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Long-Term Effects of Phosphorus on Dynamics of an Overseeded Natural Grassland in Brazil☆,☆☆

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ABSTRACT

Fertilization can affect vegetation dynamics and natural grassland diversity. This study evaluated the vegetation dynamics of a natural grassland 16 years after the initial fertilization, discussing the long-term effects of addition of triple superphosphate (TP) or Gafsa rock phosphate (RP) sources, as well as the effect of exotic species introduction on the inter seasonal dynamic of floristic composition. Phosphate (P) was applied in 1997, 1998, 2002, 2010, and 2012 at the quantities of 78.6, 39.3, 43.7, 43.7 and 43.7 kg·ha^{-1}, respectively, totaling 249 kg·ha^{-1} P. Total herbage mass production (THM) with RP (13 485 kg·ha^{-1}) and TP applications (14 668 kg·ha^{-1}) was higher than in the Control (11 291 kg·ha^{-1}). There was a higher warm tussock perennial grasses C_4 contribution on herbage mass (HM) during the summer season (1 106 kg·ha^{-1}), whereas it was similar between treatments. In summer, the warm-season prostrate perennial grasses C_4 group contribution for HM was on average 48% higher when RP was used (1 590 kg·ha^{-1}) in relation to the other treatments. The HM contribution from the cool season annual grasses C_3 group (CAG) in the total HM, over spring 2012, winter and spring 2013 in TP treatment, was 17% higher than the other treatments. The changes in the seasonal botanical composition dynamics mainly by inducing modifications in the proportion of *Paspalum notatum* A. H. Liogier ex Flüggé on RP treatment and *Paspalum urvillei* Steud. and *Lolium multiflorum* Lam. on TP treatment. However, no significant effects were observed in species richness, which ranged from 19–24 species among growth seasons. In the same way, the Shannon Diversity Index and Pielou Equitability Index were not modified by historical P sources. These results indicate that phosphorus fertilization has lower effects on natural grasslands diversity and could be used as a tool with important implications for livestock.

Introduction

The Campos grasslands (Allen et al., 2011) lie between 24° and 35°S including in its area southern Brazil, southern Paraguay, northeastern Argentina, and the whole territory of Uruguay, covering approximately 500 000 km^2 (Pallarés et al., 2005). In Brazil, Campos grasslands occupy 176 496 km^2 of the national territory and it is the main forage source for approximately 18.8 million domestic ruminants (IBGE, 2012). This environment presents approximately 3 000 plant species; however, considering forage for animal production, the most relevant are the grasses, with approximately 450 species (Boldrini, 2009). Most of these species are perennial with a C_4 metabolic pathway, and some of these have a C_3 metabolic pathway (warm- and cool-season growth, respectively) (Overbeck et al., 2007). Most of the Southern Brazil Campos grasslands grow on acidic soil with low availability of phosphorus (P) (Pallarés et al., 2005). The soil pH typically ranges from 4.4–5.1, and the available P in the soil ranges from 0.1–7.6 mg·kg^{-1} (de Oliveira et al., 2011; Rheinheimer et al., 1997). Despite that, native grass species are adapted to this environment and produce aboveground biomass ranging from 3 760 kg·ha^{-1}·yr^{-1} (Soares et al., 2005) to 9 820 kg·ha^{-1}·yr^{-1} of dry matter (Pellegrini et al., 2010) without any fertilization. Even so, a low-production potential is attributed to this natural forage resource. Due to this, according to Hasenack et al. (2010), there was a conversion from the natural vegetation to cultivated areas in the order of 58%,

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where the Campos grasslands were replaced by crops and forest plantations (Overbeck et al., 2007).

In order to preserve and avoid the Campos grasslands' replacement by croplands and forestation, it is necessary to develop alternatives that allow improvement in the utilization of these grasslands and/or increase herbage production to livestock production. On one hand, Barbieri et al. (2014) showed the possibility to increase forage utilization efficiency through the use of rotational grazing management considering plant morphogenetic traits (duration of leaf expansion of major grasses) to manage the natural grassland, mostly in warm seasons. On the other hand, increases in herbage production can be achieved through oversowing of cool season exotic species and adding fertilizers (Gatiboni et al., 2000; Tiecher et al., 2013). *Lolium multiflorum* and *Trifolium* spp. are the main exotic species introduced in natural grasslands to increase the herbage production in southern Brazil. Among the nutrients limiting the growth of C_3 and C_4 grasses, nitrogen and phosphorous are those having the largest impact on forage productivity (Rubio et al., 2010).

Fertilization with N and P may demand high investments; however, it is necessary to estimate the increases in terms of herbage productivity to evaluate its profitability in financial terms. Another important fact is that the fertilization could affect vegetation dynamics and grassland diversity. Interseasonal dynamics of grasslands' floristic composition is changed by P availability, favoring the group of plants that has high responsiveness to fertilization (Hejcman et al., 2007; Rodríguez et al., 2007). This could occur if the nutrient availability provides a reduction in the grasslands' biodiversity benefiting one species or a group of species that presents fast relative growth rate (Gaujour et al., 2013). In this way, the fertilization can allow species of fast relative growth rate (e.g., *Paspalum notatum* and *Axonopus affinis*; Machado et al., 2013) and others representing resources' capture functional groups (Cruz et al., 2010) to become the dominant species on grasslands. However, these possible changes on dynamics and diversity are poorly understood in Campos grasslands, mostly due to the lack of long- term experiments in South American grasslands.

Considering the particular characteristics of local soils, as mentioned previously, it was designed as an experiment (in 1997) to analyze grasslands productivity when soil P availability is improved by the addition of different P sources (Gatiboni et al., 2000). This study characterized changes in 1) the interseasonal forage production (Gatiboni et al., 2000); 2) the botanical composition (Bandinelli et al., 2005); and 3) the biogeochemical cycle of P in the soil and some soil chemical attributes (Rheinheimer et al., 2008; Tiecher et al., 2013). In this way, this paper presents results from grassland dynamics 16 years after the beginning of P fertilization, discussing the long-term effects of adding different phosphate sources and the introduction of exotic species over the dynamics and diversity of Southern Brazil Campos grasslands. Besides, the results were used to simulate livestock production under these conditions.

Methods

Site Description

The experiment was established in a natural area of the Campos grasslands (Allen et al., 2011), with Ultisol soil type at the Universidade Federal de Santa Maria, Rio Grande do Sul State, Southern Brazil (lat 29°45′S, long 53°42′W). Climate of the site is classified as humid subtropical Cfa according to Köppen classification, with 95 m o.s.l. and an average annual rainfall of 1 769 mm. The mean monthly air temperature in summer (December–March) is 24.2°C and in winter (June–September) is 14.5°C with few frost occurrences between May and August. Before starting the experiment, the 0- to 20-cm soil layer showed the following attributes: 170 g·kg^{-1} clay, 10.4 g·kg^{-1} total organic carbon, soil pH in water (1:1 v/v) 4.5, 2.5 and 60 mg·kg^{-1} available P and K, respectively, extracted by Mehlich-1, 1.30, 1.17, and 0.75 cmol$_c$·kg^{-1} exchangeable Al, Ca, and Mg extracted by 1.0 M KCl, respectively. When the experiment starts, the native grass species that had the major contribution in the biomass yield were *P. notatum* (45.3%), *Andropogon ternatus* (1.0%), *P. plicatulum* (0.5%), *Eustachys ulignosa* and *Schizachyrium microstachyum* (0.3%), and *Aristida laevis, Piptochaetium montevidense,* and *Saccharum angustifolius* (0.2%). The Umbelliferae family presented *Eryngium ciliatum* (21.2%) and *E. horridum* (0.2%). Senescent material (29.2%) was the second most yielding component (Bandinelli et al., 2005).

Experimental Design, Treatments, and History

The experiment was established in May 1997. The experimental design used was a complete randomized blocks with three treatments and three replications, with plots measuring 3.3 m wide and 3 m long. The treatments were the application of triple superphosphate (TP); Gafsa rock phosphate (RP), and no-phosphorus fertilizer (Control). In treatments with P application, 78.6 kg·ha^{-1} P were applied. All treatments received potassium (108 kg·ha^{-1} K), nitrogen (70 kg·ha^{-1} N using urea as N source), and introduction of cool season species. The cool season species overseeded were Italian ryegrass (*L. multiflorum* cv. "Comum"—MAPA, 2013) and arrowleaf clover (*Trifolium vesiculosum* cv. Yuchi—Frame, 2005). These species were overseeded in lines on natural grassland, at the amounts of 30 and 12 kg·ha^{-1} seeds, respectively, without using herbicides. To ensure adequate distribution of fertilizers, replicates were delimited with ropes and then the fertilizers were applied through manual application. Fertilizers (P, K, N) used in the treatments followed the Brazilian Soil Association Manual recommendation (CQFS-RS/SC, 2004).

The P was reapplied in winter of 1998 (39.3 kg·ha^{-1}), 2002 (43.7 kg·ha^{-1}), 2010 (43.7 kg·ha^{-1}) and 2012 (43.7 kg·ha^{-1}), totaling 249 kg·ha^{-1} P applied over time. Besides, in the winter season of 2002, plots were mowed and ryegrass and clover were reintroduced in the natural grassland by oversowing at the same previous amounts.

The grassland was clipped to 5 cm height in May 1997 (before the beginning of the experiment), October 1997, November 1997, February 1998, and April 1998 (for more details, see Gatiboni et al., 2000). Subsequently, the grassland was left in fallow. In June 2002, December 2002, and February 2003, the grassland was clipped again to 5 cm height (for more details, see Rheinheimer et al., 2008). In January 2009 and August 2010, the pasture was clipped again (for more details, see Tiecher et al., 2013). After each clipping, the cut biomass was removed from the experimental area.

Experimental Period Management

The results presented in this article were collected between September 2012 and November 2013 (Fig. 1), totaling eight sampling periods: October 2012, November 2012, January 2013, February 2013, April 2013, July 2013, September 2013, and November 2013. Along this period, at each sampling date, grassland was clipped to 5 cm height and biomass

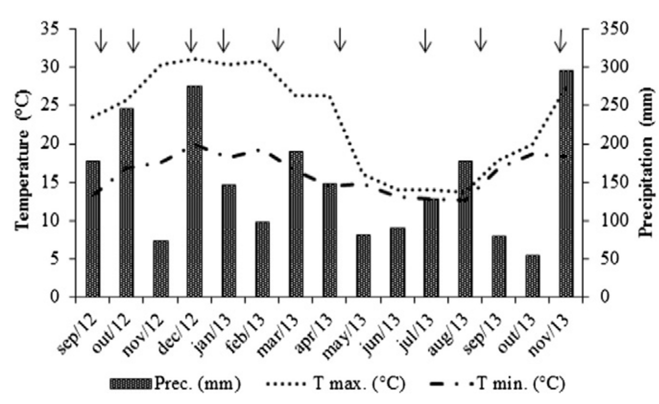

Fig. 1. Precipitation (Prec.) and maximum and minimum temperature (T max., T min.) during the experimental period. The arrows indicate grassland's sampling periods.

was removed from soil surface. Besides, after each mowing, 40 kg·ha^{-1} of N, in urea form, were applied at all treatments, totaling 320 kg·ha^{-1} of N during this period.

Vegetation Sampling

On 28 August 2012, before the beginning of samplings, the experimental area was mowed to 5 cm and the biomass was removed. Next, the grassland dry matter yield and composition of vegetation were evaluated in the following dates: 11 October 2012 and 23 November 2012 (Spring); 5 January 2013 and 22 February 2013 (Summer); 18 April 2013 (Autumn); 3 July 2013 and 3 September 2013 (Winter); and 1 November 2013 (Spring) corresponding to 44, 43, 43, 49, 55, 76, 62, and 59 days of growth between each evaluation. The criterion used to define sampling dates was *P. notatum* leaf life span, which had a thermal sum of approximately 810 degree days (DDs) (Machado et al., 2013). This species was chosen due to the high participation and its presence in all treatments (or plots). Thermal sum was calculated using 135 DD as phyllochron multiplied by six green leaves (Machado et al., 2013). To calculate thermal sum, mean daily temperature was used.

The botanical composition of the experimental area was evaluated by the BOTANAL method (Tothill et al., 1992) on five replications using fixed frames of 0.25 m^2 in a diagonal transect, into each experimental plot. The fixed frames area used was defined as an appropriate size sample unit to obtain representative vegetation samples by Girardi-Deiro and Gonçalves (1989). The harvested herbage was dried at 55°C in a forced-draught oven until constant weight to calculate the dry matter yield per hectare. Jointly with the botanical composition, the proportion of uncovered soil (% SOIL) was estimated by visual sampling, considering quadrat area. The senescent material (SM) proportion was also visually estimated, and the results were expressed in kg of dry matter per ha.

For the evaluation of forage dry matter yield, in each plot, two frames were cut at the ground level and visual estimates were made in the other three frames. The estimative was made according to a comparative yield varying from 1–5, as proposed in the BOTANAL method, and adjusted through linear regressions. The total herbage mass production (THM) was obtained with the sum of the herbage mass of each period, totaling eight evaluations in a total of 385 days.

Plant Functional Group

Plant species were classified using a hierarchical approach (Lavorel et al., 1997) according to growth form (grasses, legumes, forbs, sedges); life history (annual, perennial); photosynthetic pathway (C_3 or C_4); morphology (tussock, prostrate); and growing season (cool or warm season). The following functional groups were warm-season C_4 tussock perennial grasses (WTPGs), warm-season C_4 prostrate perennial grasses (WPPGs), warm-season C_4 annual grasses (WAGs), cool-season C_3 annual grasses (CAGs), cool-season C_3 perennial grasses (CPGs), cool-season legumes (CLs), warm-season legumes (WLs), forbs, and sedges (species in each group were indicated in Table S1).

Species Diversity

Species diversity was characterized by three components: richness, diversity, and dominance (equitability). Species richness was estimated as the total number of species in each plot in an area of 1.25 m^2 (five frames of 0.25 m^2). Species diversity was estimated by Shannon index (H′) calculated by the following equation: $H\prime = -\sum_{i=1}^{S} \frac{ni}{N} \ln \frac{ni}{N}$; where *S* is the number of species sampled (1.25 m^2); *ni* is the herbage mass of each species; *N* is the total herbage mass of the community; and *ln* is the natural logarithm. Equitability was estimated by the Pielou index (J) by the following equation: $J = \frac{H\prime}{SR}$; where H′ is the Shannon diversity index and SR′ is the species richness.

Statistical Analyses

Data from herbage mass, group mass, species richness, Shannon diversity index, and Pielou equitability index were subjected to nonparametric randomization tests with Euclidean distance as similarity measure. The effects of phosphorus sources (Control, RP, and TP); growth seasons (spring/12, summer, autumn, winter, and spring/13); and their interactions were tested. When there was interaction between sources × growth seasons ($P < 0.05$), analysis of variance was performed to separate phosphate sources means within growing seasons.

Species' mass contribution was subjected to ordination multivariate analysis using the principal coordinates method (PCoA). The means of sampling units were submitted to Euclidean distance as similarity measure using species as variables. The species plotted in the PCoA were those that had correlations > 60% with the *x* and/or *y* axis. Afterwards, herbage mass (HM), SM, and % SOIL variables were plotted according to its simple correlation with ordination axis scores. We used the PCoA method because our focus was on the sampling units receiving the treatments, not in variables (species), according to Pillar (2004).

After the identification of phosphate sources trajectory in the ordination diagram, the similarity between trajectories was tested by multivariate analysis of variance (MANOVA) randomization tests. The effects of treatments (Control, RP, and TP), growth seasons (spring/12, summer, autumn, winter, and spring/13), and their interactions were tested using Euclidean distance as similarity measure. When there was interaction between sources × growth seasons ($P < 0.05$), we performed analysis of variance to separate phosphate sources means within growing seasons. All statistical comparisons were considered significant below 5% probability. All analyses were performed with the software MULTIV (Pillar, 2004).

Results

Herbage Mass

Total herbage mass production (THM), during 385 days, was 11 291 kg·ha^{-1} in the Control (Table 1). When RP was applied, the THM production was higher than in the Control (13 485 kg·ha^{-1}) but lower than in TP (14 668 kg·ha^{-1}). During seasons of low temperature (autumn and winter), THM in TP treatment was 21% greater than in RP treatment. In the same way, the application of RP produced 21.6% more THM than the Control treatment. Meanwhile, in spring 2013 the TP application produced 10% more THM than RP and 45% more THM than Control. In the same season, the RP application increased the production around 31% when compared with Control. Over the summer 2013, THM was similar between RP and TP treatments and greater than Control (see Table 1). In spring 2012, there were no differences in the THM between treatments.

Functional Groups Mass

The herbage contribution of warm-season C_4 tussock perennial grasses group (WTPG) in the HM was similar independently from the treatments ($P = 0.090$; see Table 1). There was a higher WTPG contribution in the HM during the summer season (1 106 kg·ha^{-1}) and lower contribution in the other periods (see Table 1).

The warm-season C_4 prostrate perennial grasses group (WPPG) contribution was similar among the treatments in spring 2012 and 2013, with 595 and 659 kg·ha^{-1}, respectively. In summer, WPPG contribution in the HM was, on average, 48% greater when applied RP (1 590 kg·ha^{-1}) in relation to the other treatments. In summer, the WPPG group presented a similar mass in Control and TP. However, during the low-temperature seasons (autumn and winter), WPPG contribution mass

Table 1
Total herbage mass (THM), seasonal herbage mass (HM), and functional groups mass during the experimental period

Groups	Sources (S)	Growth season (GS)[1]					THM	*P* value	
		Spring 12	Summer	Autumn	Winter	Spring 13	Mean		
		——— kg ha^{-1} ———							
Herbage mass	Control	1765 a	2186 b	1504 c	704 c	1260 c	11291 c	S	0.001
	RP[2]	1880 a	2662 a	1706 b	979 b	1653 b	13485 b	GS	0.001
	TP	1885 a	2800 a	2038 a	1211 a	1829 a	14668 a	S × GS	0.019
	Mean	1843	2549	1749	965	1581			
Warm-season C_4 tussock perennial grasses	Control	846	1088	845	423	524	745	S	0.090
	RP	765	943	519	380	578	637	GS	0.001
	TP	776	1286	927	399	537	785	S × GS	0.418
	Mean	796 B	1106 A	763 B	401 D	546 C			
Warm-season C_4 prostrate perennial grasses	Control	639 a	1003 b	567 b	225 b	498 a	586	S	0.002
	RP	714 a	1590 a	1052 a	482 a	736 a	915	GS	0.005
	TP	433 a	1156 b	905 a	413 a	744 a	730	S × GS	0.001
	Mean	595	1250	841	373	659			
Cool-season C_3 annual grasses	Control	1 b	0 a	0 a	3 c	2 c	1	S	0.001
	RP	1 b	0 a	0 a	26 b	57 b	17	GS	0.004
	TP	158 a	0 a	0 a	271 a	395 a	165	S × GS	0.001
	Mean	54	0	0	100	151			
Cool-season C_3 perennial grasses	Control	12	0	0	0	27	8 bc	S	0.015
	RP	30	0	1	3	63	19 ab	GS	0.001
	TP	5	0	0	0	17	4 c	S × GS	0.282
	Mean	16 A	0 B	0 B	1 B	36 A			
Warm-season C_4 annual grasses	Control	0 a	0 b	0 a	0 a	0 a	0	S	0.002
	RP	0 a	0 b	0 a	0 a	0 a	0	GS	0.033
	TP	2 a	73 a	9 a	0 a	0 a	17	S × GS	0.021
	Mean	1	24	3	0	0			
Cool-season legumes	Control	0	0	0	0	0	0 b	S	0.001
	RP	1	0	0	8	24	7 a	GS	0.004
	TP	0	0	0	0	4	1 b	S × GS	0.132
	Mean	1 BC	0 C	0 C	3 AB	9 A			
Warm-season legumes	Control	11	14	9	2	8	9	S	0.408
	RP	20	20	10	0	11	12	GS	0.001
	TP	20	17	11	2	6	11	S × GS	0.940
	Mean	17 A	17 A	10 AB	1 C	8 B			
Forbs	Control	218	66	72	38	148	108	S	0.356
	RP	330	77	95	62	165	146	GS	0.001
	TP	295	166	77	53	81	134	S × GS	0.503
	Mean	281 A	103 B	82 C	51 D	131			
Sedges	Control	38 b	14 c	10 c	12 b	55 a	26	S	0.001
	RP	20 b	33 b	29 b	18 b	19 a	24	GS	0.025
	TP	196 a	102 a	108 a	74 a	44 a	105	S × GS	0.007
	Mean	85	50	49	35	39			

1 Lowercase letters compare phosphorus sources between lines, and capital letters compare seasons between columns by randomization tests at 5% probability.
2 RP indicates rock phosphate; TP, triple superphosphate.

was similar between RP and TP and, on average, 80% higher than Control.

There was no contribution of the CAGs in the HM during the summer and autumn seasons. During the other seasons, CAGs presented a higher contribution when TP was applied. In spring 2012 and winter and spring 2013, CAG contribution in the HM was 8.4% (158 kg·ha^{-1}), 22% (271 kg·ha^{-1}) and 22% (395 kg·ha^{-1}), respectively, in the TP treatment. CPG contribution was 4.8 times greater in RP treatment than in Control. The CPG group had the greater contribution in the HM along the two springs. WAG contribution was greater in TP during summer (73 kg·ha^{-1}), but it was negligible along the other seasons. Cool season legumes group (CSL) had the lowest contribution in the HM, and besides, its contribution was greater in the RP treatment. Warm-season legumes (WSLs) and forbs contributions in the HM were not influenced by treatments, differing only among seasons. Sedges group contribution had the greater contribution in the TP treatment relative to RP and Control, except in the spring of 2013.

Botanical Composition Dynamics

Ordination analysis allowed us to show the dynamic of vegetation in response to treatments, according to the contribution of 75 species predominant in the HM (Fig. 2). In the ordination diagram, the major part of variability of the treatments (77%) was synthesized in the first two axes (62% axis x; 15% axis y), in function of the contribution from the species in the HM (variables).

Species with the highest correlations *(r)* with axis *x* were *P. notatum* (0.99), *Desmodium incanum* (0.82), *Aeschynomene falcata* (0.71), *S. angustifolius* (0.66), *Eragrostis alopecuroides* (0.66), and *Paspalum plicatulum* (0.65). Species with the highest correlations with axis *y* were *Paspalum urvillei* (0.85), *L. multiflorum* (0.76), *Relbunium richardianum* (0.71), *S. microstachyum* (0.68), and *E. ulignosa* (0.63).

There was interaction phosphorus sources × seasons for the botanical composition dynamics ($P = 0.010$; Table 2). Although the trajectory from dynamic of vegetation followed a similar trend in the ordination diagram, botanical composition was different between treatments and along the seasons. In spring 2012, botanical composition differed between TP and the other treatments ($P = 0.006$; Table 2). During this season, according to the ordination diagram, *L. multiflorum* and *Relbunium richardianum* were the species with the greatest correlations with the dynamic observed in the treatment TP (see Fig. 2).

In summer, the composition of Control only differed from the RP treatment ($P = 0.007$), but it was similar to TP ($P = 0.282$; Table 2). In this season, RP treatment showed high mass contribution of the

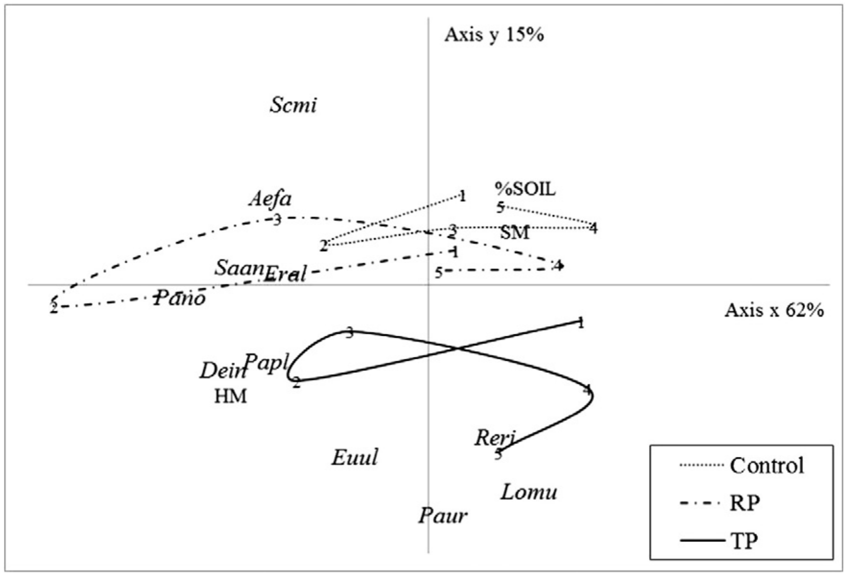

Fig. 2. Ordination diagram of sampling units trajectories [Control, Gafsa rock phosphate (RP) e triple superphosphate (TP) treatments] as a function of growth seasons: spring/2012, summer, autumn, winter, and spring/2013 represented as 1, 2, 3, 4, and 5, respectively. Species presented had correlations above 0.6 with one of the axis. *Pano* indicates *Paspalum notatum; Dein, Desmodium incanum; Papl, Paspalum plicatulum; Aefa, Aeschynomene falcata; Scmi, Schizachyrium microstachyum; Reri, Relbunium richardianum; Saan, Saccharum angustifolius; Paur, Paspalum urvillei; Eral, Eragrostis alopecuroides;* Euul, *Eustachys uliginosa;* Lomu, *Lolium multiflorum*; HM, herbage mass; SM, senescent material; and %SOIL, bare soil percentage.

P. notatum grass (see Fig. 2). In the opposite, when TP was used as fertilizer, species that had greater contribution in the herbage mass were *Paspalum plicatulum, P. urvillei, Eustachys uliginosa,* and *D. incanum*.

In autumn, botanic composition remained with the same trend of summer, being different only in the RP treatment ($P = 0.001$). In RP treatment, the contribution of *P. notatum* in the herbage mass was reduced from summer to autumn and the grass *S. microstachyum* and the legume *A. falcata* increased their contribution in the HM (see Fig. 2).

In winter, TP treatment had a different botanic composition ($P = 0.024$). In this case, *L. multiflorum* was the cool-season grass with higher contribution in the HM (see Fig. 2 and Table 1). In the other treatments, this grass had an insignificant contribution. In the same way, during spring 2013, the TP treatment had a singular botanical composition ($P = 0.002$; see Table 2). Due to the historical of TP application, the grasses *L. multiflorum, P. urvillei*, and *E. ulignosa* were the species with the greatest HM contribution (see Table 2), this can also be seen by their position in the ordination diagram (see Fig. 2). In the same season, in Control and RP treatments, the legume *A. falcata* and the grasses *S. microstachyum, S. angustifolius, E. alopecuroides*, and *P.* had more relationships between them and, consequently, they had the greatest contribution in the HM.

The bare soil proportion (*% SOIL*) and senescent material proportion (SM) were positivity correlated with axis *x* (0.41 and 0.26, respectively) and with *y* (0.26 and 0.21, respectively). There was a tendency of greater *% SOIL* and SM in spring 2012 and in winter 2013. *% SOIL* was mostly correlated with the Control treatment, and SM was correlated with both Control and RP treatments. The HM was greater during summer and showed greater correlation with the TP treatment.

Species Diversity

We found 75 different plants species from 20 different families during the experimental period. Main families were Poaceae, Asteraceae, Fabaceae, and Apiaceae with 28, 15, 7, and 4 different species in each family, respectively. Species richness was not affected by the history of P source applications ($P = 0.824$; Table 3). On average, we observed 21 species in 1.25 m^2 (five squares in each repetition). However, there were significant effects among seasons ($P = 0.001$), where the species richness ranged from 19–24. The greater species number was observed on the two evaluated springs. In the same way, Shannon Diversity Index (H′) and Pielou Equitability Index (J) were not modified by historical P application, but there was a difference between growth seasons (see Table 3). These indexes were also greater in the two springs (2012 and 2013). The mean of H′ was 1.75 ranging from 2.15–1.19, and the mean of J was 0.57 ranging from 0.41–0.68.

Discussion

The historic 16 years of adding two phosphorous sources and the oversowing of exotic winter species (cool-season C3 annual grass) increased the total herbage mass (THM) production (see Table 1). This increase of forage mass was greater during the coolest season of the year

Table 2
Significance effects from the phosphorous sources (S), growth seasons (GS), and their interactions in the grassland botanic composition

Factor	*P* values
S	0.001
GS	0.001
S × GS	0.010
Spring 2012	
Control × RP[1]	0.225
Control × TP	0.004
RP × TP	0.006
Summer	
Control × RP	0.007
Control × TP	0.282
RP × TP	0.024
Autumn	
Control × RP	0.001
Control × TP	0.126
RP × TP	0.043
Winter	
Control × RP	0.127
Control × TP	0.002
RP × TP	0.024
Spring 2013	
Control × RP	0.119
Control × TP	0.002
RP × TP	0.014

[1] RP indicates rock phosphate; TP, triple superphosphate.

Table 3
Richness, number of species, Shannon Diversity Index (H'),and Pielou Equitability Index (J) in a natural grassland

Sources of variation	Richness (no. of species)	Shannon Diversity Index (H')	Pielou Equitability Index (J)
Phosphorus sources (S)			
Control	22	1.73	0.56
RP[1]	21	1.74	0.57
TP	21	1.79	0.59
Growth seasons (GS)			
Spring/12	23	2.01	0.64
Summer	19	1.60	0.55
Autumn	20	1.53	0.51
Winter	22	1.67	0.54
Spring/13	24	1.93	0.61
P value			
S	0.824	0.859	0.594
GS	0.001	0.001	0.001
S×GS	0.886	0.178	0.091

[1] RP indicates rock phosphate; TP, triple superphosphate.

(winter), mostly due to the increase in the CAG group, which, in turn, was uniquely represented by *L. multiflorum*, that is the exotic overseeded specie (see Table S1 - Supplementary file).

P fertilization management, added to oversowing hibernal species, changed the dynamic of seasonal botanical composition of the natural grassland, mainly by inducing modifications in the proportion of each species in the community (see Fig. 2). Nevertheless, these dynamics modifications did not decrease the diversity of species (see Table 3). Therefore we can infer that fertilization or introduction of exotic species can be used to increase herbage production with insignificant effects on the natural ecosystems diversity.

These findings are in agreement with Tiecher et al. (2013). The authors found that surface application of soluble phosphate fertilizers combined with overseeding with cool-season species are simple management interventions that could be effective in minimizing the adverse effects of low forage availability during the cool season. By these means, the South American Campos could be preserved as productive grasslands, thereby avoiding its replacement by croplands, afforestation, and cultivated pastures.

Our results show that, even in the Control treatment, the THM production was greater than the results in experimental studies in the Southern America Campos previously cited (11 290 $kg \cdot ha^{-1} \cdot yr^{-1}$; Pellegrini et al., 2010). This difference in THM probably indicates that the pasture management by its phyllochron respects the "growth rhythm" of the grass, it is a suitable way to manage pastures with maximum production. Furthermore, THM production in the TP (14 600 $kg \cdot ha^{-1}$) and RP treatments (13 500 $kg \cdot ha^{-1}$) were greater than the results obtained in a similar natural grassland and treatments (control or with lime and P application and cool season species introduction) (4 100 kg $ha^{-1} \cdot yr^{-1}$ to 11 100 $kg \cdot ha^{-1} \cdot yr^{-1}$, Gatiboni et al., 2000). These results were attempted in our experiment where 320 $kg \cdot ha^{-1}$ N were applied. It was clearly demonstrated that when N and P are applied, these natural grasslands increase their potential of production.

It is usually accepted that P fertilization increases forage production through the increase in annual grasses (Gatiboni et al., 2000, 2003) or through the increased legume productivity of cultivated pastures and natural grasslands (Henkin et al., 1996; Osman et al., 1999; Whitehead, 2000). In our case, the increases of 30% (TP) and 19% (RP) in forage production relative to Control (see Table 1) were mainly a consequence of *L. multiflorum* (see Fig. 2) (CAG group) response to the P fertilization (see Table 1), once there was no increase of CSL or WSL mass (see Table 1). During the winter period, 100% of CAG mass was due to the *L. multiflorum* contribution. Besides, on average, during winter and spring of 2013, CAG represented 3% of the THM in RP and increased to 22% in the TP treatment. On the other hand, there was no contribution of the CAG group in Control.

The increase in soil P availability with TP historical application (for more details, see Tiecher et al., 2013) provided favorable conditions for the exotic grass *L. multiflorum* to grow and develop within the plant community. Thus *L. multiflorum* presented a competition with native species and, then, this contribution may cause differences in the botanical composition of the natural grassland in TP treatment, during winter and spring compared with the other treatments (see Fig. 2). In this way, the increase in herbage production during the cool season, which is the period with lower herbage production in natural grasslands, could be achieved by applying soluble phosphate and overseeding cool season annual species. These results are in agreement with Tiecher et al. (2013), which hypothesized that *L. multiflorum* would be considered as a key species for increasing production in Southern Brazil natural grassland.

Concerning to the composition dynamics, our results indicate that the trend of plant community was differently affected by the P sources over the seasons. The greater contribution of *L. multiflorum* in the HM, during winter and spring (2012 and 2013), in the treatments RP and TP caused different effects in botanical composition on natural vegetation over the other seasons (see Fig. 2). Due to the *L. multiflorum* growth in the treatments with P application, there may have been a shading effect on prostrated C_4 grass species. Such effect can explain the lower mass contribution of WPPG group during the summer in TP treatment relative to RP (see Table 1). The grass *P.* was the main species in the WPPG group, which had the major variation in the ordination diagram (axis *x*) (see Fig. 2). Therefore the greater contribution of *P. notatum* over the summer in RP treatment (trajectory in the ordination diagram longer and close to *P. notatum*) could be explained by the lower contribution of *L. multiflorum* in the HM, during spring 2012.

Similar trends were found for *P. urvillei* in TP treatment (see Fig. 2). This grass has a growing habit like tussock, with a relative faster growing rate and larger leaves than the other species. These attributes allowed *P. urvillei* to compete for light with *L. multiflorum* during spring, explaining its high contribution in the TP during summer.

Mass contribution of WPTG decreased from 44% in summer to 35% in spring 2013, with the concomitant increase of CAG mass contribution. The lower mass contribution of WPTG is not a simple effect of its lack of consistent responsiveness to P addition, as demonstrated by Gatiboni et al. (2000). Instead, our results clearly showed that there is a complex proportional substitution in the contribution of the main species (see Fig. 2). The contribution of WPTG group is given by *P. urvillei* in TP treatment and by species such as *S. microstachyum* and *S. angustifolius* in Control. This fact may occur due to the greater soil P availability in TP treatment. This benefits species with greater relative growth rate as *P. urvillei*, which can be defined as a resource capture grass (Cruz et al., 2010; Quadros et al., 2009).

The CPG group is formed by species such as *Briza subaristata*, *Piptochaetium montevidense*, and *Calamagrostis viridiflavescens*, which do not appear in the ordination diagram (see Fig. 2). These species showed a major contribution in RP and Control treatments compared with the TP (see Table 1). In natural grasslands dominated by warm-season C_4 grasses, as in our experimental area, C_3 species tend to be a poor resource competitor, so its presence in environment could be attributed to its greater growth rate only at low temperatures. However, when the soil has a suitable nutrient status for the establishment (TP treatment), in situations of competition with introduced species, such as *L. multiflorum*, the competition effects among species determined the decrease of CPG group contribution in the HM.

According to Collantes et al. (1998), native legumes present very low cover on Campos grasslands and little response to fertilization. In this work, we found warm-season legumes species such as *Stylosanthes montevidensis, Desmanthus depressus, A. falcata, Desmodium adscendens, D. incanum*, and two exotic cool season legumes (*Vicia sativa* and *Trifolium vesiculosum*), which did not present direct responsiveness to

P fertilizers. Mainly WL are frequent in these grasslands but their contribution in forage production is scarce. Previous results have been contradictory: some authors found increases in legume mass contribution when P fertilizer was applied (Gatiboni et al., 2000; Rodríguez et al., 2007; Tiecher et al., 2013), but others found a decrease in the contribution of this group (Mendoza et al., 1983). In our study, there was a reduction of the legume species contribution in HM, mostly due to the high quantities of N applied, which promoted the sward domination by C_4 tall grasses. Thus this impact in the WL group may be important considering that legume species improve forage quality and increase the soil N pool via symbiotic fixation, a crucial mechanism in these grasslands to maintain a positive balance of nutrient and ensure sustainability (Chaneton et al., 1996; Rubio et al., 1997).

Ceulemans et al. (2013) indicated that increasing the soil P availability from 01 to 20 mg·kg^{-1} (P Olsen) reduced from 30 to 15 species in 135 experimental plots at European continent (C_3 metabolic path). As a consequence, it could reduce the species' richness and diversity (Hejcman et al., 2007; Planteureux et al., 2005; Venterink, 2011). Similarly, Blanck et al. (2011) demonstrated that P content in plants is negatively correlated with diversity in South American natural grasslands. However, in this experiment, the increase in soil P availability (for details, see Tiecher et al., 2013) did not change the species' richness (see Table 3). This could be attributed to relatively low P quantities in this trial, as well as to larger application intervals along 16 years of trial. Besides, low soil pH provided conditions for rapid adsorption of applied P and this situation reduced P availability for levels near original 200 days after application (Oliveira et al., 2014).

Recorded stability in species richness and floristic diversity of phosphate-fertilized plots can be attributed to species replacement, and this process could change the botanical composition without changing the floristic diversity. This change could be attributed to increase in P availability and competition due to annual ryegrass in TP treatment. Both effects in this treatment promoted *P. urvillei* and *Uroclhoa plantaginea* contributions, which were absent in RP and Control treatments. Besides, average species' richness of 21 plants for all treatments (see Table 3) were lower when comparing with other South American grasslands (Rodriguez and Jacobo, 2010). Reduced number of species can be attributed to the historic management, with larger rest periods or even no cutting along growth seasons, causing a competitive exclusion of species with lower ability to compete for light (Duru et al., 2014).

Implications

In a simulation, using our data and applying the stocking rate adjustment method described by Soares et al. (2005) using beef heifers' performance in our results of HM and accumulation rate, the livestock production calculated could be 751, 876, and 932 kg of body weight (BW) ha^{-1}·yr^{-1} for Control, RP, and TP, respectively. These results, when comparing with the livestock production obtained in the usual farm management (70 kg·BW·ha^{-1}) (SEBRAE/SENAR/FARSUL, 2005) and from experiments with stocking rate adjustment (236 kg·BW·ha^{-1}, Soares et al., 2005), demonstrated an increment of four times on livestock production per area. Besides, considering that TP treatment has a greater cool-season species mass contribution, we calculated a potential livestock production from livestock data obtained in a similar fertilized natural grassland with cool-season species overseeded (Garagorry et al., 2008) and we used the same method for stocking rate adjustment. These simulations indicate a possibility to reach a value of 1319 kg BW·ha^{-1}·yr^{-1} of livestock production. This value is 5.6 times greater than the values reported by Soares et al. (2005), and besides, this value represents a 75% increment in relation to the result obtained when we used the same methodology for Control's data (751 BW·ha^{-1}·yr^{-1}). Even so, the simulated value with Control's data is 10 times higher than the mean results of local farmers (Nabinger et al., 2009). The same authors, also simulating a similar scenario, affirmed that livestock production per area could reach 1 000 kg·LW·ha^{-1}. In this sense, our results demonstrate that fertilization management and oversowing promote an increase on primary and livestock production on natural grasslands. These results allow us to conclude that surface application of soluble phosphate fertilizers, combined with cool-season species overseeding, are simple interventions on management that could be effective in minimizing the adverse effects of low herbage availability during the cool season and bare soil proportion.

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