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Chemical Pattern of Vegetation and Topsoil of Rangeland Fertilized Over 21 Years with Phosphorus Sources and Limestone

Abstract

The Pampa's rangeland in South America has been underused or replaced by annual crops or forest cultivation with higher economic returns. However, fertilization and liming are suitable strategies to improve rangeland dry matter production and avoid the replacement of these natural areas. Nevertheless, it is necessary to analyze and understand the long term consequences of the use of different fertilizers on soil chemical characteristics and the response of endemic vegetation. Thus, our goal was to test whether, in rangeland, the long-term surface application of phosphorus fertilizers and limestone allow for an increase in phosphorus availability and aluminum neutralization in deep soil layer and increase in rangeland productivity. For this, a 21-year, long-term field trial with limestone and phosphorus fertilizers was used. The treatments were different phosphorus sources (triple superphosphate, single superphosphate, and phosphate rock), applied in three rates over time (118, 205, and 249 kg ha⁻¹ of P until the years of 1998, 2010, and 2012, respectively), combined or not with limestone. The dry matter (between 2016 and 2019) and nutrient accumulation in plant shoot were measured. The topsoil was divided in four layers (0–2.5, 2.5–5.0, 5.0–7.5, and 7.5–10.0 cm), and chemical properties were determined. The use of phosphate rock in soil surface provided a deepening of phosphorus content at 7.5 cm after 21 years in a rate of 249 kg ha⁻¹ of P. After 21 years of liming, the reacidification was not significant and the neutralizing front reached depths greater than 10 cm. In these conditions, the contents of exchangeable Ca, Mg and Al and pH are greater than, and Al saturation is lower than without limestone. The effect of fertilization and limestone amendments are however limited in time and there was no response of rangeland dry matter production after more than four years after the last fertilization.

Keywords: Legacy—P, Dry matter production of Pampa Grassland, Neutralizing front

1. Introduction

The Pampa ecosystem of South America is an ecological region consisting of grasslands and rangelands, covering 500,000 km² in Argentina, Brazil, Uruguay, and Paraguay (Pallarés et al., 2005). The Pampa ecosystem is composed of more than 3,000 plant species, of which approximately 450 have economic interests (Boldrini, 2009). The great potential of the Pampa natural vegetation leads it to be used as the main source of animal food in these regions (rangeland) (Carvalho et al., 2006). In Brazil, the Pampa rangelands are the alimentary base for almost 13 million head of cattle (IBGE, 2016) which are predominantly managed in the extensive system. To this point, maintaining or increasing forage productivity is extremely important to sustaining agricultural production systems and the biodiversity of the ecosystem.

Nevertheless, historically, landowners in Pampa have not made investments to improve pasture productivity. Since the end of the 18th century, grasslands have been used to produce beef on large farms without the application of any agricultural inputs; hence, nowadays, these areas are cultivated in an inadequate way, resulting in overgrazing, low productivity, and consequently, low economic returns (Borges et al., 2016; Carvalho and Batello, 2009; Fedrigo et al., 2018).

In the last 50 years, problems related to the chemical quality of the soils were responsible for a decrease in 26% of the native vegetation area (Borges et al., 2016; Oliveira et al., 2017; Ruviano et al., 2016). Therefore, farmers ended up leaving pasture production and gave place to annual crops (e.g., soybean and corn) and cultivated forests (pine and eucalyptus) with better financial return (Oliveira et al., 2017). Thus, to avoid the conversion of native areas to other production systems, it is necessary to increase the economic efficiency of cattle production in order to make the activity economically competitive (Borges et al., 2016).

The improvement of the economic efficiency of cattle production in rangelands implies an increase in dry matter production. Well managed rangelands of Pampa can reach a dry matter production around 14 Mg ha⁻¹ year⁻¹ (Oliveira et al., 2015); in contrast, in areas with inadequate management, the productions achieve averages between 3.7 Mg ha⁻¹ year⁻¹ (Soares et al., 2005) and 9.8 Mg ha⁻¹ year⁻¹ (Pellegrini et al., 2010). The principal causes of low productivity is high soil acidity (pH ~ 4.4) and low fertility, such as reduced available P (2.6 to 7.6 mg of P kg⁻¹), especially in soils of Southern Brazil (Oliveira et al., 2011; Rheinheimer et al., 1997; Tiecher et al., 2014). In the scenario of low fertility, an increase of nutrients availability by limestone and phosphorus fertilizers are important strategies to support plant development (Gatiboni et al., 2000; Prestes et al., 2017, 2016).

The dolomite rock (magnesian limestone) is abundant and is almost the unique material used to neutralize soil potential acidity in Southern Brazil. The very low solubility of dolomite demands its incorporation into the soil profile. Phosphorus fertilizers should also be incorporated into the soil because of very low phosphorus mobility. However, in grasslands, liming and fertilization are carried out by deposition on the soil surface. Nevertheless, the fertilization in soil surface can restrict the improvement of soil fertility to topsoil layers and, consequently, rangeland productivity.

Surface application limits the neutralization of aluminum and the increase of available soil P to the topsoil layer (Nunes et al., 2011; Rodrighero et al., 2015; Soltangheisi et al., 2018), which may lead to a below-expected response of vegetation to soil fertility increase. Nevertheless, long term improvement of soil chemical characteristics can be also expected in-depth, as in agricultural areas, due to laminar water flow through a porous medium and turbulent water flow in macropores (Czachor, 2011; Rheinheimer et al., 2018; Vargas et al., 2019).

Finally, monitoring the soil chemical changes in the soil profile and the impact in dry matter production in a long-term experiment with limestone and phosphorus fertilizer application in the soil surface is crucial to showing a possible preservation of Pampa vegetation and make the farm

economically viable. The objective of this study was to test whether long-term surface application of phosphorus fertilizers and limestone in an rangeland allow for an increase in the phosphorus availability and aluminum neutralization in deep soil layer and increase in the rangeland dry matter production.

2. Material and Methods

2.1. Local and soil properties

The field trial was established in Rio Grande do Sul state in Southern Brazil (29°43'05.7"S 53°42'33.7"W) on a soil classified as Ultisol. The regional climate was humid subtropical, type Cfa according to Köppen classification, with an average annual precipitation of 1,769 mm year⁻¹ distributed over all months. The average monthly temperature of the hottest months (December–February) is 24.2°C and the coldest months (June–August) is 14.5°C. In 1997 the chemical properties of the soil were characterized at the layer of 0–20 cm and are shown in Table 1.

2.2. Field trial treatments

The field trial is a two-factorial experiment with randomized complete block design in a split-plot and three replications. The main factor is P sources, applied in a plot of 56 m², and the secondary factor is three total rates of phosphorus, applied in a split-plot of 18.5 m². Briefly, the beginning of field trial was in 1997 with different P sources application, magnesian limestone, and the over-seeding of *Lolium multiflorum* cv. “Comum” and *Trifolium vesiculosum* cv. “Yuchi”. The main plots correspond to triple superphosphate + limestone + over-seeding (TPCa); triple superphosphate + over-seeding (TP); single superphosphate + limestone + over-seeding (SPCa); phosphate rock of Gafsa + over-seeding (RP); without P but with over-seeding (OS), and control without P neither over-seeding (Control). The split-plot consist in the total application of 118, 205, and 249 kg ha⁻¹ of P, with the last P fertilization occurring in 1998, 2010, and 2012, respectively. The general

fertilization scheme is presented in Figure 1. The liming was carried out only in 1997 at the rate of 3.2 Mg ha⁻¹ on the soil surface (the amount necessary to achieve the pH 5.5).

All treatments, except the Control, were fertilized with potassium (108 kg ha⁻¹ of K) and nitrogen (70 kg ha⁻¹ of N in urea form) superficially. The over-seeding of *L. multiflorum* and *T. vesiculosum* were carried out in line onto the surface of grassland in the winter of 1997 and 2002 with 30 and 12 kg ha⁻¹ of seeds, respectively. During the 21 years of the experiment, the rangeland was periodically harvested. Because it is a natural biome, no pesticide has ever been used. There is no need to control invasive plants. Botanical modifications resulting from the use of natural pasture are being periodically evaluated. Field trial and managements detail can be found in Gatiboni et al. (2000), Gatiboni et al. (2008), Tiecher et al. (2014) and Oliveira et al. (2015).

2.3. Soil sampling and analyses

Soil sampling was performed at layers of 0–2.5, 2.5–5.0, 5.0–7.5 and 7.5–10 cm in October 2018. The soil was oven dried at 65°C and passed through a 2 mm mesh sieve for posterior chemical characterization. The soil pH H₂O was measured with a 1:1 soil:solution ratio. The potential acidity (H + Al, cmol_c kg⁻¹) was calculated according to SBCS (2016). The available soil P was quantified using acid extraction with Mehlich-1 solution (0.05 mol L⁻¹ HCl + 0.0125 mol L⁻¹ H₂SO₄) (Mehlich, 1953). The available soil P content was also quantified by extraction with anion exchange resin - AER (AR 103 QDP 434 Ionics Inc. plates). Briefly, the AER membrane of 2 cm² was saturated with 0.5 mol L⁻¹ NaHCO₃ and washed with osmosed H₂O. The membrane was then maintained in contact with 0.5 g of soil + 10 ml of osmosed H₂O for 16 h in an end-over-end agitator at 25° C. The membrane was collected, rinsed to remove the adhering soil particles, and then eluted with 10 ml of 0.5 mol L⁻¹ HCl. The P content from Mehlich-1 and AER extractions were both determined by colorimetry (Murphy & Riley 1962). The concentrations of exchangeable Ca, Mg and Al in the soil were quantified after extraction with 1 mol L⁻¹ KCl solution (Tedesco et al., 1995). The Ca and Mg

contents were quantified by atomic absorption spectrophotometry and Al content was quantified by titration with 0.0125 mol L⁻¹ NaOH. The effective cations exchange capacity (CEC_{ef}, cmol_c kg⁻¹), Al saturation (Al_{sat}, %), and base saturation (V, %) were calculated.

2.4. Dry matter production and chemical characterization

Between 2016 and 2019, the annual dry matter production was quantified by regular cutting of vegetation aboveground in an area of 0.25 m². The vegetation samples were oven dried at 60°C for 48 h. Soon after each vegetation sampling for dry matter quantification, all field area was mowed, and the plant material was removed from the site. The analysis of rangeland productivity was performed by the annual sum of the dry matter sampled in each plot. The values of annual dry matter production were normalized by the treatment without application of P as $RDm = (\Sigma Dm_x / \Sigma Dm_{OS}) * 100$, with RDm (%) being the relative dry matter production; ΣDm_x (kg ha⁻¹) the annual sum of dry matter of treatment x, and ΣDm_{OS} (kg ha⁻¹) the annual sum of dry matter of treatment OS.

The chemical composition of plant shoot was determined in the dry matter sampled in March 2019, after a time growth of 150 days. P, Ca, and Mg contents in plant shoot were quantified after nitric-perchloric digestion (Embrapa, 2009). The Ca and Mg were quantified by atomic absorption spectrophotometer and P by colorimetry (Murphy and Riley, 1962). Carbon (C) and nitrogen (N) were quantified by elemental analysis (Flash EA, Thermo Electron Corporation, Bremen, Germany).

2.5. Statistical analysis

An exploratory principal component analysis (PCA) was performed to identify the variables with the highest variance explanation and to guide other analysis. For PCA analysis, the data were transformed into a standardized normal distribution. To perform the PCA, R software, version 3.5.0, was utilized.

The analysis of variance of vegetation variables were performed as a two-factorial experiment with randomized complete block design in a split-plot. The main factor (plot) corresponds to the P sources and the secondary factor (split-plot) corresponds to the rate of phosphorus applied.

Differently of vegetation variables, the analysis of variance of soil variables were carried out as a three-factorial experiment with complete randomized block design in a split-split plot. The main and secondary factors also correspond to the P sources and the rate of phosphorus applied. The third factor (split-split plot) correspond to the topsoil layers.

When the effect of treatments and/or rate of phosphorus applied was significant by F test ($p \leq 0.05$), the differences between means were tested by Tukey or orthogonal contrast tests ($p \leq 0.05$). When the soil layer effect was significant by F test ($p \leq 0.05$), the means were submitted to regression analysis.

3. Results

3.1. Exploratory analysis

By exploratory PCA analysis (Figure 2), treatments were grouped mainly by the use of limestone and later by the use of P sources. The variance explained in the first two axes was 68.8%. The soil pH_{H_2O} , Ca, Al, P-AER, and P-Mehlich-1 contents and V and Al_{sat} were the variables with the highest contribution to variance explanation. The treatments were separated mainly by PC1, which explain 51.4% of the total variance. The RP and TP were differentiated from the others treatments (OS, Control, SPCa, and TPCa) (PC2, 17.4%) due to the soil and plant variables related to P (Figure 2).

3.2. Soil chemical characteristics

3.2.1. Limestone addition

The soils with limestone addition (TPCa and SPCa) have the highest values of $\text{pH}_{\text{H}_2\text{O}}$, exchangeable Ca and Mg, V, and CEC_{ef} and lower contents of exchangeable Al and Al_{sat} in the layers 2.5–5.0, 5.0–7.5, and 7.5–10 cm (Table 2 and Figure 3). The use of 3.2 Mg ha⁻¹ of magnesian limestone in the soil surface was sufficient to deepen the neutralization front, increase the $\text{pH}_{\text{H}_2\text{O}}$ and V, and reduce exchangeable Al and Al_{sat} (Figure 3). For all topsoil layers, the difference between the groups with (TPCa and SPCa) and without (Control, OS, RP, and TP) limestone application was significant by the method of orthogonal contrasts (Figure 3).

The exchangeable Ca content in the RP treatment was similar to the levels of the treatments without limestone in all evaluated layers (Table 2), conversely to the expected result due to the RP dissolution on soil over the years.

3.2.2. Phosphorus addition

For all treatments with P fertilization, available P contents in the 0–2.5 cm layer were higher in soils fertilized with 249 kg ha⁻¹ of P (last fertilization in 2012) than with the other rates (Table 3). In this layer, the highest value of P was obtained in the treatment RP (42.1 and 51.8 mg kg⁻¹ of P extracted by AER and Mehlich-1, respectively). For the other two rates, in the 0–2.5 cm layer, soils fertilized with TPCa and TP had greater available soil P contents than the others treatments (Table 3). In these cases, TPCa (118 kg ha⁻¹ of P) had 28.1 and 40.6 mg kg⁻¹ of P extracted by AER and Mehlich-1, respectively, and TP (205 kg ha⁻¹ of P) had 33.1 and 41 mg kg⁻¹ of P extracted by AER and Mehlich-1, respectively.

In deeper layers, although there was a decrease of the P-AER and P-Mehlich-1 contents (Figure 4), the rate of 249 kg ha⁻¹ of P provided by RP resulted in the highest values of P-AER in the layers

up to 7.5 cm (Table 3). For P-Mehlich-1, there was no significant difference between the P sources (Table 3), but the RP always had the highest absolute values.

The available P-Mehlich-1 values recommended for forage production in rangeland are above 15 mg kg⁻¹ in the 0–10 cm soil layer (SBCS, 2016). However, when topsoil layers were analyzed for rates of 118 and 205 kg ha⁻¹ of P, the recommended value of 15 mg kg⁻¹ was generally attained only up to the depth of 4.5 cm. Nonetheless, at a rate of 249 kg ha⁻¹ of P, this value was attained up to 10 and 7.4 cm in RP and TP, respectively, and up to 4.9 cm in the SPCa and TPCa treatments.

3.3. Dry matter production and plant shoot nutrient concentration

The RDm of rangeland developed under soils fertilized with different P sources was not significantly different for the four years of sampling (2016–2019) (Table 4). Differences in plant shoot P content and in P exportation by dry matter occurred only for the treatment fertilized with 249 kg ha⁻¹ of P (2012). The RP and SPCa treatments had the highest levels of plant shoot P content (1.63 and 1.73 g kg⁻¹, respectively) and SPCa had the highest P exportation (7.8 kg ha⁻¹), although not statistically different from the TPCa and TP treatments (Table 5). Differences between the concentrations of C, N, K, Ca, and Mg in plant shoot were not significant ($p \leq 0.05$) and had an overall mean of 418, 10.9, 6.3, 5.8, and 4.5 g kg⁻¹, respectively (data not shown).

4. Discussion

4.1. Soil properties

4.1.1. Limestone addition

The higher pH_{H2O} values and lower exchangeable Al and Al_{sat} observed in the 0–2.5 cm topsoil layer (Figure 3) are explained by the higher content of organic matter and root exudation in the soil superficial layers (Chen et al., 2017; Scavo et al., 2019).

In the 2.5–10 cm layer, the higher values of $\text{pH}_{\text{H}_2\text{O}}$, exchangeable Ca and Mg, V and CEC_{ef} , as well as lower contents of exchangeable Al and Al_{sat} , denote a downward movement of the limestone neutralization effect. After 21 years of the limestone application, the neutralizing front reached a soil layer deeper than 10 cm. The deepening of the neutralizing front observed in the rangeland is similar to that observed in soil with annual crops under a no-tillage system, where the downward movement is governed by mass flow (Rheinheimer et al., 2018; Vargas et al., 2019). For agricultural areas with the same soil as in this experiment, the neutralization front exceeds 20 cm 12 years after liming (Vargas et al., 2019) and reaches 60 cm after 18 years (Rheinheimer et al., 2018). In the last case, the authors explained the variation of the chemical characteristics in soil depth by the high rainfall. The percolation of fine limestone particles is favored by water movement, such as a laminar water flow through a porous medium and turbulent water flow in macropores (Czachor, 2011). In the analyzed area, the presence of macropores is favored by the texture (170 g kg^{-1} of clay) and the absence of soil plowing.

In long-term, the difference in $\text{pH}_{\text{H}_2\text{O}}$ values in the 0–10 cm layer between treatments with and without limestone has increased from 0.3 units, one year after liming (1998), to 1.2 units, 12 years after liming (2009) (Tiecher et al., 2014), and then, it reduced to 0.6 units, 21 years after liming (2018). This demonstrates the existence of a slow soil surface reacidification that is mainly due to the constant (re)cycling and plants exportation of Ca, Mg, and even Si and the downward migration with waterflow. However, the inexistence of surface erosion favors the permanence of limestone on the surface soil and prevents its loss by runoff.

The principal component analysis differentiated two groups on the principal component (PC1), with acidity variables and exchangeable Ca and Mg contents of the soil at a depth greater than 2.5 cm being the main contributors. This confirms that the application of limestone is the factor that most differentiated the treatments. The higher levels of exchangeable Ca content in all soil depths is attributed to the application of limestone in TPCa and SPCa. However, higher levels of Ca were also

expected in the first soil layer with the RP treatment due to the dissolution of RP minerals in the soil. RP is composed of minerals of the apatite group ($\text{Ca}_3(\text{PO}_4)_2(x)$) as the main P source. Therefore, Ca is a product of apatite dissolution that, besides the increase P content, also favors an increase in Ca content. The calcium content increase due to phosphate rock dissolution is possible and was demonstrated in flooded soils (Gonçalves et al., 2008). However, in our experiment, no significant difference in Ca content was observed in the RP treatment compared to the control.

4.1.2. Phosphorus addition

The application of soluble P sources in soil surface (as in TPCa, SPCa, and TP) with or without limestone did not favor the nutrient downward movement in the soil profile. The available soil P gradients observed (Figure 4) may be due to nutrient cycling by plants (Tian et al., 2017) and the application of all the fertilizers onto soil surface. The rapid dissolution and release of P by soluble sources favor its adsorption by reactive functional groups of clay minerals and oxides of Fe and Al, that are abundant in tropical and sub-tropical soils (Arai and Sparks, 2001; Bolan et al., 2003; Bortoluzzi et al., 2015). Therefore, it is likely that the P from the soluble sources has been absorbed by the plants or by the soil colloids in the superficial layers. This limited the effect of fertilization in increasing P contents in depth. Higher levels of nutrients in deeper layers are crucial to increase the root system development and drought tolerance by plants.

In contrast, the use of RP at the highest rate (249 kg ha^{-1} of P) over the 21 years provided an increase in available soil P contents to a depth of 7.5 cm, contributing to the legacy-P of this soil. High P levels at depths greater than 2.5 cm were the factors that most contributed to the variance explained by the PC2 axis of PCA (Figure 2). This confirms the differentiation of RP from treatments without limestone application (Control, OS, and TP) due to the deepening of P in the soil profile.

The increase of P content to a depth of 7.5 cm, non-associated with an increase of soil Ca content, suggests low apatite dissolution rate (minerals present in phosphate rock fertilizer). The dissolution of apatite minerals occurs only when there are favorable thermodynamic conditions. In soil, the dissolution of apatite is controlled by factors like soil pH (Chien, 1977), water content (Heindel et al., 2018), soil organic matter content (Alloush, 2003), and mainly by the Ca contents (Robinson and Syers, 1990). Thus, when these factors are not favorable to the dissolution of mineral, apatite remains in its crystalline form (Kumar et al., 1994). In this case, the downward movement of P is possible by mass flow, in the same way that occurs with limestone, and also verified in the experiment (Figure 3).

However, agronomically, the absence of RP dissolution is not desired because it limits the release of P to the soil solution and plant uptake. In this experiment, the difference between P-AER and P-Mehlich-1 suggests some recalcitrance of the apatite minerals in the rangeland soil. Therefore, further studies are encouraged to better understand the apatite mineral recalcitrance in rangelands soils and its P availability to plants.

4.2. Vegetation analysis

The content of P in plant shoot corroborates the historic amount of P applied to the soil and to the available soil P content. In work carried out in the same experimental area, Oliveira et al. (2014) quantified the P content in the plant shoot in the order of 0.9, 1.5, and 1.9 g kg⁻¹ (respectively for OS, RP, and TP) after 186 days of fertilization, in 2010. For these treatments, in our study, after approximately nine years of fertilization, lower values of P concentration were obtained even in the RP treatment (0.99 g kg⁻¹). This demonstrates that for all P sources tested, there is a reduction in P uptake with increasing time after fertilizer application. This is mainly due to the reduction of available soil P content, either through the uptake and export of the nutrient by forrage (Oliveira et

al., 2014; Pavlů et al., 2016) or by adsorption by functional groups of clays minerals and oxides present in the soil (Bortoluzzi et al., 2015; Kim et al., 2011).

On the other hand, in the treatment with fertilization at a rate of 249 kg ha⁻¹ of P performed until 2012 (seven years before the sampling and quantification of P in plant shoot for this study), the levels of P in shoot were higher in the RP and lower in TP compared to the results obtained by Oliveira et al. (2014). The highest levels in the RP are due to the higher rate (249 kg ha⁻¹ of P). However, in this case, there is also the possibility of the long-term effect of RP dissolution. This behavior was not identified after nine years of RP application (rate of 205 kg ha⁻¹ of P) but possibly still exists seven years after the last application (rate of 249 kg ha⁻¹ of P).

The P exportation obtained for the treatments was similar to values available in the literature for rangeland P fertilized and for the same growing season (March) but with a shorter period after the last fertilization (186 days) (Oliveira et al., 2014). Treatments with phosphorus fertilizers resulted in higher P exportation due to a greater soil P availability, P uptake and P contents in plant shoot. However, when the SPCa treatment was applied at a rate of 249 kg ha⁻¹ of P, high dry matter production during the evaluated period also played a key role to the P exportation. The greater P exportation in SPCa contributes to a greater recovery of P applied. Therefore, SPCa does not have the highest levels of P in any soil layer analyzed. In addition, the increase in soil fertility levels may favor, in the long term, the alteration of the occurrence of plant species in the areas (Harpole et al., 2017, 2016; Oliveira et al., 2015). Among these species are legumes, which have greater nutritional requirements for their development.

Even with the significant difference in plant shoot P content, in response to the distinct soil available P levels (Table 3 and Table 5), there was no differences in rangeland dry matter production. Differences in dry matter production due to P fertilizer application were reported in previous studies carried out in the same experiment. In these cases, the treatments SPCa and TPCa had higher dry matter production (Gatiboni et al., 2000; Oliveira et al., 2015; Tiecher et al., 2014). Though, even

without a statistical difference, it is possible to observe a pattern in the RDm values between 2016 and 2019. The lowest RDm was obtained in the treatment Control, OS, and RP. The productivity of rangeland grown on soils with soluble fertilizers was higher, reaching up to 27% of superiority in comparison to the other treatments.

Differences in Ca and Mg contents in the plant shoot were expected, especially in SPCa and TPCa, where soil Ca and Mg contents were increased due to the use of magnesian limestone. An increase in the concentration of Ca^{2+} in the soil solution often leads to an increase in the Ca concentration in the leaves (Hawkesford et al., 2012). However, in this experiment, differences in the content of Ca and Mg in plant shoot were not observed.

5. Conclusions

This study investigated the chemical properties of rangeland fertilized over 21 years with different phosphorus sources and liming. The highest rate of phosphorus (249 kg ha⁻¹ of P applied until 2012) provides the highest levels of nutrient availability in the soil and in the plant shoot, but there is no more response in dry matter production of rangeland after more than four years since the last fertilization. That indicates the necessity of periodic phosphorus fertilization in a time-space lower than four years.

The use of phosphate rock in the soil surface provides a greater deepening of phosphorus in the soil profile by mass flow phenomenon (achieving 7.5 cm after 21 years and a rate of 249 kg ha⁻¹ of P) compared to soluble fertilizers. Moreover, further studies are needed to understand the apatite mineral dissolution in rangelands soils and the P availability to plants.

After 21 years of surface deposition of limestone in rangeland, the reacidification was not significant, and the neutralizing front reached depths greater than 10 cm. In these conditions, the levels of exchangeable Ca and Mg, pH, exchangeable Al, and the saturation by Al are better than without limestone for plant development.

6. Author contributions

LCG and DR led the implementation of the experiment field. AS, DR, and LC participated in the conception of the work. AS, MC, LM, IBS, and GL participated in data acquisition and data interpretation. AS led the writing of the manuscript. All authors contributed to the manuscript correction, discussion, and interpretation of results.

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9. References

- Alloush, G.A., 2003. Dissolution and effectiveness of phosphate rock in acidic soil amended with cattle manure. *Plant Soil* 251, 37–46. <https://doi.org/10.1023/A:1022987915057>
- Arai, Y., Sparks, D.L., 2001. ATR-FTIR spectroscopic investigation on phosphate adsorption mechanisms at the ferrihydrite-water interface. *J. Colloid Interface Sci.* 241, 317–326. <https://doi.org/10.1006/jcis.2001.7773>
- Bolan, N.S., Adriano, D.C., Naidu, R., 2003. Role of Phosphorus in (Im)mobilization and Bioavailability of Heavy Metals in the Soil-Plant System, in: *Reviews of Environmental Contamination and Toxicology*. Springer, New York, p. 177. https://doi.org/10.1007/0-387-21725-8_1
- Boldrini, I.I., 2009. *A flora dos campos do Rio Grande do Sul, Campos Sulinos - conservação e uso sustentável da biodiversidade*. Brasília.

- Borges, J.A.R., Tauer, L.W., Lansink, A.G.J.M.O., 2016. Using the theory of planned behavior to identify key beliefs underlying Brazilian cattle farmers' intention to use improved natural grassland: A MIMIC modelling approach. *Land use policy* 55, 193–203. <https://doi.org/10.1016/j.landusepol.2016.04.004>
- Bortoluzzi, E.C., Pérez, C.A.S., Ardisson, J.D., Tiecher, T., Caner, L., 2015. Occurrence of iron and aluminum sesquioxides and their implications for the P sorption in subtropical soils. *Appl. Clay Sci.* 104, 196–204. <https://doi.org/10.1016/j.clay.2014.11.032>
- Carvalho, P.C. d F., Batello, C., 2009. Access to land, livestock production and ecosystem conservation in the Brazilian Campos biome: The natural grasslands dilemma. *Livest. Sci.* 120, 158–162. <https://doi.org/10.1016/j.livsci.2008.04.012>
- Carvalho, P.C.F., Fisher, V., Dos Santos, D.T., L Ribeiro, A.M., F De Quadros, F.L., S Castilhos, Z.M., E C Poli, C.H., G Monteiro, A.L., Nabinger, C., Cristina Genro, T.M., A Jacques, A. V., 2006. Produção animal no bioma Campus Sulinos. *Brazilian J. Anim. Sci.* 35, 156–202.
- Chen, Y.T., Wang, Y., Yeh, K.C., 2017. Role of root exudates in metal acquisition and tolerance. *Curr. Opin. Plant Biol.* 39, 66–72. <https://doi.org/10.1016/j.pbi.2017.06.004>
- Chien, S.H., 1977. Dissolution of phosphate rocks in a flooded acid soil. *Soil Sci. Soc. Am. J.* 41, 1106–1109.
- Czachor, H., 2011. Laminar and turbulent flow in soils, in: *Encyclopedia of Earth Sciences Series*. Springer Netherlands, p. 413. https://doi.org/10.1007/978-90-481-3585-1_80
- Embrapa, 2009. Manual de análises químicas de solos, plantas e fertilizantes, 2. ed. ed. Brasília.
- Fedriço, J.K., Ataíde, P.F., Filho, J.A., Oliveira, L. V., Jaurena, M., Laca, E.A., Overbeck, G.E., Nabinger, C., 2018. Temporary grazing exclusion promotes rapid recovery of species richness and productivity in a long-term overgrazed Campos grassland. *Restor. Ecol.* 26, 677–685. <https://doi.org/10.1111/rec.12635>
- Gatiboni, L.C., Kaminski, J., Pellegrini, J.B.R., Aquino, J.E.R., 2008. Efeito da adubação fosfatada e da calagem sobre a qualidade bromatológica da forragem de pastagem natural com introdução de espécies forrageiras de inverno. *Rev. Bras. Agrociência* 14, 125–134.
- Gatiboni, L.C., Kaminski, J., Pellegrini, J.B.R., Brunetto, G., Saggin, A., Flores, J.P.C., 2000. Influência da adubação fosfatada e da introdução de espécies forrageiras de inverno na oferta de forragem de pastagem natural. *Pesqui. Agropecu. Bras.* 35, 1663–1668. <https://doi.org/10.1590/S0100-204X2000000800020>
- Gonçalves, G.K., Oliveira De Sousa, R., Vahl, C., Bortolon, L., 2008. Solubilização dos fosfatos naturais Patos de Minas e Arad em dois solos alagados, *Revista Brasileira de Ciência do Solo*. <https://doi.org/10.1590/S0100-06832008000500036>
- Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T., Chase, J., Fay, P.A., Hautier, Y., Hillebrand, H., Macdougall, A.S., Seabloom, E.W., Williams, R., Bakker, J.D., Cadotte, M.W., Chaneton, J., Chu, C., Cleland, E.E., Antonio, C. D', Davies, K.F., Gruner, D.S., Hagenah, N., Kirkman, K., Knops, J.M.H., La Pierre, K.J., Mcculley, R.L., Moore, J.L.,

- Morgan 25, J.W., Prober, S.M., Risch, A.C., Schuetz, M., Stevens, C.J., Wragg, P.D., 2016. Addition of multiple limiting resources reduces grassland diversity. *Nat. Publ. Gr.* 537, 28. <https://doi.org/10.1038/nature19324>
- Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T., Chase, J., Fay, P.A., Hautier, Y., Hillebrand, H., Macdougall, A.S., Seabloom, E.W., Bakker, J.D., Cadotte, M.W., Chaneton, E.J., Chu, C., Hagenah, N., Kirkman, K., Pierre, K.J. La, Moore, J.L., Morgan, J.W., Prober, S.M., Risch, A.C., Schuetz, M., Stevens, C.J., 2017. Out of the shadows: multiple nutrient limitations drive relationships among biomass, light and plant diversity. *Funct. Ecol.* 31, 1839–1846. <https://doi.org/10.1111/1365-2435.12967>
- Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Møller, I.S., White, P., 2012. Functions of Macronutrients, in: Marschner, P. (Ed.), *Mineral Nutrition of Higher Plants*. Elsevier, London, pp. 135–189. <https://doi.org/10.1016/B978-0-12-384905-2.00006-6>
- Heindel, R.C., Lyons, W.B., Welch, S.A., Spickard, A.M., Virginia, R.A., 2018. Biogeochemical weathering of soil apatite grains in the McMurdo Dry Valleys, Antarctica. *Geoderma* 320, 136–145. <https://doi.org/10.1016/j.geoderma.2018.01.027>
- Instituto Brasileiro de Geografia e Estatística, I., 2016. *Produção da Pecuária Municipal*. Rio de Janeiro.
- Kim, J., Li, W., Philips, B.L., Grey, C.P., 2011. Phosphate adsorption on the iron oxyhydroxides goethite (α -FeOOH), akaganeite (β -FeOOH), and lepidocrocite (γ -FeOOH): A 31P NMR Study. *Energy Environ. Sci.* 4, 4298–4305. <https://doi.org/10.1039/c1ee02093e>
- Kumar, V., Gilkes, R.J., Armitage, T.M., Bolland, M.D.A., 1994. Identification of residual P compounds in fertilized soils using density fractionation, X-ray diffraction, scanning and transmission electron microscopy. *Fertil. Res.* 37, 133–149. <https://doi.org/10.1007/BF00748554>
- Mehlich, A., 1953. Determination of P, Ca, Mg, K, Na and NH₄ by North Carolina Soil Testing Laboratoris. Raleigh Univ. North Carolina.
- Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chem. ACTA* 27, 31–36. [https://doi.org/10.1016/S0003-2670\(00\)88444-5](https://doi.org/10.1016/S0003-2670(00)88444-5)
- Nunes, R.S., Sousa, D.M.G., Goedert, W.J., Vivaldi, L.J., 2011. Distribuição De Fósforo No Solo Em Razão Do. *R. Bras. Ci. Solo* 877–888. <https://doi.org/10.1021/JP047021Q>
- Oliveira, T.E. de, Freitas, D.S. de, Gianezini, M., Ruviaro, C.F., Zago, D., Mércio, T.Z., Dias, E.A., Lampert, V. do N., Barcellos, J.O.J., 2017. Agricultural land use change in the Brazilian Pampa Biome: The reduction of natural grasslands. *Land use policy* 63, 394–400. <https://doi.org/10.1016/j.landusepol.2017.02.010>
- Oliveira, L.B., Soares, E.M., Jochims, F., Tiecher, T., Marques, A.R., Kuinchtner, B.C., Rheinheimer, D.S., De Quadros, F.L.F., 2015. Long-Term Effects of Phosphorus on Dynamics of an Overseeded Natural Grassland in Brazil. *Rangel. Ecol. Manag.* 68, 445–452. <https://doi.org/10.1016/j.rama.2015.07.012>

- Oliveira, L.B., Tiecher, T., de Quadros, F.L.F., Trindade, J.P.P., Gatiboni, L.C., Brunetto, G., dos Santos, D.R., 2014. Formas de fósforo no solo sob pastagens naturais submetidas à adição de fosfatos. *Rev. Bras. Cienc. do Solo* 38, 867–878. <https://doi.org/10.1590/S0100-06832014000300018>
- Oliveira, L.B., Tiecher, T., Quadros, F.L.F., Santos, D.R., 2011. Fósforo microbiano em solos sob pastagem natural submetida a queima e pastejo. *Rev. Bras. Cienc. do Solo* 35, 1509–1515. <https://doi.org/10.1590/S0100-06832011000500005>
- Pallarés, O.R., Berretta, E.J., Maraschin, G.E., 2005. The South American Campos ecosystem, in: Suttie, J., Reynolds, S.G., Batello, C. (Eds.), *Grasslands of the World*. Rome, pp. 171–219.
- Pavlů, L., Gaisler, J., Hejzman, M., Pavlů, V. V., 2016. What is the effect of long-term mulching and traditional cutting regimes on soil and biomass chemical properties, species richness and herbage production in *Dactylis glomerata* grassland? *Agric. Ecosyst. Environ.* 217, 13–21. <https://doi.org/10.1016/j.agee.2015.10.026>
- Pellegrini, L.G., Nabinger, C., Neumann, M., Carvalho, P.C. de F., Crancio, L.A., 2010. Produção de forragem e dinâmica de uma pastagem natural submetida a diferentes métodos de controle de espécies indesejáveis e à adubação. *Rev. Bras. Zootec.* 39, 2380–2388. <https://doi.org/10.1590/S1516-35982010001100010>
- Prestes, N.E., Do Amarante, C.V.T., Pinto, C.E., Prestes, G., Zanini, G.D., De Medeiros-Neto, C., Sbrissia, A.F., 2016. Forage production in a natural grassland with limestone and phosphorus dosages. *Semin. Agrar.* 37, 3265–3275. <https://doi.org/10.5433/1679-0359.2016v37n5p3265>
- Prestes, N.E., Do Amarante, C.V.T., Pinto, C.E., Prestes, G., Zanini, G.D., Zanella, P.G., Sbrissia, A.F., 2017. Limestone and phosphorus application and forage production in natural pastures with sodseeding of cool-season species. *Semin. Agrar.* 38, 3681–3693. <https://doi.org/10.5433/1679-0359.2017v38n6p3681>
- Rheinheimer, D. dos S., Tiecher, T., Gonzatto, R., Santanna, M.A., Brunetto, G., da Silva, L.S., 2018. Long-term effect of surface and incorporated liming in the conversion of natural grassland to no-till system for grain production in a highly acidic sandy-loam Ultisol from South Brazilian Campos. *Soil Tillage Res.* 180, 222–231. <https://doi.org/10.1016/j.still.2018.03.014>
- Rheinheimer, D.S., Santos, J.C.P., Kaminski, J., Mafran, A.L., 1997. Crescimento de leguminosas forrageiras afetado pela adição de fósforo, calagem do solo e micorrizas, em condições de casa de vegetação.pdf. *Ciência Rural* 27, 571–576. <https://doi.org/10.1590/S0103-84781997000400008>
- Robinson, J.S., Syers, J.K., 1990. A critical evaluation of the factors influencing the dissolution of Gafsa phosphate rock. *J. Soil Sci.* 41, 597–605.
- Rodrighero, M.B., Barth, G., Caires, E.F., 2015. Aplicação superficial de calcário com diferentes teores de magnésio e granulometrias em sistema plantio direto. *Rev. Bras. Cienc. do Solo* 39, 1723–1736. <https://doi.org/10.1590/01000683rbc20150036>
- Ruviaro, C.F., da Costa, J.S., Florindo, T.J., Rodrigues, W., de Medeiros, G.I.B., Vasconcelos, P.S., 2016. Economic and environmental feasibility of beef production in different feed management

systems in the Pampa biome, southern Brazil. *Ecol. Indic.* 60, 930–939.
<https://doi.org/10.1016/J.ECOLIND.2015.08.042>

SBCS, 2016. Manual de adubação e calagem para os estados do Rio Grande do Sul e Santa Catarina.

Scavo, A., Abbate, C., Mauromicale, G., 2019. Plant allelochemicals: agronomic, nutritional and ecological relevance in the soil system. *Plant Soil*. <https://doi.org/10.1007/s11104-019-04190-y>

Soares, A.B., Carvalho, P.C. de F., Nabinger, C., Semmelmann, C., Trindade, J.K. da, Guerra, E., Freitas, T.S. de, Pinto, C.E., Fontoura Júnior, J.A., Frizzo, A., 2005. Produção animal e de forragem em pastagem nativa submetida a distintas ofertas de forragem. *Ciência Rural* 35, 1148–1154. <https://doi.org/10.1590/S0103-84782005000500025>

Soltangheisi, A., Rodrigues, M., Coelho, M.J.A., Gasperini, A.M., Sartor, L.R., Pavinato, P.S., 2018. Changes in soil phosphorus lability promoted by phosphate sources and cover crops. *Soil Tillage Res.* 179, 20–28. <https://doi.org/10.1016/j.still.2018.01.006>

Tedesco, M.J., Gianello, C., Bissani, C.A., Bohnen, H., Volkweiss, S.J., 1995. Análises de solo, plantas e outros materiais, 2 Ed. ed. Porto Alegre.

Tian, J., Boitt, G., Black, A., Wakelin, S., Condrón, L.M., Chen, L., 2017. Accumulation and distribution of phosphorus in the soil profile under fertilized grazed pasture. <https://doi.org/10.1016/j.agee.2017.01.022>

Tiecher, T., Oliveira, L.B., Rheinheimer, D.S., Quadros, F.L.F., Gatiboni, L.C., Brunetto, G., Kaminski, J., 2014. Phosphorus application and liming effects on forage production, floristic composition and soil chemical properties in the Campos biome, southern Brazil. *Grass Forage Sci.* 69, 567–579. <https://doi.org/10.1111/gfs.12079>

Vargas, J.P.R., dos Santos, D.R., Bastos, M.C., Schaefer, G., Parisi, P.B., 2019. Application forms and types of soil acidity corrective: Changes in depth chemical attributes in long term period experiment. *Soil Tillage Res.* 185, 47–60. <https://doi.org/10.1016/j.still.2018.08.014>

Years	P fertilizer (kg ha ⁻¹ of P)		
1997	79	79	79
	↓	↓	↓
1998	39	39	39
	↓	↓	↓
2002	NA*	44	44
	↓	↓	↓
2010	NA	44	44
	↓	↓	↓
2012	NA	NA	44
	↓	↓	↓
Total	118	205	249
2018	Sampling		
Last fertilization	1998	2010	2012

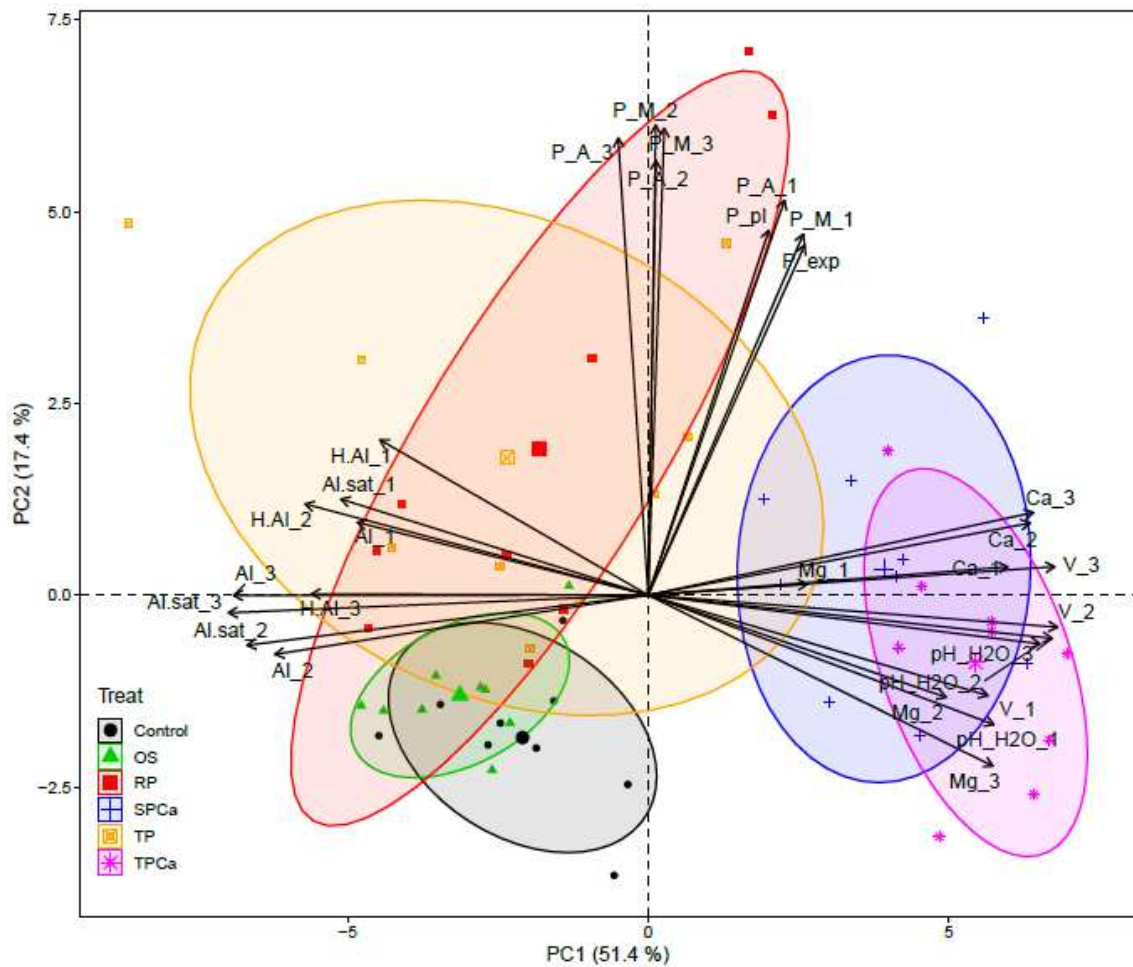
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2 Figure 1—Rates and years of phosphorus fertilization. *Not phosphorus fertilizer
 3 application.

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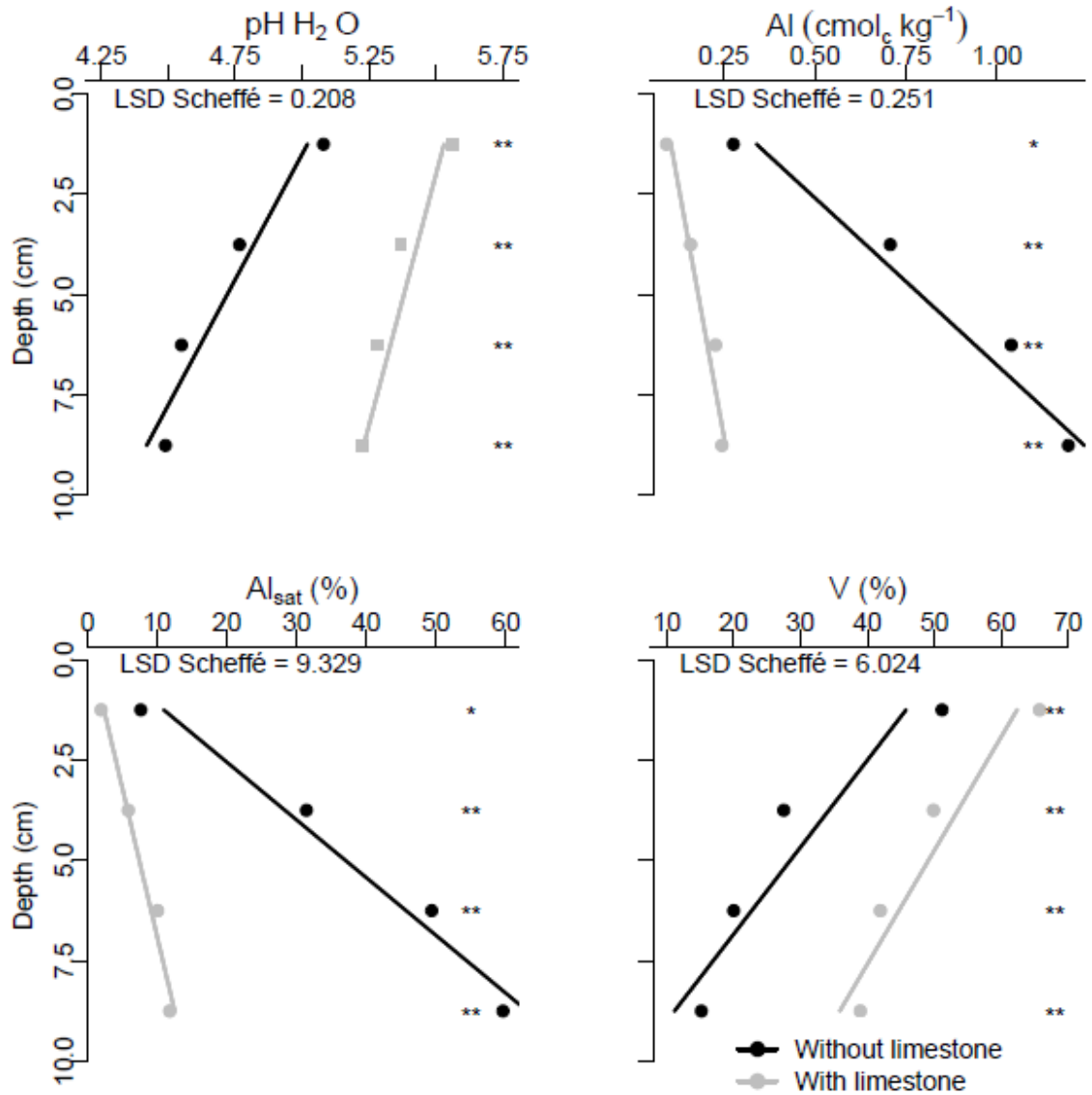
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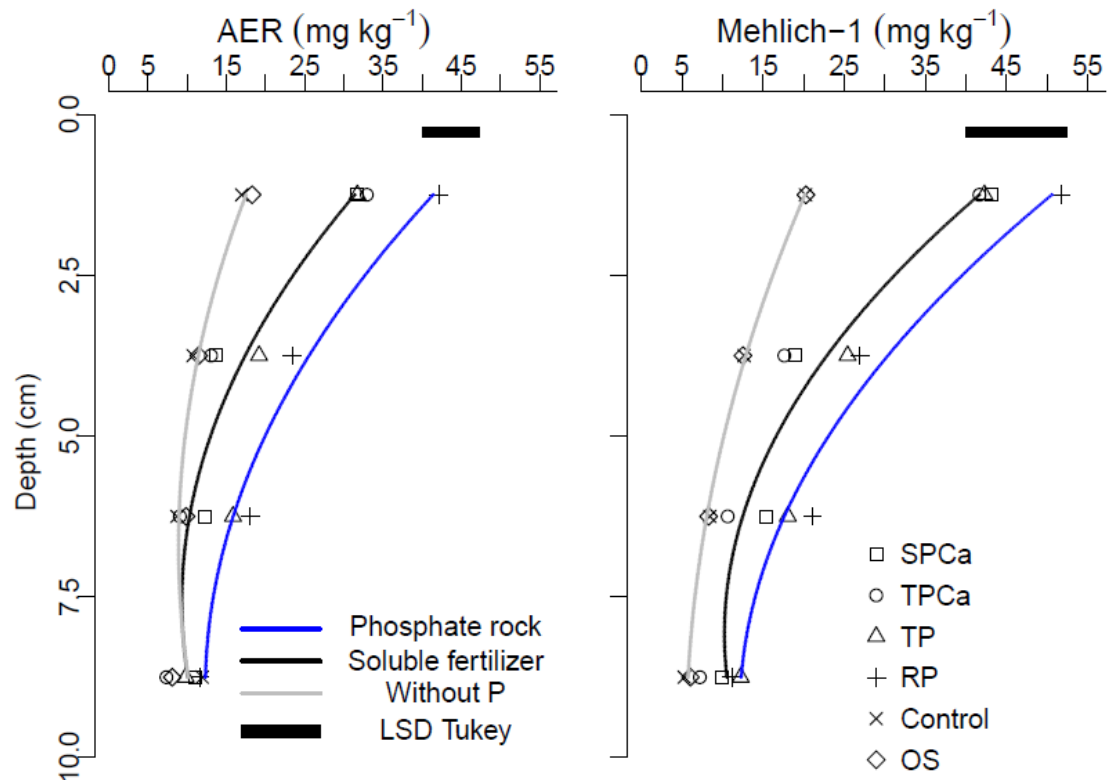
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Figure 2—Exploratory principal component analysis of soil and plant dataset for the different P sources. Treatments: Control—control without P neither over-seeding; RP—Gafsa phosphate rock + over-seeding; SPCa—single superphosphate + limestone + over-seeding; OS—without P but with over-seeding; TPCa—triple superphosphate + limestone + over-seeding; TP—triple superphosphate + over-seeding. Variables: P_M and P_A refer to P-Mehlich-1 and P-AER, respectively; Al.sat—aluminum saturation; H.AI—potential acidity; V—base saturation; The index 1, 2, and 3 refer to chemical characteristic in the layers of 0–2.5, 2.5–5.0, and 5.0–7.5 cm, respectively; P_exp—phosphorus exportation.



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Figure 3—Chemical characteristics of soil due to the use of limestone 21 years before. Al_{sat}—Aluminum saturation and V—Base saturation. *significant by orthogonal contrast test (p≤0.05). **significant by orthogonal contrast test (p≤0.01).



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Figure 4—Phosphorus available contents by anion exchange resin (AER) and Mehlich-1 in soil cultivated with rangeland over 21 years and fertilized with 249 kg ha⁻¹ of P. RP—Gafsa phosphate rock + over-seeding; SPCa—single superphosphate + limestone + over-seeding; OS—without P but with over-seeding; TPCa—triple superphosphate + limestone + over-seeding; TP—triple superphosphate + over-seeding. LSD Tukey—Least significant difference by Tukey test ($p \leq 0.05$).

1 **Table 1—Topsoil layer of 0-20 cm characterization of the field trial establishment in 1997.**

Factor	Values
pH*	4.5
Phosphorus (mg kg ⁻¹) **	2.5
Potassium (mg kg ⁻¹) **	60
Calcium (cmolc kg ⁻¹) ***	1.17
Magnesium (cmol _c kg ⁻¹) ***	0.75
Aluminum (cmol _c kg ⁻¹) ***	1.3
Carbone (g kg ⁻¹)	10.4
Clay (g Kg ⁻¹)	170

2 *water pH (1:1 v/v); ** Extracted by Mehlich-1; *** extracted by 1 mol L⁻¹ KCl.

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5 Table 2—Depth chemical characterization of soil cultivated with rangeland after 21 years of
6 phosphorus fertilization and liming.

Depth (cm)	Control ¹		OS		RP		SPCa		TPCa		TP		LSD ²
	----- pH _{H2O} -----												
1.25	5.21	b ³	5.03	b	5.05	b	5.53	a	5.6	a	5.03	b	0.29
3.75	4.77	b	4.77	b	4.7	b	5.25	a	5.48	a	4.83	b	
6.25	4.56	b	4.52	b	4.54	b	5.15	a	5.41	a	4.6	b	
8.75	4.48	b	4.44	b	4.52	b	5.09	a	5.36	a	4.5	b	
	----- Al (cmol _c kg ⁻¹) -----												
1.25	0.2	a	0.37	a	0.23	a	0.1	a	0.09	a	0.3	a	0.35
3.75	0.75	a	0.75	a	0.69	a	0.21	b	0.11	b	0.64	a	
6.25	1.07	a	1.1	a	1.01	a	0.3	b	0.16	b	0.98	a	
8.75	1.21	a	1.24	a	1.16	a	0.33	b	0.16	b	1.16	a	
	----- Ca (cmol _c kg ⁻¹) -----												
1.25	1.89	bc	1.52	c	2.03	bc	2.29	ab	2.81	a	1.63	c	0.52
3.75	0.81	b	0.82	b	0.96	b	1.55	a	1.91	a	0.94	b	
6.25	0.54	b	0.53	b	0.66	b	1.3	a	1.59	a	0.66	b	
8.75	0.42	b	0.39	b	0.5	b	1.14	a	1.4	a	0.5	b	
	----- Mg (cmol _c kg ⁻¹) -----												
1.25	1.44	abc	1.33	bc	1.6	ab	1.77	a	1.48	abc	1.2	c	0.37
3.75	0.52	b	0.51	b	0.48	b	0.95	a	0.95	a	0.46	b	
6.25	0.42	a	0.36	a	0.33	a	0.54	a	0.52	a	0.33	a	
8.75	0.34	a	0.27	a	0.24	a	0.49	a	0.45	a	0.26	a	
	----- H+Al ³ (cmol _c kg ⁻¹) -----												
1.25	3.19	ab	3.57	a	3.02	ab	2.48	b	2.25	b	3.76	a	1.04
3.75	3.91	ab	4	ab	4.28	a	3	bc	2.7	c	4.36	a	
6.25	4.53	a	4.33	a	4.55	a	3.07	b	2.77	b	4.2	a	
8.75	4.57	a	4.62	a	4.66	a	3.13	b	2.67	b	4.76	a	
	----- CEC _{ef} (cmol _c kg ⁻¹) -----												
1.25	3.94	bc	3.55	c	4.23	ab	4.48	ab	4.76	a	3.42	c	0.61
3.75	2.26	c	2.24	c	2.29	bc	2.88	ab	3.11	a	2.17	c	
6.25	2.15	a	2.08	a	2.1	a	2.24	a	2.37	a	2.08	a	
8.75	2.06	a	1.98	a	1.99	a	2.03	a	2.09	a	2	a	
	----- Al _{sat} (%) -----												
1.25	5.15	a	10.04	a	5.8	a	2.273	a	1.72	a	9.697	a	13.00
3.75	32.83	a	33.21	a	30.11	a	8.182	b	3.586	b	29.48	a	
6.25	49.4	a	53.04	a	48.63	a	13.69	b	6.485	b	46.42	a	
8.75	58.82	a	62.9	a	58.8	a	16.2	b	7.44	b	57.82	a	
	----- V (%) -----												
1.25	54.7	cd	47.4	ef	56.7	bc	64.0	ab	67.4	a	45.8	f	8.41
3.75	28.3	b	27.6	b	27.5	b	47.1	a	52.7	a	26.7	b	
6.25	19.6	b	19.0	b	19.9	b	39.1	a	44.7	a	21.7	b	
8.75	15.8	b	13.8	b	15.1	b	35.6	a	42.3	a	16.2	b	

7 ¹RP—Gafsa phosphate rock + over-seeding; SPCa—single superphosphate + limestone + over-seeding; OS—without P
8 but with over-seeding; TPCa—triple superphosphate + limestone + over-seeding; TP—triple superphosphate + over-
9 seeding. ²LSD—Least Significant Difference (p≤0.05). ³Means followed by the same letter, in a row, did not differ
10 statistically by Tukey test (p≤0.05). ³H+Al—potential acidity; CEC_{ef}—effective cations exchange capacity; Al_{sat}—
11 aluminum saturation; V—base saturation.
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13 Table 3—Soil phosphorus contents by anion exchange resin (AER) and Mehlich-1 in soil cultivated with rangeland after 21 years of
 14 phosphorus fertilization and liming.

Depth (cm)	Control ¹						RP						SPCa					
	118 ² (1998)		205 (2010)		249 (2012)		118 (1998)		205 (2010)		249 (2012)		118 (1998)		205 (2010)		249 (2012)	
P- Anion Exchange Resin - AER																		
0-2.5	21.53	Ab ³	21.56	Abc	16.95	Ac	24.33	Bab	27.18	Bab	42.1	Aa	21.94	Bab	23.87	Bb	31.63	Ab
2.5-5.0	11.97	Aa	12.06	Aa	10.73	Ac	16.23	Ba	16.05	Ba	23.4	Aa	15.67	Aa	16.36	Aa	13.72	Abc
5.0-7.5	10.03	Aa	8.62	Aa	8.68	Ab	11.53	Ba	10.61	Ba	18.02	Aa	8.31	Aa	12.99	Aa	12.21	Ab
7.5-10.0	8.29	Aa	6.37	Aa	11.92	Aa	8.73	Aa	7	Aa	11.64	Aa	5.28	Aa	8.58	Aa	10.98	Aa
P - Mehlich-1																		
0-2.5	29.23	Aab	26.03	Ab	20.24	Ab	31.95	Bab	33.54	Bab	51.76	Aa	36.22	Aba	32.34	Bab	43.24	Aa
2.5-5.0	14.29	Aa	12.17	Aa	12.67	Ab	14.56	Ba	16.48	Ba	26.94	Aa	17.83	Aa	16.81	Aa	19.03	Aab
5.0-7.5	9.39	Aa	6.45	Aa	8.43	Aab	8.68	Ba	11.19	ABa	21.1	Aa	13.14	Aa	10.31	Aa	15.34	Aab
7.5-10.0	6.15	Aa	4.49	Aa	5.29	Aa	9.32	Aa	6.43	Aa	11.18	Aa	4.94	Aa	5.75	Aa	10.01	Aa
<hr/>																		
Depth (cm)	OS						TPCa						TP					
	118 (1998)		205 (2010)		249 (2012)		118 (1998)		205 (2010)		249 (2012)		118 (1998)		205 (2010)		249 (2012)	
P- Anion Exchange Resin - AER																		
0-2.5	20.22	Ab	16.03	Ac	18.27	Ac	28.08	ABa	25.23	Bb	32.92	Ab	20.47	Bb	33.09	Aa	31.71	Ab
2.5-5.0	15.09	Aa	11.84	Aa	11.46	Ac	14.03	Aa	11.37	Aa	13.03	Abc	15.63	Aa	15.06	Aa	19.17	Aab
5.0-7.5	9.6	Aa	8.48	Aa	9.84	Ab	9.43	Aa	7.64	Aa	9.06	Ab	10.85	Aa	11.81	Aa	15.84	Aab
7.5-10.0	6.93	Aa	5.98	Aa	8.08	Aa	7.2	Aa	5.64	Aa	7.33	Aa	6.61	Aa	10.37	Aa	9.81	Aa
P - Mehlich-1																		
0-2.5	26.41	Ab	23.65	Ab	20.29	Ab	40.65	Aa	33.5	Aab	41.67	Aa	28.94	Bab	41.02	Aa	42.29	Aa
2.5-5.0	14.48	Aa	13.9	Aa	12.53	Ab	11.48	Aa	12.98	Aa	17.63	Aab	15.46	Aa	20.45	Aa	25.42	Aa
5.0-7.5	7.99	Aa	7.24	Aa	8.28	Ab	9.41	Aa	6.94	Aa	10.65	Aab	9.96	Aa	12.29	Aa	18.06	Aab
7.5-10.0	4.07	Aa	5.57	Aa	6.06	Aa	5.03	Aa	5.55	Aa	7.22	Aa	5.76	Aa	8.86	Aa	12.25	Aa

15 ¹RP—Gafsa phosphate rock + over-seeding; SPCa—single superphosphate + limestone + over-seeding; OS—without P but with over-seeding; TPCa—triple
 16 superphosphate + limestone + over-seeding; TP—triple superphosphate + over-seeding. ²Kg ha⁻¹ of P. ³Means followed by the same letter, upper case letter between rate
 17 in the same treatment and lower-case letter between treatments, did not differ statistically by Tukey test (p≤0.05).
 18

19 Table 4—Analysis of variance of relative dry matter production between 2016 and 2019.

	2016	2017	2018	2019
	----- % -----			
Treatments				
Control ¹	91	86	91	84
OS	100	100	100	100
RP	105	94	99	113
SPCa	117	105	114	127
TPCa	125	108	113	122
TP	123	101	110	116
Rate of phosphorus applied				
118	111	98	104	110
205	108	99	104	109
249	112	101	105	112
Effect				
Treat ²	ns	ns	ns	ns
Rate ³	ns	ns	ns	ns
Treat*Rate	ns	ns	ns	ns
CV1(%)	41.6	32.3	23.0	31.3
CV2(%)	13.9	16.0	9.6	21.1

20 ¹RP—Gafsa phosphate rock + over-seeding; SPCa—single superphosphate + limestone + over-seeding; OS—without P
 21 but with over-seeding; TPCa—triple superphosphate + limestone + over-seeding; TP—triple superphosphate + over-
 22 seeding. ²Treat—main plot (phosphorus sources); ³Rate—split-plot (Rate of phosphorus applied). *significant at p≤0.05;
 23 ns—not significant at p≤0.05.
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Table 5—Phosphorus contents in plant shoot and P exportation by dry matter of one cut carried out in mar/2019 after growth time of 150 days.

Treat	118 kg ha ⁻¹ of P (1998)		205 kg ha ⁻¹ of P (2010)		249 kg ha ⁻¹ of P (2012)	
	P shoot (g kg ⁻¹)	P export (kg ha ⁻¹)	P shoot (g kg ⁻¹)	P export (kg ha ⁻¹)	P shoot (g kg ⁻¹)	P export (kg ha ⁻¹)
Control ¹	0.89 Aa	2.4 Aa	0.80 Aa	2.3 Aa	0.89 Ab	2.3 Ab
OS	0.88 Aa	3.5 Aa	1.00 Aa	3.0 Aa	0.84 Ab	2.5 Ab
RP	0.87 Ba	3.0 Ba	0.99 Ba	4.0 ABa	1.63 Aa	5.6 Aab
SPCa	0.84 Ba	3.3 Ba	1.00 Ba	3.8 Ba	1.73 Aa	7.8 Aa
TPCa	0.77 Ba	3.1 Ba	1.18 ABa	4.2 ABa	1.26 Aab	5.4 Aab
TP	1.03 Aa	4.2 Aa	1.11 Aa	3.9 Aa	1.27 Aab	4.9 Aab

28 ¹RP—Gafsa phosphate rock + over-seeding; SPCa—single superphosphate + limestone + over-seeding; OS—without P
29 but with over-seeding; TPCa—triple superphosphate + limestone + over-seeding; TP—triple superphosphate + over-
30 seeding. ²Means followed by the same letter, upper case letter in a line and lower-case letter in a column, did not differ
31 statistically by Tukey test (p≤0.05).