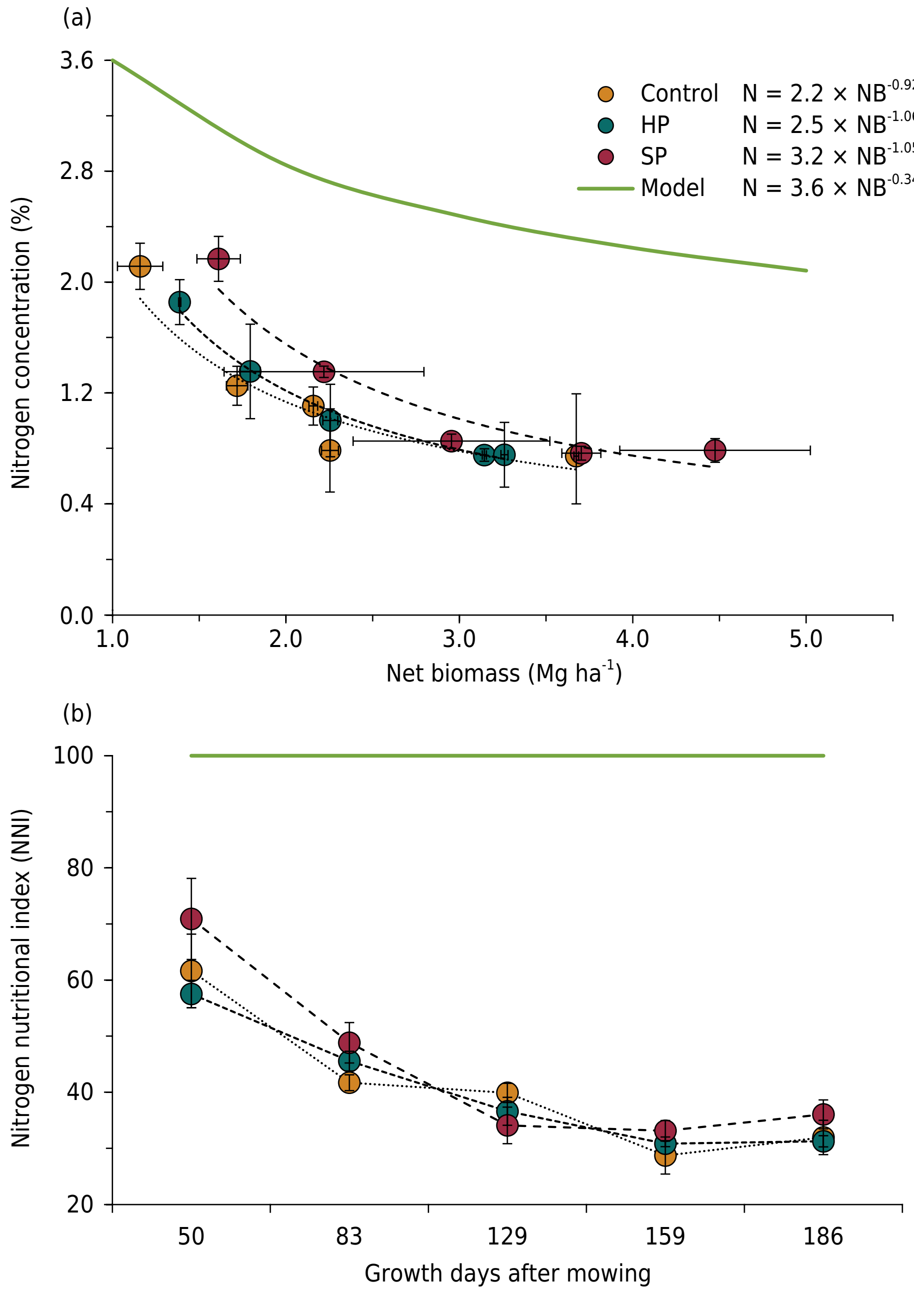

Figure 4. Nitrogen dilution curve in the aboveground biomass net accumulated (a); nitrogen nutrition index (NNI) over time (b) in a natural grassland from Southern Brazil with the application of different P sources. Control: without P addition; HP: hyperphosphate; SP: superphosphate; Model: model suggested by Bélanger and Gastal (2000).

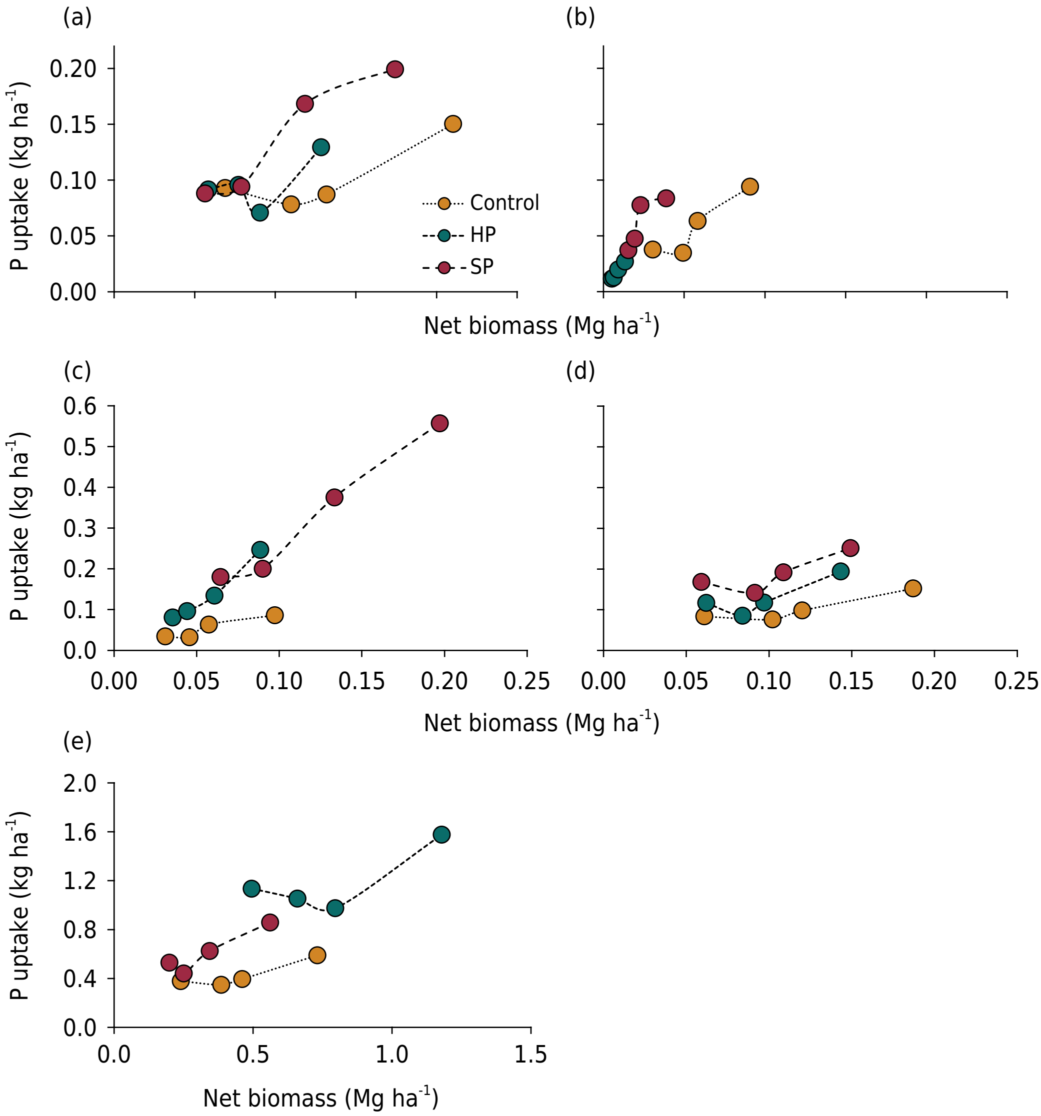

Figure 5. Relationship between P uptake and accumulated dry matter for different native species (a) *Aristida laevis*, (b) *Dichantelium sabulorum*, (c) *Eusthachys uliginosa*, (d) *Paspalum plicatulum* and (e) *Paspalum notatum* of a natural grassland from Southern Brazil with application of different P sources Control: without P addition; HP: hyperphosphate; SP: superphosphate.

Table 2. Biomass of native species in a natural grassland from Southern Brazil affected by the application of different P sources

Species	Biomass			p-values		
	Control	HP	SP	P sources (P)	Days (D)	P × D
	Mg ha^{-1}					
Dichantelium sabulorum	0.06 ^{a}	0.01 ^{c}	0.03 ^{b}	*0.001*	*0.001*	*0.928*
Eustachys uliginosa	0.06 ^{b}	0.06 ^{b}	0.13 ^{a}	*0.001*	*0.001*	*0.943*
Paspalum notatum	0.46 ^{b}	0.85 ^{a}	0.36 ^{b}	*0.001*	*0.001*	*0.254*
Paspalum plicatulum	0.12 ^{ns}	0.10	0.11	*0.889*	*0.001*	*1.000*
Aristida laevis	0.13 ^{ns}	0.10	0.11	*0.381*	*0.001*	*0.987*
Others species	1.37 ^{b}	1.24 ^{b}	2.25 ^{a}	*0.001*	*0.001*	*0.353*

Control: without P addition; HP: hyperphosphate; and SP: superphosphate.

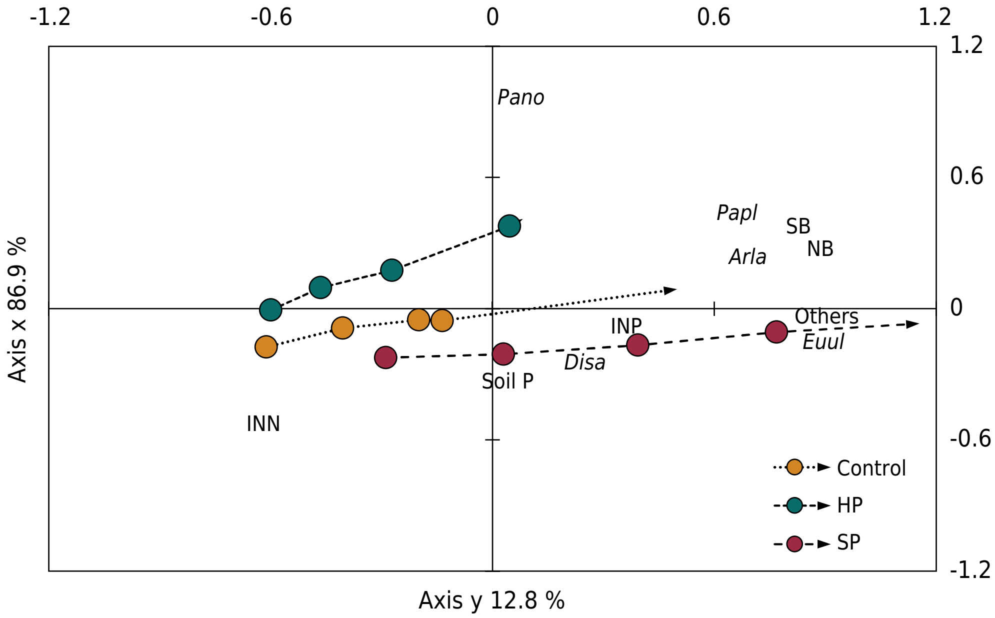

Figure 6. Ordering diagram of the variables available soil P resine (Soil P), nitrogen nutritional index (NNI), phosphorus nutritional index (PNI), net biomass (NB), senescent biomass (SB) and biomass of the main contributing species in the grassland: *Dichantelium sabulorum* (*Disa*), *Eustachys uliginosa* (*Euul*), *Paspalum notatum* (*Pano*), *Paspalum plicatulum* (*Papl*), *Aristida laevis* (*Arla*) and (Others) other species. Control: without P addition; HP: hyperphosphate; and SP: superphosphate.

DISCUSSION

Soil fertilization for increasing forage production

The application of SP in 1997, 1998, 2002 and 2010 increased the availability of P in the soil (Figure 1) and the production of aboveground biomass in 2010/2011 (Figure 2), evidencing that the growth of natural grassland species is limited by the low natural availability of P (Oliveira et al., 2011). However, the application of the same quantities of P in the form of HP did not result in the same increase in the soil available P (Figure 1). As a consequence, there was not adequate P nutrition of the natural grassland (Figure 3a), even in this soil with low Ca and pH (Table 1), probably due to the dominance of minerals such as apatite in the fertilizer, which has very low dissolution in soil even under acidic conditions (Somavilla et al., 2021b).

Furthermore, the response of the natural grassland species to the application of P may be limited by the availability of soil N (Duru and Ducrocq, 1996; Bélanger and Richards, 1999). The results of INN presented in figure 4 are distant from the literature model that describes the maximum growth rate. Although the models used here were not developed for natural grasslands in southern Brazil, this indicates that the N supplied naturally by the soil plus the addition of 30 kg ha^{-1} of N via fertilization were probably not sufficient to supply the plants' needs, resulting in an average INN of 63 (Figure 4b).

Forage nutrition and soil nutrient availability in the Pampa

The relationship of P and N in aboveground biomass (Figure 3b) demonstrated that adequate soil P availability is closely dependent on N availability, which means that high N availability needs greater P availability. Under the conditions of N supply of this study, the ideal content of soil available P, estimated by the anion exchange resin, necessary to avoid P limitation was 17.0 mg of P kg^{-1} of soil (ranging from 14 to 20 mg kg^{-1}). This result is very similar to the critical level of 20 mg kg^{-1} of P used to estimate doses of fertilizers in the region (CQFS-RS/SC, 2004).

P uptake by different forage species

All forage species had higher P uptake with the increase in the availability of soil P with the application of HP and SP (Figure 5), indicating that they responded to soil P availability, probably with different strategies regarding the P acquisition. For example, *P. notatum* in all three treatments was the species with the highest dry matter production and the highest P absorption per hectare. These observations corroborate the fact that this species, along with other species of this genus, such as *P. pumilum*, *P. urvillei* and, *P. plicatulum*, had high P use efficiency in leaves and roots for dry matter production (Marques et al., 2020b). However, *D. sabulorum* and *E. uliginosa* had the highest P concentration in the biomass, probably due to very efficient strategies of P uptake, but with low efficiency in the use to produce plant biomass.

The patterns regarding the uptake and use of P by forage species in this study corroborated the grouping patterns observed by Marques et al. (2020a, b). They found that some forage species of the Pampa are functionally grouped as specialists in acquiring soil P and accumulating more P in their tissues; whereas other plants are specialists in using more efficiently the P absorbed and produce more biomass per unity of P. By studying the mechanisms behind these patterns, Marques et al. (2021) found that forage species from the South American native grasslands with more efficient P uptake strategies also have larger root area and length per unit of root dry matter. These forage species have a higher potential to increase growth by applying P fertilizers. On the other hand, species that have more conservative strategies have thicker and denser roots (smaller root area and length per unity of root dry matter), and thus, they have a lower capacity to absorb P and to respond to increased P availability in the soil after fertilization (Marques et al., 2021).

Applying P to the naturally P-poor soil of the Pampa native grasslands can change the botanical composition due to the differences mentioned above of each plant species in acquiring and using nutrients in that environment. The functional group species specialized in capturing P may have higher rates of relative growth and higher responses to P application, whereas the functional group specialist in saving resources may have a low response to P application (Oliveira et al., 2014; Marques et al., 2020b). This hypothesis was confirmed by the contrast in the growth patterns of the species *E. uliginosa* (grew more in the SP treatment) and *D. sabulorum* (grew more in the control). The explanation could be the fact that *D. sabulorum*, which is short and small, may sprout fast in open spaces and poor soils (Quadros and Pillar, 2001), but species of larger size outcompete it in more fertile conditions (Boldrini and Eggers, 1997).

P. plicatulum and *A. laevis* are two clumping grass species with a high accumulation of senescent material, low relative growth rate, and high adaptability to poor environments. Similar biomasses of these forage species in all treatments may be due to their uniform occurrence throughout the grassland area, as observed by Bandinelli et al. (2005) and Tiecher et al. (2014). The lower relative growth rate of *P. plicatulum* and *A. laevis* compared with other native species (Machado et al., 2013) may represent a greater adaptation to the deficit in nutrient supply and, consequently, less responsiveness to the increase in nutrient availability, as in the treatment with SP.

CONCLUSIONS

The best strategy to increase soil available P and forage production in natural grassland from Pampa biome is to use soluble phosphate. Despite the acidic condition and low content of soil available P and Ca, that would be ideal for the solubilization of natural phosphates, the use of the same rate of P via Gafsa hyperphosphate produced only intermediate results between the soluble phosphate and the treatment without P.

The nutritional indexes indicate that the ideal P availability in the soils of natural grasslands is 17.0 mg kg^{-1}. However, the species *P. plicatulum* and *A. laevis* produce less aboveground biomasses and accumulate less P in the biomass than *D. sabulorum* and *E. uliginosa*, and *P. notatum*. Therefore, natural grasslands dominated by *D. sabulorum* and *E. uliginosa* and *P. notatum* demand greater P availability in the soil to maintain high aboveground biomass yields, which should be further investigated in future research.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from the Federal University of Santa Maria Graduate Support Program (UFSM/PROAP, Santa Maria, Brazil); the scholarships for L. B. Oliveira from the Coordination for the Improvement of Higher Education Personnel (CAPES, Brazil).

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