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The effect of phosphorus fertilization on a Brazilian subtropical grassland intercropped with winter grass

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Abstract: Brazilian subtropical grasslands present generally low yield and quality due to low soil fertility and, as their forage production is concentrated on the spring and summer period, present a winter feed gap problem for producers. To overcome this feed gap, producers can intercrop their subtropical native pastures with winter active grasses. In order to establish the optimal P application rate for subtropical grassland intercropped with winter active grasses (oats - *Avena sativa* L.) and Italian ryegrass (*Lolium multiflorum* Lam.), five rates of P were applied (0, 45, 90, 180 and 360 kg ha^{-1} P_2O_5), in autumn 2010. The yield and quality (nutritional values of forage and mycorrhizal colonization of the roots of the winter active grasses) and botanical composition of the forage were monitored for 4.5 years. Grassland and winter forage yields increased average 2.6- and 6.0-fold, respectively, decreasing seasonality of feed supply. Phosphorus application increased concentrations of P, K, Ca, Mg and Cu in the winter active grasses. Conversely, only a small increase in P concentration was observed in the native, summer active grasses. Mycorrhizal colonization of the winter active grasses was sensitive to P application, reducing from 90 and 50% at control, to 50 and 15% at highest phosphorus rate, for oats and ryegrass, respectively. The phosphate fertilization affected species composition of the pasture, decreasing the number of species, but, did not compromised the ecological diversity index (Shannon-Wiener (H'), Simpson diversity and the Pielou equitability (J') indices). The capacity of Mehlich-1 extractor to predict soil P availability was discussed.
Keywords: Native pasture, forage yield, mineral supply, livestock, nutrition, winter grass

1. Introduction

Approximately 95.090 km² the subtropical native grasslands of southern Brazil are used as pasture for cattle raising (IBGE, 2017). After centuries of use, native grasslands under low input management have seen a reduction in productivity and have been converted to more profitable crops such as soybeans or forest (Hermann, Lang, Gonçalves, & Hasenack, 2016; Landau, Elena & Simeão, 2020). Improving the productivity of native grasslands using fertilizers could be a way to increase profitability, avoiding the conversion of this grassland to annual crops or commercial forest.

One of the major problems of raising animals in an extensive way, under Brazilian native subtropical grasslands, is the paucity of feed available from May-October (autumn and winter) (Brunetti et al., 2020; Malaguez et al., 2017). The cultivation of hibernal forages such as black oats (*Avena sativa* L.) and ryegrass (*Lolium multiflorum* Lam.) has been used to provide fodder in the autumn/winter months, decreasing the winter feed gap (Dillard et al., 2018; Jurjanz et al., 2012). The other benefit of adoption of hibernal forage, it is potential to reduce greenhouse gas emission from cattle rise (Dick et al., 2015; Ruviaro et al., 2015).

The adoption of hibernal forages intercropped with Brazilian native subtropical grasslands unlikely can be implemented without improvement of soil pH and fertility, since annual forage is generally more sensitive to acidity and nutrient supply that the native grasses (Prestes et al., 2017). Soil P deficiency is considered one of the biggest limiting factors to the implementation and maintenance of pastures in Brazil (Pavinato et al. 2020). In poor soil, P application can stimulate root growth of pastures (Tiecher et al., 2014; Tiecher et al., 2015), which can boost nutrient uptake, but can decrease mycorrhizae root colonization (Miao et al., 2023; Bhantana et al.,2021). Additionally, there are many reported negative interactions involving P application and the uptake of nutrients like Zn, Cu, Fe and others by plants (Yang et al., 2011; Arriaga et al., 2012; Malhotra et al., 2018). So, phosphate fertilization can have positive effect by enhancing P uptake and negative effect over Zn which is very important nutrient for cattle given by the occurrence of deficiency for tropical and subtropical forage.

Additionally, P application can change the botanical composition through influences on the competitiveness of native and established species (Blanck et al., 2011; Bedaso et al., 2022). In practical terms, achieving high yield is paramount, however, it is plausible in environmental terms that any increase in yield obtained in native pastures does not cause negative effects on its biodiversity. Thus, for environments such as a native pasture, after the use of practices that aim to increase the yield (such as P fertilization), the concomitant evaluation of yield and biodiversity gains relevance. Biodiversity can be assessed through the identification and quantification of co-existing species, such as the BOTANAL analysis, proposed by Tothill et al., (1992). In addition, ecological indices such as Simpson's diversity (S'), Pielou's equability (J') and Shannon-Wiener diversity (H') (Biondi & Bobrowski, 2014; Casella & Júnior, 2013).

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In the Southern Brazil the Mehlich-1 extractor is routinely used to determine P availability in the soil (SBCS-NEPAR, 2017; SBCS-NRS, 2016). However, for clayey and acidic soils, P recovery by the extractor reduces considerably over time after the P fertilization. This reduction in P recovery occurs due to the increase of the phosphate retention force by soil colloids, which in theory would be reducing the theoretical availability of the nutrient for plants (Freitas et al., 2013; Novais, Smyth, & Nunes, 2007). However, studies have verified that in soils fertilized with P is low or null the relationship between P content extracted by Mehlich-1 and responsiveness to P fertilization (Freitas et al., 2013; Novais et al., 2015). Consequently, it is understood that the time after P fertilization can lead to forms not detectable by the extractor, but still obtainable by plants. Thus, in order to verify if this effect would occur for the native grass/winter forage succession system after P fertilization, the concomitant monitoring of the yield obtained as a function of the fertilization and the P level in the soil extractable by Mehlich-1 becomes crucial.

The aim of this work was to evaluate the effects of phosphorus fertilization on yield, nutritional quality and botanical composition of the native grassland and yield, root mycorrhizae colonization and nutritional quality of winter grass pastures (oats and ryegrass). The efficiency of Mehlich-1 as extractor for residual P availability was also evaluated. It is expected that the knowledge generated in this study could help maintain productive systems that preserve the subtropical grassland biome.

2. Material e Methods

The experiment was established in April 2009, at Pinhais county, Paraná State, Brazil (25°23'25.0"S 49°07'44.5"W), with 908 m of altitude. The climate is classified as Cfb (Subtropical Humid without dry season with temperate summer), according to Köoppen (Alvares et al., 2013). The soil was formed from Smectite Guabirotuba clay stone formation and classified as Inceptisol according to the Soil Taxonomy (Soil Survey Staff, 2014). The soil chemical analysis of the experimental site at the start of the experiment is presented in table 1.

Table 1 – Soil attributes in the experimental site, Southern Brazilian native grassland. Pinhais, PR state, Brazil.

pH	K^+	Ca^{2+}	Mg^{2+}	H + Al	Al^{3+}	BS	CEC
$CaCl_2$	$cmol_c\ dm^{-3}$						
4.1	0.13	2.0	1.7	9.7	3.4	3.83	13.53
V	m	P	OC	Sand	Silt	Clay	
%		$mg\ dm^{-3}$	$g\ dm^{-3}$	$g\ kg^{-1}$			
28	47	1.6	40.9	180	320	500	

BS: base sum; CEC: cationic exchange capacity; V: base saturation; m: Al^{3+} saturation; OC: organic carbon.

Two hectares of native grassland (the principal species are Paspalum urvilei, P. paniculatum, P. plicatulum, P. notatum var. saurae, P. dilatatum, Brachiaria decumbens, Desmodium incanum, Dichondra repens, Setaria geniculata., Eragrostis sp., Axonopus jesuiticus, A. compressus, Panicum miliaceum, Digitaria insularis), with no history of fertilization or liming, was selected. The area was previously managed by mowing and removal of bushes only.

After experimenting establishment, the native pastures did not grow more spontaneously during the autumn and winter months. Instead of that, during this period, sowing and cultivation of intercropped winter forages (oats and ryegrass) were carried out. So, for the experiment, a native grassland area was converted into a succession system: native grassland spontaneously developing in the spring and summer seasons, and the cultivation of oats and ryegrass in the autumn and winter months. But, in the early autumn of 2013, there were heavy frosts, which spoilt the winter grass in the field and made them unable to establish over the 2013 autumn and winter. So then remarkably through autumn/winter 2013 period, the grassland developed and was evaluated instead of winter grass.

In April 2009, dolomite lime (Effective Calcium Carbonate Equivalent = 70%) was applied at a dose of 3 Mg ha^{-1} and incorporated by plowing. In May 2009 phosphorus fertilizer was hand broadcasted. A disk was then carried at all plots, including the ones which no received P fertilization.

The design was a randomized block design with five replications and five treatments, corresponding to rates of 0, 45, 90, 180 and 360 kg ha^{-1} P_2O_5, in the form of triple superphosphate (TS), totalling 25 plots of 50 m² each (10 x 5 m). A 1.0 m aisle between plots was kept within each block and a 2.0 m aisle between blocks.

Mean daily temperature values of 15.8, 20.0, 18.5 and 13.2 °C and 340, 710, 475 and 390 mm of accumulated rainfall for spring, summer, autumn and winter, respectively, were recorded during the experiment (Figure 1). The ryegrass or ryegrass and oats were sowed mechanically by double disk, using equivalent to 80 kg ha^{-1} of seeds. When ryegrass and oats were sowed together it was kept a proportion of 75% oats and 25% ryegrass.

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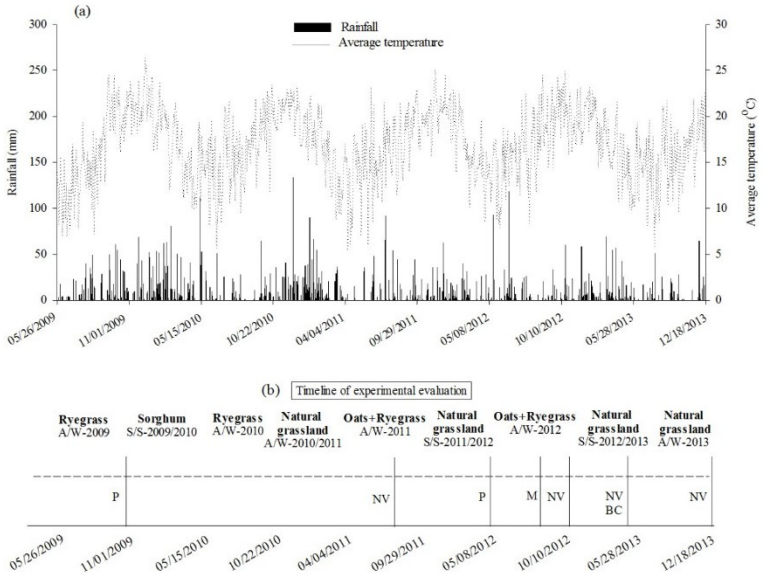


Figure 1 – Rainfall and average temperature (SIMEPAR, Pinhais – PR) (a) and experimental procedures developed (b). [NV]Nutritional values of forage, [P]P available in soil measured for Mehlich-1, [BC]Botanical Composition of pastures, [M]Winter grass roots mycorrhizal colonization. Yield forage was evaluated for all sessions.

Each year was divided into two periods: spring/summer (SS), comprised of November or December to April or May, and autumn/winter (AW) comprising the months of May or June to October or November (Figure 1).

At the end of each period, the experimental area was mowed to ground level with a manual cutter. Each period started in the first ten days of said month. A 0.5 x 0.5 m quadrat (0.25 m²) was thrown randomly four times in each plot and pasture samples were taken using scythe, cutting the plants at ground level. The samples of plant material were washed with tap water, rinsed with deionized water and dried in a forced-air circulation oven at 60°C until constant mass. The samples were then weighed to obtain the dry matter (DM) content.

For the AW-2009, AW-2013 and for all the SS periods, the yield represents plant growth from beginning to the end of the growing season, conducting only one sampling at the end of each period. For the AW-2010, AW-2011 and AW-2012 periods, the total yield was the sum of multiples harvests. During these periods, the plots of each treatment were monitored weekly, and every time the plants reached the height of 0.4 m a harvest of plant material was sampled, thus the treatments that had higher forage growth were harvested more times. After each harvest, the whole plot was mowed. At the end of the growing season, a final harvest was carried out in all treatments, regardless of plant size. In the end of all growing seasons a last mowing was performed.

After the fresh weight was measured in the lab, a sub sample of approximately 300 g of pasture was collected. The sub sample was washed with tap water and rinsed with deionised water, oven dried, weighed again, ground and stored. The samples of the periods which present multiple harvests were combined for the chemical analysis. The chemical analysis was performed for P, K, Ca, Mg, Cu, Fe, Mn and Zn after 1.0 g DM of plant sample was ashed at 500 °C in muffle furnace. The plant samples were then digested with HCl 3 mol L^{-1}. The P content was determined using the vanadate/molybdate colorimetric method via UV/VIS spectrophotometer; K by flame photometry, and the other elements by atomic absorption spectrometry (Martins and Reissmann, 2007).

2.1. Mycorrhizal colonization and oats/ryegrass ratio

At 84 days after sowing of winter fodder in the AW-2012 period, two monoliths 10 x 20 x 10 cm (width, length and height) were collected per plot, keeping the plants intact. Then, in the lab, the soil from the monoliths was removed by immersion in 4 L dispersive solution ($NaPO_3$ 3.75 g L^{-1} solution with the pH adjusted to seven ± 0.1 units, using Na_2CO_3) and rinsed with tap water. This methodology used for soil dispersion of the monoliths was adapted from the methodology used for the dispersion of soil samples in granulometric analysis (EMBRAPA, 2011). The plants with interlaced root system were gently manually separated from each other under the water. The plants were separated by species (black oat and ryegrass). The shoots of each species were oven dried and the roots were kept in 70 % alcohol.

The root samples were then stored in 70 % alcohol for subsequent staining and mycorrhizal colonization percentage determination by the methodology proposed by Giovannetti and Mosse (1980).

2.2. Botanical composition of natural grassland

The botanical composition of native grassland was determined at the end of the SS-2012/2013 period (26 May 2013), through the BOTANAL method proposed by Tothill et al., (1992). The botanical composition was used to calculate the Shannon-Wiener

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(H'), Simpson diversity and the Pielou equitability (J') indices. The diversity indices were calculated using the Past v2.17c application (Hammer, Harper, & Ryan, 2001).

2.3. Phosphorus concentration in soil over time

The available soil P concentration was obtained by extraction with Mehlich-1 solution at 6 and 36 months after sowing of the experiment. In both evaluations, a Dutch auger was inserted to a depth of 20 cm, and 10 samples were removed per plot. These were subsequently bulked, resulting in one composite sample per plot. The samples were dried in a forced-air oven at 45 °C and available P was extracted using P-Mehlich I extractor (1/10 soil/solution ratio). P concentration was determined by UV/VIS spectrophotometer using the molybdate colorimetric method.

All the data obtained were submitted to an analysis of variance (ANOVA). In cases where the ANOVA result was significant at 95% confidence level, the data were analysed by regression using the Sisvar software (Ferreira, 2011). For the variables obtained in duplicate the mean was used in the statistical analyses.

3. Results and Discussion

3.1. Forage yield

The autumn/winter (AW) yield increased until ten-fold under P fertilization while spring/summer (SS) showed a three-fold increase (Figure 2), indicating that the sown winter active grasses are more responsive to P application than the native, summer active grasses. This is beneficial regardless of livestock productivity, which is limited by the lower availability of pasture in the autumn/winter months. The SS yield/AW yield ratio may be indicative of the magnitude of this limitation. So, the phosphate fertilization reduces the autumn winter feed gap by providing larger yield for AW forage compared to SS. In the control treatment, the SS mean yield was 4.3-fold higher than AW mean yield, while the difference between the SS means yield and the AW when 360 kg ha^{-1} P_2O_5 was applied was smaller (2.3-fold).

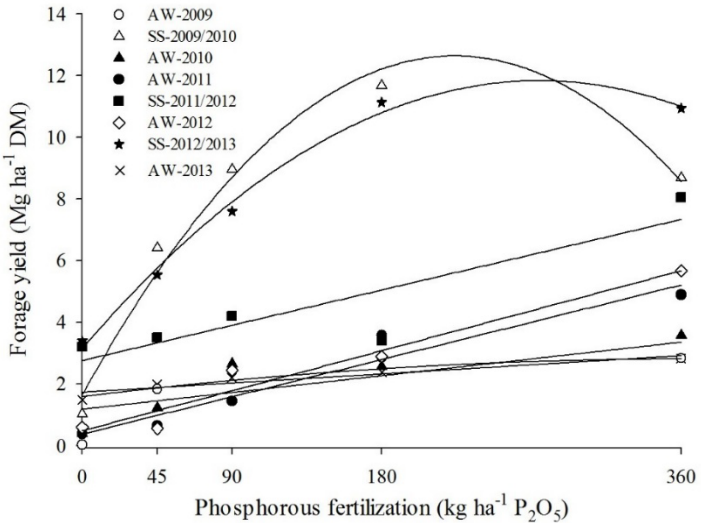


Figure 2 – Southern Brazilian native grassland intercropped with winter grass species (Oats and Ryegrass) system: Forage yield (Mg ha^{-1} DM) of pastures under different phosphorus rates (kg ha^{-1} P_2O_5-ST*). Pinhais, PR state, Brazil. *P_2O_5 rate on triple superphosphate form.

The natural grassland (SS periods) presented satisfactory yield, even when compared with selected genotypes of *Paspalum sp*. growing under optimal conditions (Batista and Godoy, 2000). However, winter forages (cultivated in the AW periods) showed higher demand for P and did not reach the productive potential of more than 6 Mg ha^{-1} yr^{-1} DM by winter forage species (Restelatto et al., 2014). Thus, phosphate fertilization would have the potential to further decrease the SS yield/AW yield ratio.

3.2. Elemental composition

Under all treatments, native grasses showed P concentrations below 1 g kg^{-1} (Table 2), which is below the minimum P requirement of a lactating cow (1.7 g kg^{-1} of P), and for growing and finishing cattle (1.2 to 1.6 g kg^{-1}; NRC, 2001; Gadberry, 2010).

Plant species that occur in native grasslands in Southern Brazil have low P concentrations (Senger et al., 1996; Wunsch et al., 2006) and low capacity to increase their P concentration when it is available. Therefore, P supplementation of animals is necessary in these native grasslands, in both grasslands with and without P fertilizer addition.

Table 2 – Nutritional values (g kg^{-1} DM) (Macro minerals) of forage (natural grassland) due phosphorus rates (kg ha^{-1} P_2O_5-ST*). Pinhais, PR state, Brazil.

[1]Growing season	P_2O_5 rates					Equation	R^2
	0	45	90	180	360		

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			P				
SS-2012/2013	0.44	0.49	0.45	0.45	0.66	y = 0.42 + 0.00056x**	0.75
AW-2013	0.61	0.77	0.67	0.87	0.98	y = 0.65 + 0.001x***	0.83
			K				
SS-2012/2013	3.95	5.08	5.57	5.81	4.99	y = 4.14 + 0.018x* – 0.00005x²	0.94
AW-2013	17.47	18.93	16.80	20.55	19.43	y = 18.6	-
			Ca				
SS-2012/2013	4.89	5.32	4.07	3.59	3.78	y = 4.85 – 0.004x**	0.54
AW-2013	4.39	5.43	4.44	4.19	4.15	y = 4.54	-
			Mg				
SS-2012/2013	2.02	2.38	2.23	1.92	2.22	y = 2.15	-
AW-2013	2.15	2.49	2.58	2.72	2.71	y = 2.36 + 0.0013x*	0.58

****p<0.01* ***p<0.05* **p<0.10*, P_2O_5-ST P_2O_5 rate in triple superphosphate form, [1]Autumn and Winter (AW), Spring and summer (SS), DM Dry matter.

Therefore, in the AW period with P fertilization, it was possible to reach almost all the lactating dairy cow's P requirement from the forage (1.7 g kg^{-1} DM of P according to NRC (2001)) (Table 4). The dose demanded to achieve a concentration close to this value (1.6 g kg^{-1} DM of P) was a minimum of 180 and 360 kg ha^{-1} P_2O_5, for the evaluations performed after 2.5 and 3.5 years after P supply, respectively (Table 4).

Table 4 – Nutritional values (g kg^{-1} DM) (Macro minerals) of forage (winter grass species) due phosphorus rates (kg ha^{-1} P_2O_5-ST*). Pinhais, PR state, Brazil.

[1]Growing season	P_2O_5 rates 0	45	90	180	360	Equation	R^2
			P				
AW-2011	0.97	0.96	1.20	1.59	2.28	y = 0.88 + 0.004x***	0.99
AW-2012	0.53	0.46	0.83	0.84	1.60	y = 0.44 + 0.003x***	0.92
			K				
AW-2011	16.75	17.70	20.92	23.16	24.51	y = 17.7 + 0.022x***	0.85
AW-2012	9.08	9.51	19.39	16.63	21.87	y = 10.8 + 0.034x***	0.68
			Ca				
AW-2011	0.62	0.69	0.73	1.18	1.60	y = 0.58 + 0.003***	0.97
AW-2012	1.61	1.53	1.92	1.93	2.71	y = 1.50 + 0.003***	0.92
			Mg				
AW-2011	1.58	1.54	1.90	2.08	2.50	y = 1.56 + 0.003x***	0.95
AW-2012	0.82	0.75	1.00	1.06	1.62	y = 0.74 + 0.002x***	0.94

The equations represent the correlation between nutritional values (g kg^{-1} DM) (Macro minerals) of forage (winter grass species) due phosphorus rates (kg ha^{-1} P_2O_5-ST*), ****p<0.01*, P_2O_5-ST P_2O_5 rate on triple superphosphate form, [1]Autumn and Winter (AW), DM Dry matter.

Application of phosphorus fertilizer increased Ca, Mg, K and Cu concentration for winter grasses in both seasons evaluated (AW-2011 and 2012) (Table 4 and 5). However, P application influenced concentration of Fe, Mn and Zn in the pasture, but the influence of P application on concentrations of these nutrients varied under different rainfall patterns (Table 5); during the AW-2011 period, the rain was constant while for the AW-2012 was sparse and mild, especially at the end of growing season. The differences we found in Fe, Mn and Zn concentrations between the different years may be due to differences in soil moisture, which has been shown to affect metal diffusion rates and bioavailability for plants (McBeath et al., 2012; Novo & Gonzalez, 2013). Furthermore, soil moisture also affects soil concentrations of Fe and Mn, because these elements are subjected to biological oxi-redox reactions (SBCS, 2007). The soil in this study had a very low level of Zn available by Mehlich-1 extractor (<0.4 mg dm^{-3}) (Table 1), which likely made the bioavailability of Zn more susceptible to the changes of greater magnitude than the concentrations of Fe and Mn (the soluble fractions of these elements present soil levels in order of 100 and 10 mg dm^{-3}).

Table 5. Nutritional values (mg kg^{-1} DM) (Trace elements) of forage (winter grass species) due phosphorus rates (kg ha^{-1} P_2O_5-ST*). Pinhais, PR State, Brazil.

[1]Growing season	P_2O_5 rates 0	45	90	180	360	Equation	R^2
			Fe				
AW-2011	98.77	68.52	95.82	65.90	81.76	y = 82.15	-
AW-2012	48.47	50.65	62.41	47.80	89.67	y = 45.8 + 0.10x***	0.70
			Mn				
AW-2011	140.38	96.45	92.40	79.22	73.96	y = 130.77 – 0.5x** + 0.001x²	0.88

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[1]Growing season	P_2O_5 rates 0	45	90	180	360	Equation	R^2
AW-2012	154.8	160.1	145.0	138.2	134.1	y = 146.4	-
			Zn				
AW-2011	15.24	18.66	19.27	28.61	33.17	y = 16.1 + 0.05x***	0.93
AW-2012	50.42	18.00	12.70	10.09	16.72	y = 42.7 – 0.37x* + 0.0008x²	0.80
			Cu				
AW-2011	7.95	6.78	9.36	8.93	9.74	y = 7.74 + 0.006x*	0.52
AW-2012	9.49	8.98	10.88	12.47	14.32	y = 9.22 + 0.015x***	0.93

****p<0.01 **p<0.05 *p<0.10*, P_2O_5-ST P_2O_5 rate on triple superphosphate form, [1]Autumn and Winter (AW), DM Dry matter.

Lactating dairy cows require minimal mineral concentrations in forage in order to present a satisfactory development; these values are 7.0, 2.6 and 2.7 g kg^{-1} for K, Ca and Mg, respectively (NRC, 2001). Therefore, winter grass forage has a satisfactory concentration of K (from 16 to 25 g kg^{-1} DM of K for AW-2011 period, and from 9 to 22 g kg^{-1} DM of K for AW-2012 period) (Table 4). Nonetheless, Ca and Mg concentrations were below requirement concentrations, which suggests it would be necessary to provide mineral supplementation by mineral salt addition in the fodder in order to supply lactating dairy cows with the Ca and Mg they require.

The minimum requirement for lactating cows is the values of 10, 50, 40 and 30 mg kg^{-1} for Cu, Fe, Mn, and Zn in forage, respectively (NRC, 2001). During the two years of our study, winter forage had adequate concentrations of Fe and Mn in order to achieve these requirements (Table 5). However, application of P fertilizer decreased the Fe values in forage of natural grassland to level below the animal requirement (Table 3). In general, a large amount of Fe can be consumed from soil contamination of the pasture (Jurjanz et al., 2012), therefore below requirement levels of Fe in forage may not be a problem.

Table 3 – Nutritional values (mg kg^{-1} DM) (Trace elements) of forage (natural grassland) due phosphorus rates (kg ha^{-1} P_2O_5-ST*). Pinhais, PR state, Brazil.

[1]Growing season	P_2O_5 rates 0	45	90	180	360	Equation	R^2
			Fe				
SS-2012/2013	171.2	113.6	59.4	70.5	51.3	y = 93.20	-
AW-2013	39.2	48.1	47.3	18.2	10.5	y = 46.9 – 0.11x***	0.76
			Mn				
SS-2012/2013	355.8	380.6	361.5	386.8	271.9	y = 355.11	-
AW-2013	400.6	381.7	333.1	480.5	332.2	y = 388.43	-
			Zn				
SS-2012/2013	42.9	30.9	28.5	26.0	26.9	y = 30.70	-
AW-2013	27.2	29.2	28.0	28.9	26.6	y = 28.02	-
			Cu				
SS-2012/2013	3.3	2.2	1.6	1.9	1.4	y = 2.64 – 0.004x*	0.57
AW-2013	3.5	4.7	5.4	5.1	4.1	y = 3.73 + 0.019x² – 0.0001*	0.85

****p<0.01 **p<0.05 *p<0.10*, P_2O_5-ST P_2O_5 rate on triple superphosphate form, [1]Autumn and Winter (AW), Spring and summer (SS), DM Dry matter.

The P treatments increased K concentration and decreased Ca concentration in natural grassland in one (SS-2012/2013) of two seasons evaluated (Table 2). Application of P fertilizer reduced Cu concentration from 3.3 to 1.4 mg kg^{-1} in the SS-2012/2013 period, however, in the subsequent growing season - AW-2013, Cu concentrations were lower in the control plots than in the P fertilized plots (Table 3). Application of P fertilizer did not affect Zn and Mn concentrations for natural grassland forage, regardless of the applied P dose (0 – 360 kg ha^{-1} P_2O_5) and the evaluated station (SS and AW). The Mn and Zn concentrations in the native pasture remained in the respective ranges 372±17 and 29±1.35 mg kg^{-1} of Mn and Zn (Table 3).

The natural grassland forage had from 4-5 g kg^{-1} DM of K when growing at SS, to 17-19 g kg^{-1} DM at AW (Table 2); these concentrations were similar to those found by Senger et al. (1996); range = 6.3 - 15.3 g kg^{-1}. The pasture Ca concentrations in our study were also similar to the 4.1 and 5.9 g kg^{-1} Ca previously recorded in the natural pastures by Wunsch et al. (2006) and Senger et al. (1996). The P fertilization increased Mg concentration in forage of native grassland by approximately 10% in SS and 20% in AW (Table 2). However, the values of Mg concentration in natural grassland forage herein observed, in any seasons evaluated (SS-2012/2013 and AW-2013), even without P fertilization (but received liming and NK fertilization), were higher (almost double) than those reported for native grasslands without any fertilization input (1.0 to 1.2 g kg^{-1} DM of Mg, on average, throughout the year) (Senger et al., 1996; Wunsch et al., 2006). The native grassland enhancement provided by liming, potassium and nitrogen fertilization probably could be responsible for increase of Mg concentration. But, it was enough to reach the level required by lactating dairy cow, indicating the necessity of mineral supplementation, regardless of adoption of winter grasses.

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3.3. Botanical composition of natural grassland

Phosphorous application increased the contribution to botanical composition by ryegrass in the AW yield (Figure 3). Although the ryegrass DM contribution was only between 4 and 12 % in the P fertilized plot, this indicates the potential of phosphorus fertilization to increase the ryegrass component in the sward.

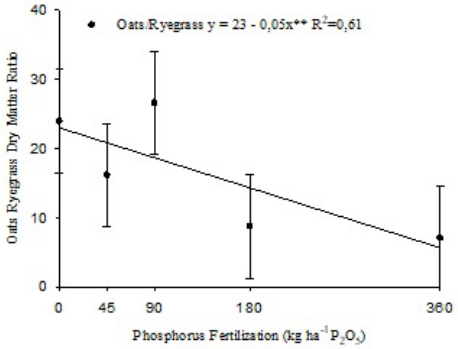


Figure 3 – The relationship between the oats:ryegrass DM ratio and phosphorus fertilization in a Southern Brazilian native grassland intercropped with winter grass species (ryegrass and oats). Data is derived from samples taken 84 days after sowing in autumn/winter 2012 (39 months after phosphorus fertilization).** denotes $p<0.05$.

Twenty-five species were found in southern Brazilian natural grassland. The species *Paspalum notatum*, *P. urvillei*, *P. paniculatum* and *P. pliclatulum* were dominant in all treatments (Table 6). *P. notatum* is known to be adaptable to several different plant growth limiting factors and for its competitiveness for resources (Giorello, et al., 2021; Pinto et al., 2013), which may explain its dominance in this study.

Table 6 – Botanical composition of natural grassland four years after phosphorus fertilization. Number of samples in which species of plant was present (10 samples per treatment) and average percentage in dry matter total (in parentheses).

	Phosphorus fertilization (kg ha^{-1} P_2O_5)				
Species	0	45	90	180	360
Paspalum notatum	7 (48.2)	7 (35.3)	9 (43.6)	9 (38.1)	9 (33.5)
Centella asiática	7 (8.6)	10 (13.6)	7 (3.0)	8 (3.8)	4 (1.5)
Cares sp.	6 (1.8)	8 (7.0)	6 (4.9)	5 (3.0)	2 (1.2)
Paspalum paniculatum	7 (12.0)	5 (8.3)	5 (4.0)	3 (10.8)	6 (22.9)
Paspalum urvillei	4 (0.9)	4 (12.0)	6 (9.5)	6 (22.3)	6 (28.6)
Eragrostis airoides	5 (1.2)	4 (1.5)	3 (1.1)	5 (1.5)	2 (3.5)
Dicondra repens	0 (0.0)	1 (0.2)	4 (2.0)	2 (0.7)	4 (1.9)
[1]N id 1	2 (0.4)	1 (0.3)	3 (1.3)	2 (1.2)	3 (1.8)
Setaria parviflora	4 (1.8)	4 (1.8)	1 (0.3)	1 (0.3)	0 (0.0)
Paspalum pliclatulum	5 (11.6)	2 (0.4)	2 (10.8)	1 (0.3)	0 (0.0)
Brachiaria decumbens	0 (0.0)	1 (9.0)	1 (1.8)	3 (14.0)	1 (0.3)
Setaria anceps	1 (0.3)	2 (1.5)	1 (0.3)	0 (0.0)	0 (0.0)
N id 3	0 (0.0)	0 (0.0)	1 (3.2)	2 (0.6)	1 (0.2)
N id 2	0 (0.0)	0 (0.0)	1 (2.7)	0 (0.0)	2 (0.5)
Desmodium sp.	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.3)
Axonopus jesuiticus	1 (0.3)	1 (0.2)	1 (3.6)	0 (0.0)	0 (0.0)
Erianthus sp. ssssssp.angustifolius	0 (0.0)	1 (7.7)	1 (7.2)	0 (0.0)	1 (3.6)
Oxalis latifolia	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.2)
Eragrostis plana	0 (0.0)	0 (0.0)	2 (0.5)	0 (0.0)	0 (0.0)
Desmodium sp. sp.ovalifolium	0 (0.0)	1 (1.0)	0 (0.0)	1 (0.3)	0 (0.0)
Solanum viarum	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)
Lantana camara	1 (1.4)	0 (0.0)	0 (0.0)	1 (3.2)	0 (0.0)
Asteracea	0 (0.0)	1 (0.2)	1 (0.3)	0 (0.0)	0 (0.0)
Axonopus affinis	1 (4.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Esporobulus sp.	1 (7.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

[1] N id denotes species not identified.

There was a negative linear relationship between phosphorus fertilization and number of species (Figure 4). However, the Shannon-Wiener (H'), the Simpson's diversity and the Pielou evenness (J') index behaved similarly to each other, which presented the highest values under application of 45 and/or 90 kg ha^{-1} P_2O_5. There was a decrease in the indices in the highest doses of P,

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which likely was a result from increase of dominance of the three main forage species (*Paspalum notatum*, *P. urvillei*, *P. paniculatum*; Table 6). To maintain diversity, it is necessary to consider application of <180 kg ha^{-1} P_2O_5, which would sacrifice pasture yield and quality but would preserve biodiversity.

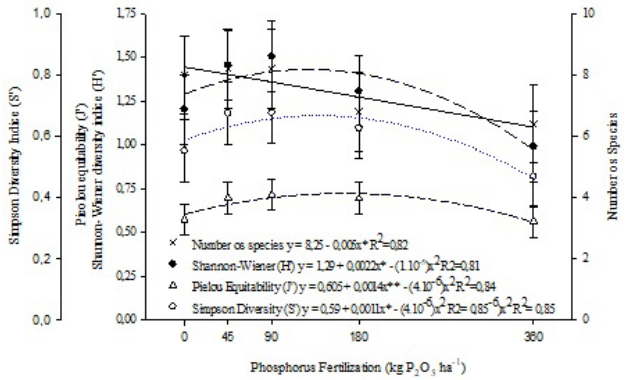


Figure 4 – The relationship between ecological indices (number of species, Shanon-Wiener índice (H'), Simpson diversity índice and Pielou equitability índice (J')) and P fertilizer rates in a Southern Brazilian native grassland intercropped with winter grass species (oats and ryegrass). Data was collected at the end of the spring/summer (SS) period of 2012/2013 (46 months after phosphorus fertilization). * denotes *p<0.10*, **denotes *p<0.05*, *** denotes *p<0.01*.

3.4. Mycorrhizal colonization

For oats, there was a high dependence of the plant on mycorrhizal fungi under low P supply. Mycorrhizae were also found to be important even under high level of P fertilization. Oats plants presented 95% colonization in the control (low P) treatment and 50% when grown under the residual rate of 360 kg ha^{-1} P_2O_5 (Figure 5), suggesting oats continue to have dependence on mycorrhizal fungi regardless of P fertilization.

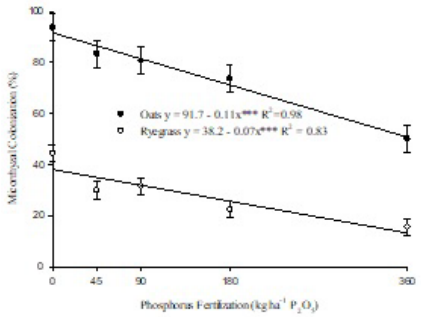


Figure 5 – The relationship between mycorrhizal colonization of winter grass species (oats and ryegrass) and phosphorus application rate in a Southern Brazilian native grassland intercropped with these winter grass species. Data is from 84 days after sowing in autumn/winter 2012 (39 months after phosphorus fertilization). ****p<0.10*.

High mycorrhizal colonization for oats was also reported by Karasawa e Takahashi (2015) who obtained 60% colonization for oats which growth under low P availability.

Ryegrass had a lower level of mycorrhizal colonization than oats; 44% and 16% in the control treatment and at the highest P rate, respectively (Figure 5), which is similar to colonization rates found by Druille et al. (2013), who recorded 50% mycorrhizal colonization of ryegrass roots 66 days after sowing. Our data suggests, that mycorrhizal colonization is more sensitive to P fertilization for ryegrass than for oats.

3.5. Phosphorous availability in soil

Application of P increased available soil P both 6 and 36 months after P application. However, available soil P decreased by more than 5-fold between 6 and 36 months (Figure 6). In the second evaluation (36 months after treatment application) available soil P concentrations for all treatments was considered "very low" according to (SBCS-NEPAR, 2017). These results agree with those obtained by Freitas et al. (2013) and Novais et al. (2015), who reported an extraction reduction as a function of contact time, especially for clay soils. The large decrease in the recovery of P by extraction contrasts with productivity maintenance (of natural grassland and winter grass), confirming the low sensitivity of the extractor in the residual effect evaluation. So, as it was observed in soybean (Palhano et al., 1983), the natural grassland as well the winter grass was able to acquire P forms in the soil which are present but are not detectable by Mehlich-1 P soil extractor.

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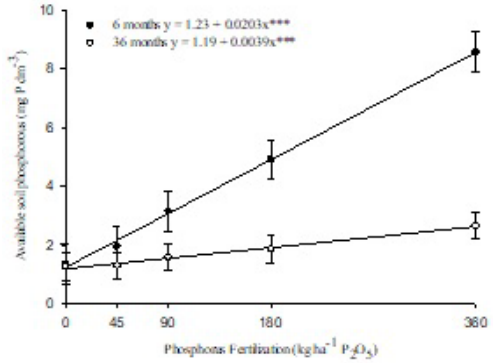


Figure 6 – The relationship between phosphorus fertilizer application rate and available soil phosphorus concentration in a Southern Brazilian native grassland intercropped with winter grass species (oats and ryegrass). Available soil P concentration was measured using a Melihch-1 extraction 6 and 36 months after phosphorus fertilization. ****p<0.10*.

Thus, there may be problems in assessing the availability of P in the soil, only by the Mehlich-1 extractor, in systems of this nature that are established and that have already received phosphate fertilization. In these cases, it would be advisable to evaluate the availability of P by factors such as productivity, since the present study demonstrates that productivity is extremely dependent on the availability of P, and also on the P content in the plant.

4. Conclusion

Without P fertilization there is no way to achieve satisfactory yields in native grassland intercropped with winter grass (Oats - *Avena sativa* and Reygrass - *Lollium multiflorum*) in southern Brazil. In this paper we reported then even with other fertility amendments (liming and NK fertilization) the annual yield is negligible (about 3.12 Mg ha^{-1} $year^{-1}$ Dm of forage), but at the same time we found an increase in mean annual yield of 3.12 to 9.15 Mg ha^{-1} $year^{-1}$ with the application of 90 kg ha^{-1} of P_2O_5, over 4.5 years evaluations.

Furthermore, winter grasses are more responsive to P application than native grassland, this increase in winter forage yield following P fertilization, from 90 kg ha^{-1} P_2O_5, could help to alleviate the winter feed gap which is present when natural grasslands alone are used for grazing. This can be seen by the contribution of winter grasses over whole annual yield which increased from 18 % without P fertilization to 24 and 30 % with amendment of 90 and 360 kg ha^{-1} de P_2O_5, respectively.

The P fertilization increases P concentration in both Winter cropped and natural grassland forage, the larger effect is on forage P concentration in winter grass than the summer grassland. However, mineral supplementation of P would still be required for dairy cattle. Application of P fertilizer increases K, Ca, Mg and Cu concentrations in the forages cultivated in the AW periods.

Phosphorus fertilization also increases the ryegrass contribution in winter grass species, as well the participation of species less tolerant to low P supply in native grassland. Still, for the native grassland, phosphate fertilizer reduced the number of species, and decreased the ecological indices in the highest P supply.

Though a decrease in mycorrhizal colonization was observed in both ryegrass and winter oat under P application, there is no indication that this causes decreases in any nutrient concentration in forage. In addition, the high level of mycorrhizal colonization of the sown winter grass suggests that the grassland soil was rich in wild mycorrhizal inoculum.

Mehlich-1 acid extraction is effective in detecting differences in P content in the soil among treatments, even after three years of fertilizer application. However, the extractor may underestimate the P availability in soil under the residual effect of fertilizer. Therefore, in order to maximize the P availability evaluation after long time of P application, it is recommended measure both the soil P concentration and forage yield.

P deficiency is a major limiting factor for the productivity of native grassland intercropped with sown winter grass. However, it is possible increase the yield and quality, decrease the natural seasonality and maintain grassland biodiversity (for at least 4.5 years) by applying less than 180 kg ha^{-1} P_2O_5.

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