Phosphate fertilization and liming in a trial conducted over 21 years: A survey for greater forage production and Pampa pasture conservation

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Abstract

Phosphorus fertilization and liming are alternatives used to increase forage production in Southern Brazil grasslands (Pampa grasslands) and to avoid its replacement by cultivated exotic crops. However, changes in natural soil fertility can significantly affect the equilibrium of natural vegetation communities. We hypothesized that triple and simple superphosphate and limestone using would lead to greater increase in dry matter production and to changes in the botanical composition and richness of Pampa grassland plant species than the use of lesser soluble P sources (phosphate rock) or no liming treatment. To test our hypotheses, we assessed a 21-year-old field trial to identify its forage production pattern, based on P sources and liming application. The vegetation was studied in 2009 and 2018, based on the following variables: Shannon and Pielou indices, species richness, dry matter and plant groups (species grouped based on growth form, life period, photosynthetic pathway, morphology and growing season). Soil chemical properties in the 0–10 cm layer were also evaluated in 2018. According to the long-term evaluation, two high dry matter yield periods were observed after P fertilization (55% and 25 % higher dry matter yield, on average). Each of these periods was followed by decreased dry matter yield, regardless of the P source. Species richness did not show changes overtime, although there was high turnover of plant species. Soluble fertilizer usage resulted in increased dry matter production and in the replacement of large proportions of species, mainly in tussock grasses and forbs groups. The contribution of legume species for dry matter production increased due to the combined effect of phosphate fertilizers and limestone. However, maintaining forage production levels higher than without phosphorus fertilization requires regular supply of soluble phosphorus fertilizers, which, in the present case, cannot exceed three years.

Keywords – Grassland, Phosphate rock, Dry matter, Hierarchical approach, Botanical composition.

1. Introduction

Phosphorus (P) corresponds to approximately 0.2% of plant dry matter (Schachtman et al., 1998); it is a key component for protein and nucleic acid production, as well as for enzymatic activation and energy transfer (Wang et al., 2017). Nitrogen and P are the macronutrients mostly limiting photosynthetic production in aquatic or terrestrial environments (Elser et al., 2007; Fay et al., 2015). Consequently, plant growth is directly linked to P availability in the soil.

Soil P availability for plants grown in natural environments results from the formation of P forms available in primary minerals during soil formation processes. Parent material composition and weathering intensity play fundamental roles in soil nutrient availability for plants (Turner et al., 2018). Overall, soils in Southern Brazil grasslands (the so-called Pampa grasslands) are intensely weathered, rich in iron oxides and kaolinite, and formed from parent materials presenting low P concentration, which implies low natural P availability to plants. Moreover, they are naturally acid soils (pH 4.4–5.1) and this feature leads to high-energy P adsorption onto soil clay minerals and oxides, as well as reduces natural soil P availability to plants (e.g., Mehlich-1 method), which often ranges from 2 mg kg^{-1} to 8 mg kg^{-1} (Oliveira et al., 2011; Rheinheimer et al., 1997).

Vegetation growing in these soils comprises species adapted to local conditions such as low P availability and soil acidity (Oliveira et al., 2018; Marques et al., 2019). Approximately 3,000 plant species can be found in the Pampa grassland ecosystem (Pampa biome), 450 of them are grass species used for forage production (Boldrini, 2009). Pampa grasslands produce from 3.7 Mg ha^{-1} $year^{-1}$ (Soares et al., 2005) to 9.8 Mg ha^{-1} $year^{-1}$ (Pellegrini et al., 2010) of dry matter under natural conditions, which enables mean potential production of 60–70 kg ha^{-1} $year^{-1}$ of cattle body weight under extensive livestock management (Carvalho et al., 2006). However, Pampa grasslands are often poorly managed due to production system intensification processes that lead to overgrazing, low forage and beef production and, consequently, to low profitability (Carvalho and Batello, 2009; Borges et al., 2016; Fedrigo et al., 2018). Pampa grassland areas have decreased by 26 % in the last 50 years to give room to annual crops and cultivated forests capable of providing higher revenues (Oliveira et al., 2017).

Nevertheless, the vegetation composing the Pampa grasslands has high potential for forage production when it is properly managed. Grassland improvement through soil fertility correction and exogenous winter species' inclusion can lead to forage dry matter production of 14 Mg ha^{-1} $year^{-1}$ and increase meat production to approximately 900 kg ha^{-1} $year^{-1}$ of cattle body weight (Oliveira et al., 2015). This means that it is possible intensifying these production systems, increasing farmers' income and protecting the natural biome, at the same time. It is necessary implementing proper plant and soil fertility management processes in order to achieve high forage production rates. Limestone and phosphate fertilizers are fundamental strategies used to improve soil fertility, increase nutrient availability and, consequently, to improve plant development (Prestes et al., 2016).

Low-solubility phosphate fertilizers, such as phosphate rock (RP), have been suggested as better alternative than soluble phosphates to increase P contents in grasslands (Tiecher et al., 2014; Oliveira et al., 2015). Slow fertilizer solubility enables the theoretical synchronism between P availability and plant demand (Szilas et al., 2007), which gradually leads to increased dry matter production in the long-term. However, RP's ability to release P is uncertain due to several factors that control its dissolution, such as soil pH (Robinson and Syers, 1990), moisture level (Heindel et al., 2018) organic matter content (Alloush, 2003) and Ca content (Robinson and Syers, 1990). Therefore, the RP effect on Pampa grasslands' dry matter production in the long-term remains unknown.

Although soil fertility improvement has beneficial effect on dry matter production, it can also change grassland' dynamics. For instance, increasing soil P availability can favor the development of more nutritionally-demanding plants, such as leguminous plants, and make them more abundant and even dominant in the vegetation community (Blanck et al., 2011;

Ceulemans et al., 2013; Harpole et al., 2016). Balanced nitrogen and phosphate fertilizer using can be an alternative to reduce the effect of grassy or leguminous species dominance on fertilized areas (Harpole et al., 2016). Species dominance is also not desired in Pampa grasslands because it can reduce the wide coexistence of plant species, which is one of the main features of Pampa vegetation communities. Changes in the botanical composition of grasslands subjected to P fertilization are contradictory. Some studies presented decreased species richness (Zhao et al., 2019), whereas other studies did not show changes in the number of plant species (Tiecher et al., 2014; Oliveira et al., 2015). These last cases can be explained by the analyzed time scale: 12 years (Tiecher et al., 2014) and 16 years (Oliveira et al., 2015). Longer follow-up time is suggested to analyze changes in botanical composition more reliably (Schellberg et al., 1999).

The current study aims to investigate (i) whether soluble P sources (triple and simple superphosphate) and limestone use result in greater dry matter production than the use of lesser soluble P sources (phosphate rock) or the use of no liming; and (ii) in which ways P fertilization and liming affects the botanical composition and grassland plant species richness.. Soil and vegetation data from a 21-year-old trial conducted in Southern Brazilian Pampa grassland managed with different phosphate sources, liming, fertilization rates and plant species' overseeding were analyzed.

2. Materials and Methods

2.1. Site location and initial soil and vegetation conditions

The trial was implemented in 1997, in soil type classified as Ultisol with natural grassland, which is commonly referred to as Pampa biome. It was conducted in the experimental area of the Soil Science Department of Federal University of Santa Maria. The grassland at the trial site is classified as Mesic Pampa Grassland subtype 5b (Andrade et al., 2019). Climatic features of the trial site comprise humid subtropical climate type with annual rainfall of approximately 1,770 mm; the mean annual temperature in the hottest months (December–February) reaches 24.2 ºC, and 14.5 ºC in the coldest months (June–August) (Fig. 1). The overall chemical features of the topsoil layer (0-20 cm) in 1997 comprised: 170 g kg^{-1} of clay (pipette method); pH (H_2O extraction) of 4.5; 10.4 g kg^{-1} of C (Walkley-Black method); 2.5 mg kg^{-1} and 60 mg kg^{-1} of P and K, respectively (extracted by Mehlich-1); and 1.30 $cmol_c$ kg^{-1}, 1.17 $cmol_c$ kg^{-1} and 0.75 $cmol_c$ kg^{-1} of Al, Ca and Mg, respectively (extracted by using 1 mol L^{-1} KCl).

The botanical composition of the area mainly comprised *Paspalum notatum* (45.3% of pasture biomass), *Eryngium ciliatum* (21.2% of pasture biomass), *Andropogon ternatus* (1.0% of pasture biomass), *Paspalum plicatulum* (0.5% of pasture biomass), *Chloris polydactyla,* and *Schizachyrium microstachyum* (0.3% of pasture biomass), as well as *Eryngium horridum*, *Aristida laevis*, *Piptochaetium montevidense* and *Saccharum angustifolius* (each with 0.2% of pasture biomass). Senescent material contribution corresponded to 29.2% of pasture biomass (Bandinelli et al., 2005).

2.2. Experimental history and treatments

The trial was carried out based on a two-factorial (6 × 3) split-plot complete randomized block design, with three replications. The main factor consisted in the application of different P sources and limestone, as well as in *Lolium multiflorum* Lam. cv. 'Comum' and *Trifolium vesiculosum* Savi. cv. 'Yuchi' overseeding, namely: TPCa—triple superphosphate + limestone + overseeding; TP—triple superphosphate + overseeding, SPCa—single superphosphate + limestone + overseeding; RP—phosphate rock from Gafsa + overseeding; OS—without P but with overseeding; and Control—without P and without overseeding (Control). The second factor (split-plot) was P fertilization rate: 118 kg ha^{-1} of P, applied until

1998; 206 kg ha^{-1} of P, applied until 2010; and 250 kg ha^{-1} of P, applied until 2012. Phosphorus inputs in each treatment over time are shown in Fig. 2.

Liming was applied to the soil surface at a rate of 3.2 Mg ha^{-1} in 1997 (the amount necessary to achieve pH 5.5). Potassium (108 kg ha^{-1} of K) and nitrogen (70 kg ha^{-1} of N, in its urea form) were applied to the soil surface in all treatments, except for the Control, in 1997. *L. multiflorum* and *T. vesiculosum* overseeding was carried out with 30 kg ha^{-1} and 12 kg ha^{-1} of seeds, respectively, in the cool season of 1997 and 2002.

2.3. Biomass production and floristic composition

Dry matter production values recorded in 1998, 2008–2010 and 2013 were taken from (Gatiboni et al., 2000; Tiecher et al., 2014; Oliveira et al., 2015) and reanalyzed in association with dry matter measurements taken between 2016 and 2018, as described below. In addition to biomass production, data about floristic composition in 2009 were taken from Tiecher et al. (2014) and reanalyzed in association with results of the botanical sampling performed in 2018 - exclusive to the present study.

Dry matter production was measured between 2016 and 2018 by cutting the shoot biomass in area covering 0.25 m^2 per plot, as carried out by (Gatiboni et al., 2000; Tiecher et al., 2014; Oliveira et al., 2015). Next, plant material was oven-dried at 60°C, for 48 h. After the cutting procedure performed to estimate dry matter production was over, all areas were mowed and plant material was removed from the site. The vegetation was not grazed by animals throughout the 21-year experiment and tractor trimmer was used to mow the pasture at 5 cm (in height), throughout the experimental period.

Data sets about quantified dry matter production were taken from the literature and normalized in comparison to the treatment without P fertilization (OS). OS was used as standard treatment because, unlike the Control treatment, it had yield data of all analyzed years available. Relative values recorded for each analysis period were achieved through the following equation:

$$RDm = \frac{Dm_x}{Dm_{OS}} 100 \qquad \text{Equation 1}$$

where *RDm* (%) is the relative dry matter, Dm_x (kg ha^{-1}) is the dry matter of treatment *x*, and Dm_{OS} (kg ha^{-1}) is the dry matter of treatment *OS*.

Floristic composition was quantified based on the BOTANAL method, in January 2009 (67-day growth period) (Tiecher et al., 2014) and in February 2018 (75-day growth period)—i.e., 12 and 21 years after the beginning of the experiment, respectively. Briefly, the three most dominant species were ranked based on dry weight, in a fixed frame (0.25 m^2), and species composition rate was estimated. All other species were identified and 1% of total dry matter was attributed to each one of them (Tothill et al., 1978).

Species identified in both botanical samplings (2009 and 2018) were classified into nine groups, according to Oliveira et al. (2015), by taking into consideration a hierarchical approach (Lavorel et al., 1997) based on growth form (grasses, legumes, forbs, sedges); life period (annual, perennial); photosynthetic pathway (C3 or C4); morphology (tussock, prostrate); and growing season (cool or warm-season) (Appendix A). These nine groups comprised warm-season C4 prostrate perennial grasses (WPPG), warm-season C4 tussock perennial grasses (WTPG), warm-season C4 annual grasses (WAG), warm-season legumes (WL), cool-season C3 annual grasses (CAG), cool-season C3 perennial grasses (CPG), cool-season legumes (CL), forbs, and sedges.

The vegetation in the plots was analyzed based on the diversity, richness, equitability and maintenance of the observed species. Species diversity was estimated based on the Shannon index (H') provided by equation 2.

$$H' = -\sum_{i=1}^{S} \frac{ni}{N} \ln \frac{ni}{N} \quad \text{Equation 2}$$

wherein S is the number of species in the sampled area, ni is the dry matter of each species (kg ha^{-1}), N is the total dry matter (kg ha^{-1}), and ln is the natural logarithm.

Species richness (SR) was based on the total number of species found in the sampled area of each plot. Species equitability (evenness) was analyzed was analyzed based on the Pielou index (J) (Pielou, 1966) provided by equation 3.

$$J = \frac{H'}{\ln(SR)} \quad \text{Equation 3}$$

The maintenance of species was analyzed by the rate of persistence (i.e., the number of species observed in both floristic composition quantification), disappearance (i.e., the number of species identified in 2009 but not identified in 2018) and emergence (i.e., the number of species not identified in 2009 but identified in 2018) of then.

2.4. Soil sampling and analysis

Soil was sampled in the 0–10 cm layer in October 2018. Sampled soil was oven-dried at 65ºC, sieved at 2 mm mesh size and stored for chemical analyses.

Soil pH was measured at soil/water ratio of 1:1 (v/v). Phosphorus (P) and K contents available in the soil were extracted through Mehlich-1 (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4) (Mehlich, 1953) and quantified through spectrophotometry (Murphy and Riley, 1962) and atomic emission techniques, respectively. Exchangeable Ca, Mg, and Al contents were extracted with 1 mol L^{-1} KCl and quantified through atomic absorption spectrophotometry (Ca and Mg), whereas Al was quantified by titration with 0.0125 mol L^{-1} NaOH. Effective cation exchange capacity (CEC_{ef}, $cmol_c\ kg^{-1}$), cation exchange capacity at pH 7.0 ($CEC_{pH\ 7.0}$, $cmol_c\ kg^{-1}$), Al (Al_{sat}, %) and cation saturation (V, %) were estimated.

2.5.Statistical analyses

Dry matter production pattern over 21 years was graphically analyzed after values were standardized, as described above. Soil variables were analyzed based on a two-factorial split-plot complete randomized block design with three replications, where each plot corresponded to P sources and split-plot corresponded to the P fertilizer rate. Analysis of variance and mean test (Scott-Knott test, $p \leq 0.05$) were carried out in the SISVAR 5.6 software (Ferreira, 2015).

Dry matter production and botanical variables of Shannon diversity index, Pielou equitability index and species richness were analyzed through randomization test ($p \leq 0.05$). In addition, principal component analysis (PCA) was applied to the same variables, which were standardized in the Multiv software (Pillar, 1997) based on their marginal total and subjected to ordination multivariate analysis in the Past software (Hammer et al., 2001).

3. Results

3.1. Soil chemical properties

Residual limestone effect persisted in the soil after 21 years. Significant effects between P sources and P fertilizer rate were only observed for available soil P content (Table 1). Liming (TPCa and SPCa) treatments decreased soil acidity, as shown through higher soil pH-H_2O, cations saturation (V) and exchangeable Ca and Mg values, as well as through low Al and Al_{sat} contents, and low potential acidity values (H+Al, Table 2).

There was no residual effect 20 years after the application of 118 kg ha^{-1} of P; mean soil P content of 15 mg kg^{-1} were observed for all treatments (Table 3). The application of additional 44 kg ha^{-1} of P in 2002 and 2010 resulted in mean increase by 13% in available soil

P content in soil subjected to treatments with P inputs, regardless of the P source. The application of additional 44 kg ha^{-1} of P in 2012 maintained the difference in available soil P content in the soil between treatments that were not treated with P (Control and OS) and those that were treated with it (RP, TP, TPCa, and SPCa) (Table 3).

3.2.Historical pattern of dry matter production

Two high yield periods identified throughout the 21 experimental years corresponded to post-fertilizer application times. Each of these periods was succeeded by decreased yield rates over time. The RDm of Pampa grassland in 1998, soon after the first fertilization with soluble P (SPCa, TPCa, and TP), was 55% higher than that of the OS treatment, on average, whereas the RDm of the control treatment was 25% lower than that of OS. Low solubility phosphate (RP) using provided RDm 34% higher than that of OS (Fig. 2).

In the period from 2016 to 2018 (i.e., 4 and 6 years after the fertilization carried out in 2012, respectively – treatment based on 250 kg ha^{-1} of P) the P fertilization did not result in significant difference in RDm between treatments (mean test not shown). Fig. 2 presents the decrease line drawn based on means recorded for treatments with soluble P in order to show yield decrease over time.

3.3. Floristic composition and plant dry matter production accessed in 2009 and 2018

Based on the evaluation performed in 2009, the experiment only consisted in two P fertilizer rates. The first one comprised the application of 118 kg ha^{-1} of P until 1998, whereas the second one encompassed the application of 162 kg ha^{-1} of P until 2002. The application of 162 kg ha^{-1} of P until 2002 - for chronological purposes - gave rise to treatments based on the application of 205 kg ha^{-1} of P until 2010 and 2012 (Fig. 2).

Dm in the 2009 botanical sampling was higher in plants treated with soluble P fertilizer, whereas RP was the less productive treatment. Dm values recorded in the 2018 botanical sampling were similar between P sources (Table 4). Overall, botanical sampling Dm values were higher in 2018 than in 2009. This pattern corroborates the significance of differences in dry matter production between 2007 and 2009 (Tiecher et al., 2014) and the non-significance of it between 2016 and 2018 (Fig. 2) (mean test not shown).

Shannon dominance index was different between RP (1.39) and TP (1.20) in 2009 (Table 4). Species richness and Pielou equitability index (J) did not show differences between treatments. These variables recorded different outcome in the 2018 botanical sampling, since Shannon index was lower in RP (0.65) and TP (0.77) and higher in OS (1.08) and SPCa (1.09). Pielou equitability (J) index was lower in RP (0.297) and higher in TP and OS (0.50 and 0.54, respectively). Species richness only differed in TP, since it recorded values lower than the ones recorded in the other treatments. Differences between P fertilizer rates were not observed in any vegetation sampling (2009 and 2018, Table 4).

The comparison between the two botanical sampling years was based on principal component analysis and it is discussed below. However, there were reductions in Shannon and Pielou indices and species richness, as well as increase in Dm between 2009 and 2018. This reduction, together with the similarity observed in 2018, may suggest homogenization in values recorded for the analyzed variables due to temporal reduction in treatments' effect. On the other hand, different P sources may lead to species turnover without causing changes in botanical indices and species richness (Oliveira et al., 2015).

3.4. Plant diversity and vegetation dynamics

Sixty-seven (67) plant species distributed in 15 families were identified throughout the experiment. *Poaceae, Asteraceae* and *Fabaceae* prevailed among them; they encompassed 25, 14 and 10 species, respectively. Overall, forbs, WTPG and WPPG were the groups of plants recording the largest number of observed species, regardless of the adopted P source (Fig. 4). Botanical sampling carried out during summer (October–March) - the season recording the highest temperatures in the year (Fig. 1) - accounted for the prevalence of warm-season species.

Forbs and WTPG were the species that mostly disappeared between the two analyzed years. Species disappearance rate ranged from 64% to 85% in forbs, and from 75% to 100% in WTPG (Fig. 4). In this plant groups (forbs and WTPG), the highest species disappearance values were observed for TP and TPCa treatments. SPCa recorded the lowest species disappearance rate. With respect to warm-season leguminous (WL) and warm-season prostrate grasses (WPPG), the lowest species disappearance rate was associated with P and limestone application. There were 50% and 67% of WL species disappearance, as well as 33% and 40% of WPPG species disappearance in plants subjected to TPCa and SPCa treatments, respectively.

The highest species emergence rate was observed for the forbs and WTPG groups; values ranged from 64% to 85% for forbs, and from 50% to 100% for WTPG. The highest species emergence rate recorded for these two groups was observed for plants subjected to TP and TPCa treatments. TP and TPCa accounted for the highest species turnover rate recorded for the forbs and WTPG groups. It resulted in forbs maintenance and in decrease (-25%) richness of WTPG plant groups in TP treatment from 2009 to 2018. TPCa treatment led to species richness increase (100%) in forbs and decrease (-50%) in WTPA plant groups from 2009 to 2018.

Overall, there was incidence of WTPG species, which led to increased species richness, mainly in treatments with PR, SPCa and TP. Plants belonging to the legume group

(WL) had a greater appearance in the RP and TPCa treatments (25 and 125%, respectively), and it increased their participation in species richness of these treatments.

Principal components analysis (PCA) was carried out to feature changes in botanical variables, plant groups and RDm from 2009 to 2018 (Fig. 5). Plant groups were represented by the dry matter produced in each treatment. The CPG, CL and CAG groups were removed from the analysis because they were not observed in any of the treatments in 2018 sampling. The first two principal components in PCA explained 64.4% of total variance.

Species richness and Shannon diversity index (H') were negatively associated with TP and SPCa, in both evaluated years. Species richness in 2009 was associated with the Control, OS and TPCa treatments, and this behavior remained the same in 2018. Nevertheless, H' was associated with RP in 2009 and presented large shift towards TPCa in 2018. Pielou equitability index (J) was positively associated with RP, TP and SPCa, and negatively associated with TPCa, Control and OS, in 2009. However, J was not positively associated with RP in 2018, but it was positively associated with SPCa and TPCa. RDm location in PCA did not change from 2009 to 2018. RDm was positively associated with SPCa, TP and TPCa, and negatively associated with PR, Control and OS, in the two evaluated years.

Groups composed of grasses and legumes (WL, WPPG and WTPG) suffered greater effect of treatments over time. WPPG was strongly correlated to TPCa in 2009, whereas its correlation to RP and TP was higher in 2018. WTPG behaved in a different way; it was positively correlated to TP and SPCa in 2009 and presented correlation to OS, TPCa and SPCa in 2018. Warm-season legumes (WL) were positively associated with SPCa and TP in 2009, although they were more closely associated with TPCa in 2018. Sedges and forbs groups were slightly affected by treatments. Overall, sedges and forbs were only associated with SPCa and TP in 2009. In 2018, they were correlated to RP, as well. Forbs and sedges groups were negatively related to TPCa in both evaluations.

4. Discussion

Phosphate fertilization addition and soil acidity correction increased dry matter production and Pampa grassland quality. These practices enable increasing the economic return of livestock activity carried out in managed natural fields, as well as avoid replacing Pampa grasslands by annual or forest crops. Concurrent with the increase in dry matter production we found that soluble phosphate fertilizer usage led to a long-term plant species replacement.

4.1. Soil chemical features

Limestone application (TPCa and SPCa) played key role to differentiate the treatments after 21 years. Even after this time, it was possible observing significant residual effect of limestone on the 0–10 cm soil layer. Similar results were observed for the same soil in cropland areas. In these cases, residual limestone effect was observed after twelve and eighteen years (Rheinheimer et al., 2018; Vargas et al., 2019). In the present case, superficial liming provided TPCa and SPCa cations saturation levels higher than the reference value (40%, SBCS, 2016) in Pampa grassland areas. This means that renewed liming application is not compulsory.

Slightly higher Ca content in RP treatment may be associated with low dissolution of apatite minerals or carbonates found in phosphate rock fertilizer. The phosphate rocks from sedimentary deposits, such as the one used in the current study, have low $CaCO_3$ contents (Robinson and Syers, 1990; Lefires et al., 2014). However, difference observed in soil Ca content is more likely associated with apatite dissolution than with $CaCO_3$ incidence in phosphate rock fertilizer, since the small increase in Ca content was not followed by changes in soil pH and Al contents.

Time was a key factor in soil P availability. There were no differences between treatments in the long-term and there was decrease in available soil P content. This reduction may explain the sharp yield decrease and differences in RDm between treatments (Fig. 3). Moreover, the reduced difference between treatments and available soil P content have evidenced P adsorption as inner-sphere complex by soil clay minerals and iron oxi-hydroxides (Kim et al., 2011; Bortoluzzi et al., 2015), as well as high nutrient exportation by vegetal biomass (Oliveira et al., 2014).

The highest available soil P content observed after the application of 250 kg ha^{-1} of P was in compliance with the higher fertilizer rate application (Fig. 3). However, RP application at rate of 250 kg ha^{-1} of P resulted in the highest available soil P content. This behavior may be indicative of gradual P dissolution. It was not observed after the application of 118 (until 1998) and 205 (until 2010) kg ha^{-1} of P, but it was observed after the application of 250 kg ha^{-1} of P (until 2012 - Table 3).

Nevertheless, higher soil P availability did not lead to higher dry matter production in RP. Thus, the highest available P values recorded for RP may be an artifact from P extraction process based on the adopted method. The Mehlich-1 extractor used to obtain available P is composed of acid solution (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4) that can promote dissolution of residual apatite minerals from RP fertilizer in the soil. In that case extracted P value may partly correspond to apatite dissolution and may not exactly correspond to bioavailable P (Freitas et al., 2013). Using acid extractors to quantify available P in soils subjected to RP fertilization is only recommended after 2-year fertilization (SBCS, 2016). Therefore, if future studies can prove that non-solubilized apatite remains in the soil 6 years after the last RP fertilization, it will be necessary recommending another method to quantify P availability in the soil.

4.2. Association between produced dry matter and P fertilization

Control and OS treatments recorded the lowest RDm, mainly in periods following fertilizer application. This feature highlights the limitations of Pampa grassland soils to achieve higher dry matter production under natural conditions, due to reduced natural P availability level in these areas, as also reported in other studies (Rizo et al., 2004; Soares et al., 2005; Pellegrini et al., 2010). Soluble phosphate fertilization is an alternative to quickly provide nutrients to soil solution. Improvements in soil chemical features due to soluble fertilizer using played key role in increasing dry matter yield (Gatiboni et al., 2000). Soluble P sources have historically led to the highest dry matter production response over time (Gatiboni et al., 2000; Tiecher et al., 2014; Oliveira et al., 2015). However, the rapid plant response to fertilization with single and triple superphosphate was followed by a decrease of forage production over time.

Yield decrease over time can be associated with negative balance in P availability in the soil. This balance is defined by leaving available soil P through dry matter exportation or immobilization by P inner-sphere adsorption onto clay minerals and/or soil oxides (P non-labile) and through no P replacement via fertilization. This process can be intensified due to low natural P availability (±13 mg kg^{-1}, Table 3) at pH ± 4.7 (Table 2) and to the presence of kaolinite, goethite and hematite at the topsoil layer (0–10 cm) (Bortoluzzi et al., 2015, 2007; Moterle et al., 2016). Thus, P availability in the soil may have shown higher exhaustion level in the first half of the investigated period (1998–2010) than in the second half of it (2010–2018); moreover, it could be the cause of greater slope in the RDm decreasing line between 1998 and 2010 (Fig. 3).

Constant P input into the system tends to saturate functional groups of inorganic constituents accounting for P sorption (Roy et al., 2017). The amount of P added to the soil until 2010 (162 kg ha^{-1} of P) may have been enough to cause small reduction in future P immobilization rates and, consequently, greater maintenance of soil P availability over time in order to avoid high dry matter production decrease rates. Therefore, periodic phosphate fertilizer applications should be conducted to counteract losses of available soil P by sorption in soil matrix and plant P exportation, as well as to maintain high dry matter production rates.

RP application has generated lower Pampa grassland yield response between P-fertilized plots due to lower P release by RP in the short-term than that observed for soluble P sources (TPCa, TP and SPCa). However, Pampa grassland response to RP was always lower than that observed for soluble P sources; the time elapsed after fertilization day led to RDm decrease. The temporal pattern of RDm reduction soon after RP application was not expected. Gradual dissolution of apatite minerals and fertilizer reapplication in the long-term were expected to keep productivity constant over time. RDm observed over time due to RP using may be associated with two main factors. The first one is the likelihood that RP is poorly solubilized in the soil, even in the long-term, and consequently, there is low P release for plant uptake purposes. Low P dissolution and maintenance in its apatite form has been evidenced in studies carried out worldwide (Kumar et al., 1994; Soltangheisi et al., 2018) and in preliminary results of the present trial. Finally, the temporal change in the botanical composition of the investigated site is the second factor, which, consequently, had effect on dry matter production.

4.3. Changes in vegetation composition

The smaller difference in vegetation indices in 2009 and larger differences in 2018 have indicated that the botanical composition has changed due to treatments applied within the evaluated period. Despite differences reported in vegetation indices, greater differences may have been avoided by cutting the vegetation on a regular basis, over 21 years. Vegetation cuts were performed in a simultaneous and uniform way throughout the treatments in order to simulate rotational grazing. Rotational grazing systems can provide plant species richness increase in the long-term, even within a mid-term period (six years) (Boavista et al., 2019). Therefore, the effect of periodic cuts may have mitigated, to some extent, the effect of soil fertility treatments on species richness and botanical indices.

However, there was small temporal variation in the Shannon (H') and Pielou (J) indices, species richness and Dm from 2009 to 2018 (Fig. 5). Significant reductions due to P fertilization over time, mainly in species richness, have been reported in grasslands investigated in the Americas, Europe and Asia (Blanck et al., 2011; Ceulemans et al., 2013; Zhao et al., 2019). Small variation in botanical features between the two surveys performed in the current study was associated with different P sources, which were capable of promoting species turnover, although without changing botanical indices and species richness, as suggested by Oliveira et al. (2015). Species turnover may lead to changes in nutrient exportation rates and such a process may affect soil nutritional levels due to changes in bromatological features of forage plants, as well as in soil organic matter mineralization and accumulation.

The largest species turnover was recorded for forbs and WTPG plant groups at TP and TPCa. Although species turnover took place, species richness only decreased in the WTPG group, regardless of the treatments. This decrease may be associated with the fact that tussock growth habit is more affected by grazing than shorter or prostrate plants (Díaz et al., 2007). Grass species grown in South American grasslands play key role in forage production due to the large number of species and efficient dry matter production (Biganzoli and Zuloaga, 2015). This feature was observed in the present study, since the largest number of identified species belonged to family *Poaceae* and accounted for more than 65% of produced dry matter, regardless of the treatment (data not shown). Accordingly, treatments associated with the WTPG and WPPG groups were also associated with higher RDm (Fig. 5).

Unlike *Poaceae* plants, *Asteraceae* and *Fabaceae* plants - which composed the forbs and WL groups, respectively - are more nutritionally demanding and frequent in Pampa grasslands, but they provide little contribution to total dry matter production (Oliveira et al., 2015). Although some results have shown increased participation of leguminous plants in total forage dry matter when P fertilizers were applied (Rodríguez et al., 2007; Tiecher et al., 2014), this increase can be easily overcome through N supplementation (Oliveira et al., 2015). The higher dry matter production of forbs and WL groups in the current study was associated with soluble P sources (triple and simple superphosphate) and limestone application, since they led to high soil fertility levels. The greater participation of leguminous species, the higher dry matter yield in the long-term, and the higher P levels in plant tissues have contributed to greater P depletion and lower availability in the soil in treatments based on soluble fertilizer and limestone application.

In addition to the fact that leguminous plants were associated with SPCa treatment and, later on, with TPCa, grass species were mostly associated with limestone-free treatments (RP, TP, OS and Control), although RP and TP presented P contents similar to that of the TPCa and SPCa treatments. This behavior indicated that the simultaneous P and limestone application is more effective in increasing legume participation in dry matter production. Increasing the proportion of legume plants, either by favoring endemic leguminous species or sowing exotic legumes, helps enriching bromatological features (e.g., digestibility *in vitro*, crude protein and phosphorus content) (Gatiboni et al., 2008) and drought resistance in forage plants (Sanaullah et al., 2012).

In addition to changes in bromatological features, P fertilization can lead to changes in plant dry matter stoichiometric ratios (C:N:P). It mainly happens due to changes in botanical composition and increased P content in plant tissue (Gatiboni et al., 2008; Oliveira et al., 2014). Studies conducted worldwide have indicated reduced soil C and N stocks in managed pastures, even after fertilization and liming application (Eze et al., 2018). Furthermore, reduced C and N stock in pasture areas is enhanced by high plant material exportation due to increased grazing intensity (Zhou et al., 2017). However, Poeplau et al. (2018) have identified increased soil C stock, in the >10 cm soil layer, of fertilized pasture areas under temperate climate. The aforementioned authors have attributed such increase to reduced root C:N stoichiometry and to consequent increase in microbial C-use efficiency. Changes in soil C stocks in subtropical climate regions, such as the Pampa biome, can be observed after soil P fertilization. However, more conclusive studies should be conducted to analyze the soil C pattern in this scenario.

Cool-season species belonging to groups CPG, CL and CAG were identified even during the season presenting the highest temperatures in 2009. None of these groups was observed in 2018 (Fig. 4). *L. multiflorum* (CAG) and *T. vesiculosum* (CL), which are exotic species that were introduced in the region by overseeding conducted in 1997 and 2002, stand out among the cool-season species identified in 2009. Species *T. vesiculosum* was only identified in SPCa and TPCa. On the other hand, *L. multiflorum* was also observed in RP. This feature highlights the nutritional demands of the two exotic species, mainly for pH, Ca and P (Tables 2 and 3). The disappearance of groups CPG, CL and CAG in 2018 cannot be exclusively attributed to the effect of P sources, because environmental factors, such as temperature, may have played key role in the development of cool-season species. Moreover, the time after CAG and CL species introduction (last sowing in 2002, 16 years earlier) may have hindered the natural re-sowing of CAG and CL species. Therefore, the non-identification of these two species emphasizes the important role played by the periodic reintroduction of both *L. multiflorum* and *T. vesiculosum* in order to maintain satisfactory stocking levels.

5. Conclusion

Phosphate fertilization and liming application in Southern Brazil Pampa grasslands soil helped improving dry matter production. However, Pampa grasslands responded to phosphorus sources in different ways over 21 years. Soluble fertilizer using, such as triple and simple superphosphate, led to higher dry matter production. Phosphate fertilization led to small changes in species richness; thus, it is possible maintaining floristic biodiversity, despite phosphorus fertilizer using. However, there was higher species turnover, mainly in the tussock grass and forbs groups, which may contribute to change in ecosystem services. Legume species' contribution to dry matter production can increase due to soluble phosphate fertilizer and limestone using.

Although species turnover and increased proportion of legumes can enable improved bromatological features, they can lead to reduced soil C stock and decrease Pampa biome' ability to adapt to climate change, in the long-term. Thus, more conclusive studies should be conducted to analyze changes in ecosystem services and soil carbon increase in fertilized Pampa biome soil.

Regular phosphorus input is necessary to maintain dry matter yield and legume species' contribution in dry matter production higher than without phosphorus fertilization; in the present case, four years after phosphorus input resulted in dry matter yields similar between treatments; in the condition of the present study (i.e., with dry matter exportation) such an input cannot surpass three years. Lower species turnover may happen under rotational grazing conditions, and the effect of phosphorus fertilization can persist for longer due to lesser selective grazing and lower phosphorus exportation.

The highest available soil P content after phosphate rock application at rate of 250 kg ha^{-1} of P (six years before soil sampling) may be an artifact occurred during P extraction process based on Mehlich-1 extractor. This assumption suggests low phosphate rock dissolution over time and justifies the low increase in Pampa grassland dry matter production response.

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Fig. 1– 21-year-olf field trial conducted in a Pampa grassland site (a); mean rainfall and temperature (1997–2018) (b) and experiment's overview.

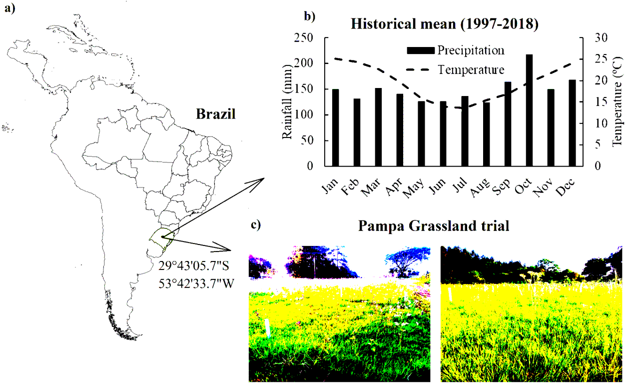
Fig. 2- 21-year-old field trial conducted in Pampa grasslands - framework of treatments and variable sampling.

Fig. 3– Dry matter production over time, normalized by OS treatment. Values recorded in 1998, 2008-2010 and 2013 were adapted from Gatiboni et al. (2000), Oliveira et al. (2015) and Tiecher et al. (2014), respectively. Arrows represent fertilization applications at the following rates: ***79 kg ha^{-1} of P, ** 44 kg ha^{-1} of P and *39 kg ha^{-1} of P. Treatments: Control— treatment without P application or overseeding; OS—without P application, although with overseeding; RP—phosphate rock of Gafsa + overseeding; TP—triple superphosphate + overseeding; TPCa—triple superphosphate + limestone + overseeding; SPCa—single superphosphate + limestone + overseeding.

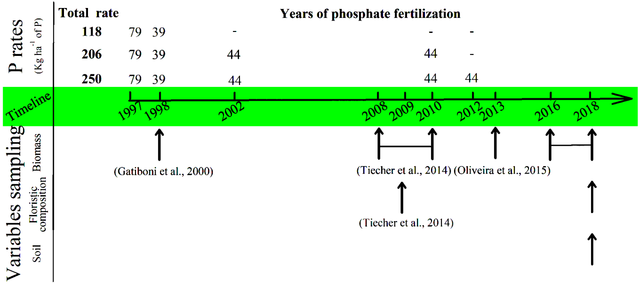
Fig. 4–Species replacement between 2009 and 2018 by functional groups. WPPG—warm-season C4 prostrate perennial grasses; WTPG—warm-season C4 tussock perennial grasses, WAG—warm-season C4 annual grasses; WL—warm-season legumes; CAG—cool-season C3 annual grasses; CPG—cool-season C3 perennial grasses; CL—cool-season legumes; forbs and sedges; Treatments: Control—treatment without P application or overseeding; OS—without P application, although with overseeding; RP—phosphate rock of Gafsa + overseeding; TP—triple superphosphate + overseeding; TPCa—triple superphosphate + limestone + overseeding; SPCa—single superphosphate + limestone + overseeding.

Fig. 5–Bi-plot of vegetation parameters and dry matter produced by the main plant groups accessed in 2009 and 2018, based on fertilization with different P sources. Arrows represent the 2009→2018 temporal change in the behavior of the assessed variables. H'—Shannon diversity index; J—Pielou equitability index; Dm—Dry matter field production; WPPG—warm-season C4 prostrate perennial grasses; WTPG—warm-season C4 tussock perennial grasses, WAG—warm-season C4 annual grasses; WL—warm-season legumes; CAG—cool-season C3 annual grasses; CPG—cool-season C3

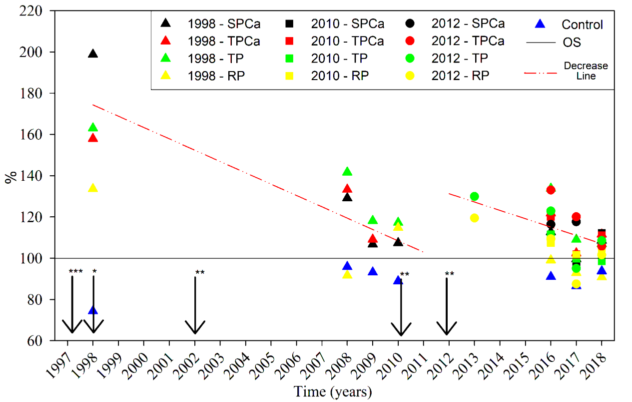
perennial grasses; CL—cool-season legumes; forbs and sedges; Treatments: Control— treatment without P or overseeding; OS—without P application, although with overseeding; RP—phosphate rock of Gafsa + overseeding; TP—triple superphosphate + overseeding; TPCa—triple superphosphate + limestone + overseeding; SPCa—single superphosphate + limestone + overseeding.



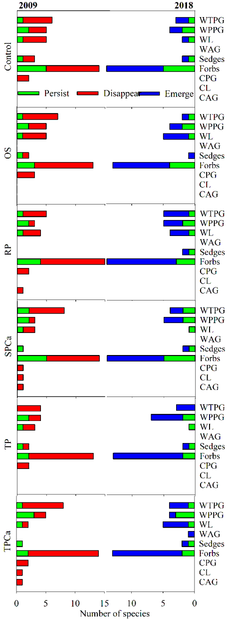
a)
Brazil
29°43'05.7"S
53°42'33.7"W
b)
Historical mean (1997-2018)
Precipitation
Temperature
Rainfall (mm)
Temperature (°C)
250
200
150
100
50
0
30
25
20
15
10
5
0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
c)
Pampa Grassland trial

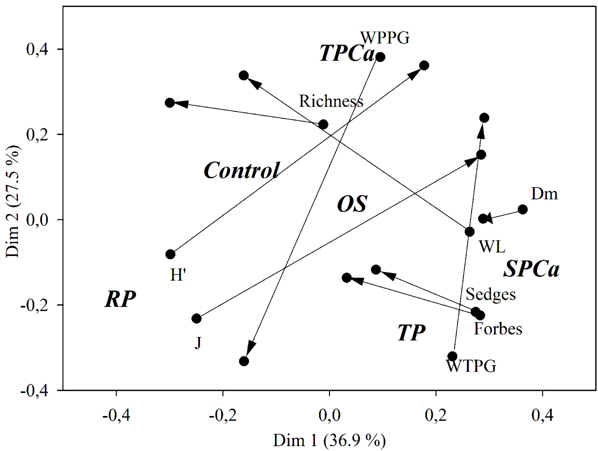


P rates (Kg ha^{-1} of P)
Total rate
Years of phosphate fertilization
118
79
39
-
-
-
206
79
39
44
44
-
250
79
39
44
44
44
Timeline
1997
1998
2002
2008
2009
2010
2012
2013
2016
2018
Variables sampling
Biomass
(Gatiboni et al., 2000)
(Tiecher et al., 2014)
(Oliveira et al., 2015)
Floristic composition
(Tiecher et al., 2014)
Soil



1998 - SPCa
1998 - TPCa
1998 - TP
1998 - RP
2010 - SPCa
2010 - TPCa
2010 - TP
2010 - RP
2012 - SPCa
2012 - TPCa
2012 - TP
2012 - RP
Control
OS
Decrease Line
%
Time (years)
***
*
**
**
**


2009
2018
Control
OS
RP
SPCa
TP
TPCa
WTPG
WPPG
WL
WAG
Sedges
Forbs
CPG
CL
CAG
Persist
Disappear
Emerge
0
5
10
15
Number of species



WPPG
TPCa
Richness
Control
OS
Dm
WL
SPCa
H'
RP
Sedges
Forbes
TP
J
WTPG
0,4
0,2
0,0
-0,2
-0,4
Dim 2 (27.5 %)
-0,4
-0,2
0,0
0,2
0,4
Dim 1 (36.9 %)

Table 1–Significance of the effects of experimental factors and their interactions on soil chemical properties, as resulting from analysis of variance (ANOVA).

FV	**pH**	**Al**	**Ca**	**Mg**	**K**	**P**	**(H+Al)**	**CEC_{ef}**	**$CEC_{pH7.0}$**	**Al_{sat}**	**V**
	H_2O	**--------- $cmol_c$ kg^{-1} ---------**			**----- mg kg^{-1} -----**		**--------- $cmol_c$ kg^{-1} ---------**			**----- % -----**	
T[a]	**	**	**	**	ns	*	**	**	ns	**	**
S	ns	ns	ns	ns	ns	**	ns	ns	*	ns	ns
T*S	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
CV 1 (%)	3.2	33.4	25.4	24.1	19.5	19.8	13.0	10.8	8.9	27.5	12.2
CV 2 (%)	3.2	24.9	15.3	14.4	15.4	17.2	10.9	8.6	5.5	23.0	12.4

[a]T—Treatments (phosphorus sources); S—Split-plot (rate of P fertilizer). *Significant at $p \leq 0.05$; ** Significant at $p \leq 0.01$; ns—Not significant at $p \leq 0.05$.

Table 2–Mean values of soil chemical properties measured in October 2018 in the 0–10 cm soil layer.

Treat	**pH H_2O**	**Al**	**Ca**	**Mg**	**K**	**(H+Al)**	**CEC_{ef}[c]**	**$CEC_{pH7.0}$[d]**	**Al_{sat}[e]**	**V[f]**
		--------- $cmol_c$ kg^{-1} ---------			**- mg kg^{-1} -**	**--------- $cmol_c$ kg^{-1} ---------**			**------ % ------**	
Control[a]	4.8 c[b]	0.81 a	0.92 c	0.68 b	76.20 a	4.05 a	2.60 b	5.84 a	36.6 a	29.6 c
OS	4.7 c	0.87 a	0.81 c	0.62 b	63.37 a	4.13 a	2.46 b	5.72 a	39.8 a	26.9 c
RP	4.7 c	0.77 a	1.04 c	0.66 b	69.18 a	4.13 a	2.65 b	6.00 a	35.8 a	29.7 c
TP	4.7 c	0.77 a	0.93 c	0.56 b	57.98 a	4.27 a	2.42 b	5.91 a	35.8 a	27.6 c
TPCa	5.5 a	0.13 b	1.93 a	0.85 a	69.08 a	2.60 b	3.08 a	5.55 a	4.8 b	51.8 a
SPCa	5.3 b	0.23 b	1.57 b	0.93 a	65.15 a	2.92 b	2.91 a	5.59 a	10.1 b	46.4 b

[a] Treatments: Control— treatment without P or overseeding; OS—without P application, although with overseeding; RP—phosphate rock of Gafsa + overseeding; TP—triple superphosphate + overseeding; TPCa—triple superphosphate + limestone + overseeding; SPCa—single superphosphate + limestone + overseeding. [b]Means followed by the same letter did not differ statistically by Scott-Knott test ($p \leq 0.05$). [c]Effective cations exchange capacity. [d]Cations exchange capacity at pH 7.0. [e]Al saturation. [f]Cations saturation.

Table 3– Available soil P contents by Mehlich-1, measured in 2018 in the 0–10 cm topsoil layer in plots that received 118, 206 and 250 kg ha^{-1} of P from different P sources.

Treat	**P[b] (mg kg^{-1})**		
	118 kg ha^{-1} (1998[d])	**206 kg ha^{-1} (2010)**	**250 kg ha^{-1} (2012)**
Control[a]	14.8 aA[c]	12.3 bA	11.7 cA
OS	13.2 aA	12.6 bA	11.8 cA
RP	16.1 aB	16.9 aB	27.7 aA
TP	15.0 aB	20.7 aA	24.5 aA
TPCa	13.4 aB	16.8 aB	20.5 bA
SPCa	18.0 aA	16.3 aA	21.9 bA

[a] Treatments: Control— treatment without P or overseeding; OS—without P application, although with overseeding; RP—phosphate rock of Gafsa + overseeding; TP—triple superphosphate + overseeding; TPCa—triple superphosphate + limestone + overseeding; SPCa—single superphosphate + limestone + overseeding. [c]Means fallowed by the same letter, upper case letters in the line and lower-case letters in the column, did not differ statistically by Scott-Knott test ($p \leq 0.05$). [d]Year of the last phosphorus fertilization.

Table 4–Randomization test to Shannon dominance (H') and Pielou equitability (J) indexes, richness and dry matter production (Dm) accessed by vegetation sampling in 2009 and 2018.

2009

Treat	Factor treatments							
	Dm (kg ha^{-1})		Shannon		Pielou		Richness	
Control[a]	2120	ab[b]	1.33	ab	0.55	ns	11.50	ns
OS	2018	ab	1.28	ab	0.56		10.00	
RP	1380	b	1.39	a	0.61		10.00	
TP	2582	a	1.20	b	0.57		10.00	
SPCa	2985	a	1.30	ab	0.53		10.50	
TPCa	2520	a	1.25	ab	0.52		10.83	
	Factor rate of P fertilization (kg ha^{-1} of P)							
118	2102	a	1.22	a	0.53	ns	10.06	ns
162	2433	a	1.37	a	0.58		10.89	

2018

Treat	Factor treatments							
	Dm (kg ha^{-1})		Shannon		Pielou		Richness	
Control	2831	b	0.95	ab	0.42	bc	9.56	a
OS	3247	a	1.08	a	0.50	ab	9.00	a
RP	3096	a	0.65	b	0.29	c	9.00	a
TP	3381	a	0.77	b	0.54	ab	7.33	b
TPCa	3547	a	1.00	ab	0.40	bc	9.22	a
SPCa	3909	a	1.09	a	0.45	b	7.78	a
	Factor rate of P fertilization (kg ha^{-1} of P)							
118	3287	ns	0.97	ns	0.45	ns	9.00	ns
206	3390		0.88		0.41		8.50	
250	3329		0.92		0.44		8.44	

[a] Treatments: Control— treatment without P or overseeding; OS—without P application, although with overseeding; RP—phosphate rock of Gafsa + overseeding; TP—triple superphosphate + overseeding; TPCa—triple superphosphate + limestone + overseeding; SPCa—single superphosphate + limestone +

overseeding. [b]Means fallowed by the same letter did not differ statistically ($p \leq 0.05$). [ns]not-significant ($p \leq 0.05$).