ORIGINAL ARTICLE

Phosphorus accumulation in soil after successive applications of swine manure: a long-term study in Brazil

José de Souza Oliveira Filho[1] · Anderson Claiton Ferrari[2] · Marcos Gervasio Pereira[2] ·
Luiz Alberto da Silva Rodrigues Pinto[2] · Everaldo Zonta[2] · Talita Santana Matos[2]

Abstract
Successive applications of swine manure (SM) in agriculture may contribute to environmental contamination by excess ofphosphorus (P). In this study, we analyzed the dynamics of P fractions in soil cultivated with *Ilex paraguariensis*, following successive applications of SM over a long period (> 40 years) in southern Brazil. Soil samples in the 0–5, 5–10 and 20–40 cm layers were collected in areas subjected to 35 (A35) and 45 (A45) years of SM application and an area with no anthropogenic intervention (M). The content of organic and inorganic P in the soil samples was quantified by sequential extraction with $NaHCO_3$ 0.5 mol L^{-1} (labile), H_2SO_4 (moderately labile), and NaOH 0.5 mol L^{-1} (recalcitrant). The potential of P adsorption was assessed using the solution of KH_2PO_4 which remained in contact with the soil for a night. Data were compared by Tukey test at 5% probability. The results showed that 35 and 45 years of successive SM application did not increase total P content in soil. In contrast, an increase in inorganic form of P was observed. In comparison to the forest area, SM application for 45 years increased the content of the labile inorganic P fraction by 168%, 162% and 290% in the 0–5, 5–10 and 20–40 cm soil layers, respectively. The accumulation of this fraction promoted the reduction of P adsorption in soil by 99%, 180% and 175% in the 0–5, 5–10 and 20–40 cm soil layers, respectively, increasing the risk of contamination of groundwater by P excess.

Keywords Manure · Phosphorus fractionation · Organic phosphorus · Inorganic phosphorus · Adsorption · Environmental contamination

Introduction

Global pork production reached 118 million tons in 2017 (FAO 2018), with an overall per capita consumption of 12.3 kg of the product (FAO 2017). In 2026, global pork consumption is projected to grow by about 9% (FAO 2017), which will be accompanied by increased production (127 million tons) and an increased volume of waste generated by production units.

About 27% of global contribution of pig production to greenhouse gas emissions is due to stock and handling waste this activity (FAO 2013). In addition, because of the reduced ability to produce the phytase enzyme seen in the monogastric system of pigs, which is responsible for the assimilation of P contained in the feed, waste with high phosphorus (P) concentrations is produced (Abioye et al. 2010; Sun and Jaisi 2018).

Brazil is the fourth largest producer of pork in the world. In 2017, the state of Santa Catarina alone (the largest Brazilian producer) produced 27% (1 million tons) of all Brazilian pork (MAPA 2018). In this region, the manure produced by swine (swine manure, SM) is typically applied to soil for cultivation in rural properties to distribute the large volume of stored waste, which also has high organic matter and nutrient content of agricultural interest.

✉ José de Souza Oliveira Filho
   jfilhomesti@gmail.com

1   Departamento de Ciências Do Solo, Centro de Ciências Agrárias, Universidade Federal Do Ceará, Campus do Pici, Bloco 807, Fortaleza, CE 60356-001, Brazil

2   Departamento de Solos, Universidade Federal Rural Do Rio de Janeiro, BR 465 km 7, Seropédica, RJ 23897-000, Brazil

SM application in soil is a common practice in the state of Santa Catarina where yerba mate (*Ilex paraguariensis*) is cultivated. This state is the largest national producer of pork and the second largest producer of yerba mate, with an estimated production of 28,853 tons of yerba mate (IBGE 2016). SM has been repeatedly applied over time, because SM application to crops totally or partially replaces the need for mineral fertilizers (Bosch-Serra et al. 2015; Penha et al. 2015; Menezes et al. 2017; Moreno-Garcia et al. 2017). This positive effect is due to an increase of P availability in the soil, among other characteristics, following successive SM application to crops (Guardini et al. 2012; Tiecher et al. 2017; Boitt et al. 2018).

Some studies have shown that most P content ($>80\%$) in SM are forms of inorganic phosphates with different degrees of solubility (Li et al. 2014; Tiecher et al. 2014; Wienhold and Miller 2004; Abioye et al. 2010). Li et al. (2014) showed that 22% of total P content in SM occurs in the form of inorganic phosphate. This form is easily extracted with water (Pi-H_2O); thus, it is highly available. Similarly, Abioye et al. (2010) estimated that the Pi–H_2O fraction represents between 54 and 75% of total P content in SM.

In contrast to the positive effect of increased P availability on soil after applying SM, some studies have demonstrated that the accumulation of highly labile P fractions in the soil increase the risk of contaminating groundwater (Guardini et al. 2012; Boitt et al. 2018). Tiecher et al. (2017) observed increases of 5.523% in available P content in the 0–5 cm soil layer, after 8 years of successive SM (80 m^3 ha^{-1}) application in a no-tillage system in southern Brazil. However, over time, the more labile fractions of P might be adsorbed into the mineral matrix of the soil, reducing its availability (Boitt et al. 2018) or converting it to little-known organic forms of P, forming a passive stock of mineralization and availability in the soil over time (Withers et al. 2018).

Estimates of the different forms of P that accumulate in soil following long-term (>40 years) application of SM remain limited in the scientific literature. This study quantified the distribution of organic and inorganic P content of different labilities (labile, moderately labile, recalcitrant and residual) and adsorption potential of P (remaining phosphorus) in soil cultivated with yerba mate in south Brazil after 35 and 45 years of successive SM application. It was hypothesized that (a) after 35 and 45 years of successive SM application, predominantly inorganic P content would accumulate in the soil, particularly the fraction with high lability (extracted with NaHCO$_3$ 0.5 mol L^{-1}), (b) the soil adsorption potential of P would decline after successive SM application and (c) causes the accumulation of the inorganic P fraction of high lability in subsurface when compared to soil of reference forest.

Materials and methods

Study area and experimental design

This study was carried out in the upper Uruguay Catarinense region, Municipality of Presidente Castello Branco (27°13′17″S, 51°48′40″W, altitude 452 m), in the state of Santa Catarina, in southern Brazil. The climate of the region, according to the Köppen–Geiger classification, is humid subtropical (Cfa), with average temperature of 20.6 °C and average annual rainfall of 1711 mm (IPARDES 2019).

In 1973, a pilot study was initiated in an area called A45, to understand the effect of applying 400 m^3 ha^{-1} SM each year to soil on which yerba mate was cultivated. The SM was obtained by each farming property to add value to the waste by transforming it to an input and promoting the circular economy. Ten years after initiating the study, yerba mate cultivation was started on a second area (A35), immediately adjacent to A45, also with the same amount of SM being applied as fertilizer each year. Physicochemical properties of SM (average of the last 35 years) were: dry matter 38 ± 15 kg m^{-3}; total carbon 17 ± 7 kg m^{-3}; total P 1.8 ± 0.5 kg m^{-3}; total nitrogen 2.9 ± 1.1 kg m^{-3}; total potassium 1.2 ± 0.3 kg m^{-3}; total calcium 1.2 ± 0.3 kg m^{-3}; total magnesium 0.86 ± 0.4 kg m^{-3}; pH 7.4 ± 0.5; and electric conductivity (CE) 1.9 dS m^{-1}. A preserved forest area (M), adjacent to A45 and A35, was used as the experimental control. In the first half of 2017, soil samples were collected in all the three areas to quantify how P accumulates in the different fractions over time. The soils of the study areas were classified as Haplic Cambisol (Santos et al. 2018).

Sample collection and soil analysis

Each experimental area had dimensions of 50 m × 50 m, totaling 0.25 ha. In each area, five soil samples were randomly collected from the 0–5, 5–10 and 20–40 cm soil layer to form a composite sample. From each area, four composite samples (replicates) were obtained, totaling eight samples per area (four composite samples × 2 depths).

The composite samples were air-dried, crushed, and passed through a 2 mm mesh sieve and analyzed for pH (H_2O), Ca^{2+}, Mg^{2+}, Al^{3+}, Na^+, K^+, H + Al, available P (Mehlich-1), organic carbon (OC), and granulometry (sand, silt, and clay content), according to the procedures proposed by Donagema et al. (2011). The characteristics of the soil are shown in Table 1.

Table 1 Soil chemical and granulometric analysis of the studied areas

Areas	pH_{water}	Ca^{2+} $cmol_c\ dm^{-3}$	Mg^{2+}	K^+	$H+Al$	Al^{3+}	Sand $g\ kg^{-1}$	Silt	Clay	Available P $mg\ kg^{-1}$	OC $g\ kg^{-1}$
0–5 cm											
A45	5.1	3.3	2.0	0.2	9.5	0.8	197	408	395	5.8	22
A35	4.5	1.5	0.7	0.3	7.2	0.9	159	291	550	5.5	20
M	5.3	5.8	2.6	0.1	7.1	0.2	290	38	328	6	25
5–10 cm											
A45	4.9	2.5	0.9	0.1	8.5	0.4	217	343	441	5.4	21
A35	4.4	1.4	0.9	0.1	12.7	1.0	146	275	579	5.5	19
M	5.4	5.2	2.8	0.1	7.8	0.2	331	328	341	6	24
20–40 cm											
A45	4.8	2.2	0.7	0.1	8.2	0.4	217	343	441	5.4	19
A35	4.3	1.2	0.8	0.1	10.7	1.0	146	275	579	5.5	17
M	5.3	5.1	2.5	0.1	6.8	0.2	331	328	341	6	23

A45 area with 45 years of consecutive application of SM, *A35* area with 35 years of consecutive application of SM, *M* native forest area used as a reference

Phosphorus fractionation

Several analytical methods for sequential extraction of P in soil are available in the scientific literature (Dean 1937; Chang and Jackson 1957; Hedley et al. 1982). These methods use extracting solutions of different compositions and extraction capacities to obtain soil P fractions with different degrees of lability. The most used method worldwide is that of Hedley et al. (1982). However, the methodology proposed by Bowman (1989) has been widely used for its simplicity, speed and precision of results (Beutler et al. 2015; Bezerra et al. 2015; Guareschi et al. 2015,2016; Rosset et al. 2016; Rossi et al. 2016).

We follow the procedures proposed by Bowman (1989) as follows: soil organic and inorganic phosphorus was extracted sequentially with $NaHCO_3$ 0.5 mol L^{-1} (labile), H_2SO_4 (moderately labile), and NaOH 0.5 mol L^{-1} (recalcitrant). For each extractor, the sample was digested with perchloric acid and magnesium chloride to obtain the total P content of the extract. The inorganic fraction of each extractor was obtained by colorimetry (Murphy and Hiley 1962). The organic fraction of each extractor was obtained by the difference between the total P content and the inorganic fraction. After the sequential extraction, we quantified the P content in residual soil (Residual P) by digestion, using perchloric acid and magnesium chloride and determination by colorimetry (Murphy and Hiley 1962). The total P content of the soil was obtained by adding the total content of P obtained in each extractor to the residual P content.

Phosphorus adsorption

The remaining P of the soil was quantified according to Alvarez and Fonseca (1990) using 20 mL of 0.01 mol L^{-1} $CaCl_2$ solution containing 60 mg of P in the form of KH_2PO_4, which remained in contact with the soil for one night. After this period, we removed an aliquot of the supernatant solution for remaining P quantification in the extract by colorimetry. The difference between the initial P content (60 mg kg^{-1}) and the remaining P content was adsorbed by the soil mineral matrix.

Statistical analysis

Statistical analysis was performed considering a completely randomized design in split plot arrangement. The plots (main factor) were composed by the three areas (A45, A35 and M) and the subplots (secondary factor) were composed by the three sampling depths (0–5, 5–10 e 20–40 cm). The split plot arrangement allows us to infer about the interaction between areas versus depths. Data were subjected to analysis of variance (ANOVA) and residues were checked for normality (Bartlett's test) and homogeneity of variances (Cochran's test). The averages of main treatments (areas), secondary treatments (depths) and their interactions (areas × depths) were compared by Tukey test at 5% significance. The analyses were performed in the software R (R Core Team 2018), using the package *EasyAnova* (Arnhold 2013).

Results and discussion

Analysis of variance

The ANOVA indicated a reduced effect of the main treatments (areas) on the results. However, among the secondary treatments (depths), the variability of the results was more pronounced. Moreover, the interaction between major

and secondary factors was not significant for most of the variables studied. Only the recalcitrant organic P fraction ($p = 0.0310$), total organic P ($p = 0.0376$) and the remaining P ($p < 0.0001$) showed significant interaction (areas × depths). This means that the effects of the areas for these variables act depending on the variation of the sampling depth (Table 2).

Normally, recalcitrant organic forms of P are associated with stable fractions of soil organic matter (SOM) and are indirectly influenced by sampling depth due to the direct effect of depth on SOM accumulation along the profile (Gonzalez Jimenez et al. 2019). Similarly, P adsorption, measured by the remaining P content, is highly dependent on the sampling depth due to the depth variation of available P levels (Bom et al. 2019) and SOM content, affecting the anion exchange potential between phosphate ions and the soil mineral matrix (Gonzalez Jimenez et al. 2019; Lemming et al. 2019). Separately, considering the variance between areas, we observed significant variance only for the inorganic labile P ($p = 0.0126$), inorganic moderately labile P ($p = 0.0030$) and total P content ($p = 0.0497$). Between depths, however, significant variance was observed for the inorganic moderately labile P ($p = 0.0216$), recalcitrant organic P ($p = 0.0120$), total organic P ($p = 0.0124$), total P ($p = 0.0161$) and remaining P ($p = 0.0252$).

Total soil P content

Total soil P values ranged from 1223 (20–40 cm layer in A35) to 2465 mg kg^{-1} (0–5 cm layer in M) (Fig. 1). Comparing the two soils submitted to the application of SM (A45 and A35) against natural soil (M) showed that the successive application of SM did not cause total P content in the soil to increase at all the depths evaluated (Fig. 1a). In the comparison between A35 and A45, only at a depth of 5–10 cm the application of SM for 45 years was statistically superior to the treatment submitted to the application of SM for 35 years ($p < 0.05$). These results differ to those reported in other similar studies (De Conti et al. 2015; Tiecher et al. 2017; Yan et al. 2017; Boitt et al. 2018; Rigo et al. 2019), which documented an increase in the P content of soil after successive applications of SM, regardless of experimental soil type. Statistical analysis between the three sampling depths inside the same area showed variation with increasing depth (from 5 to 40 cm) ($p < 0.05$), reducing by 38% of the total P content in cultivated areas (A45 and A35) and remained constant in the treatment without anthropic intervention (M) ($p = 0.1250$) (Fig. 1b).

Normally, forest soils without anthropic activity tend to maintain a balance between the inputs (leaves and dead roots) and outputs (plant absorption and leaching) of P, resulting in high values of total P in the soil (Missong et al. 2018; Zederer and Talkner 2018). First, the removal

Table 2 Analysis of variance of P fractions (inorganic and organic) and remaining P in soil subjected to swine manure application for 45 and 35 years versus the native reference forest at three sampling depths in southern Brazil

Source of variation	*F* value	*P* value
	Total P	
Areas	4.2699	0.0497
Depths	5.2346	0.0161
Areas × depths	1.0180	0.4244
	Inorganic labile P	
Areas	7.6303	0.0126
Depths	2.0767	0.1543
Areas × depths	1.1245	0.3760
	Moderately labile inorganic P	
Areas	11.8376	0.0030
Depths	4.7790	0.0216
Areas × depths	0.9610	0.4526
	Recalcitrant inorganic P	
Areas	0.6035	0.5676
Depths	1.0018	0.3868
Areas × depths	0.2727	0.8917
	Total inorganic P	
Areas	2.9317	0.1046
Depths	1.3173	0.2925
Areas × depths	0.3443	0.8444
	Labile organic P	
Areas	0.7594	0.4957
Depths	1.5982	0.2297
Areas × depths	0.7775	0.5541
	Moderately labile organic P	
Areas	4.0470	0.0558
Depths	1.5628	0.2367
Areas × depths	2.7020	0.0635
	Recalcitrant organic P	
Areas	0.9003	0.4401
Depths	5.7058	0.0120
Areas × depths	3.3936	0.0310
	Total organic P	
Areas	0.9364	0.4271
Depths	5.6575	0.0124
Areas × depths	3.2031	0.0376
	Residual P	
Areas	0.6797	0.5310
Depths	2.6050	0.1015
Areas × depths	0.2746	0.8905
	Remaining P	
Areas	1.0168	0.3998
Depths	4.5466	0.0252
Areas × depths	66.3733	<0.0001

Source of variation with *P* value < 0.05 have statistically different averages according to the *F* test
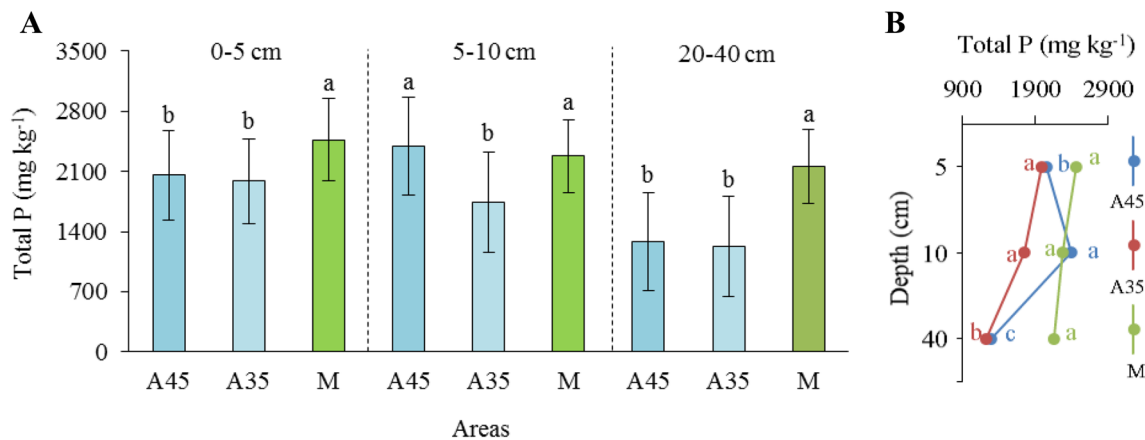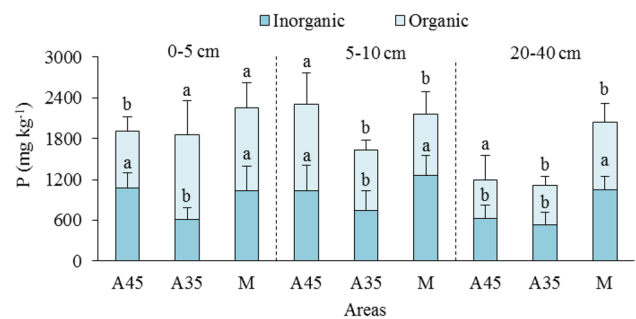
Fig. 1 Total phosphorus (Total P) of soil subjected to swine manure application for 45 (A45) and 35 (A35) years versus the native reference forest (M; control) at three sampling depths in southern Brazil. Averages followed by the same letters when comparing the three areas (**a**) and averages followed by the same letters when comparing the three depths inside the same area (**b**) are considered similar by the Tukey test at 5% significance. Each value represents the average, and the error bar in A represents the standard deviation of the average ($n = 4$)

of natural vegetation for implantation of agricultural crops tends to reduce the total P content in soil, due to an intensification of the mineralization processes (Oliveira Filho et al. 2017), leaching (Missong et al. 2018) and removal of the soil layer by erosive processes (Lourenzi et al. 2015). Second, it is expected that the application of SM to the soil increase the total P content (Tiecher et al. 2017). In our case, however, only the total P content in the 5–10 cm layer of the A45 area reached the total P level of the natural soil condition. This indicates an intense mineralization process of the added waste, with constant extraction of P from the soil by crops or lost by leaching and/or runoff.

Statistical analysis between the three areas indicated a significant reduction ($p < 0.05$) in total P content in A35 when compared with A45 and M in the 5–10 cm layer. Thus, P content increased to reflect that of the natural conditions only after 45 years of SM application. This phenomenon might be explained by the effect of considerable quantities of P being removed by the harvests. In the studied crop type, a large part of the aerial biomass, represented by leaves and thin branches, is removed by harvesting, resulting in 10.2 kg ha^{-1} of P being exported from the soil (Santin et al. 2017). In contrast, in the superficial (0–5 cm) and in the deep (20–40 cm) soil layers, 10 years difference in SM application between the two areas was not sufficient to modify the total P content.

Under natural conditions (M), there was an equal split (50/50%) between inorganic (Pi) and organic (Po) forms, with organic content decreasing in the subsurface soil layer, whereas the inorganic fraction increased (Fig. 2). In comparison, after 35 years of SM application, only the middle layer (5–10 cm) contains more organic than inorganic P. After 45 years, the inorganic fraction of P was slightly
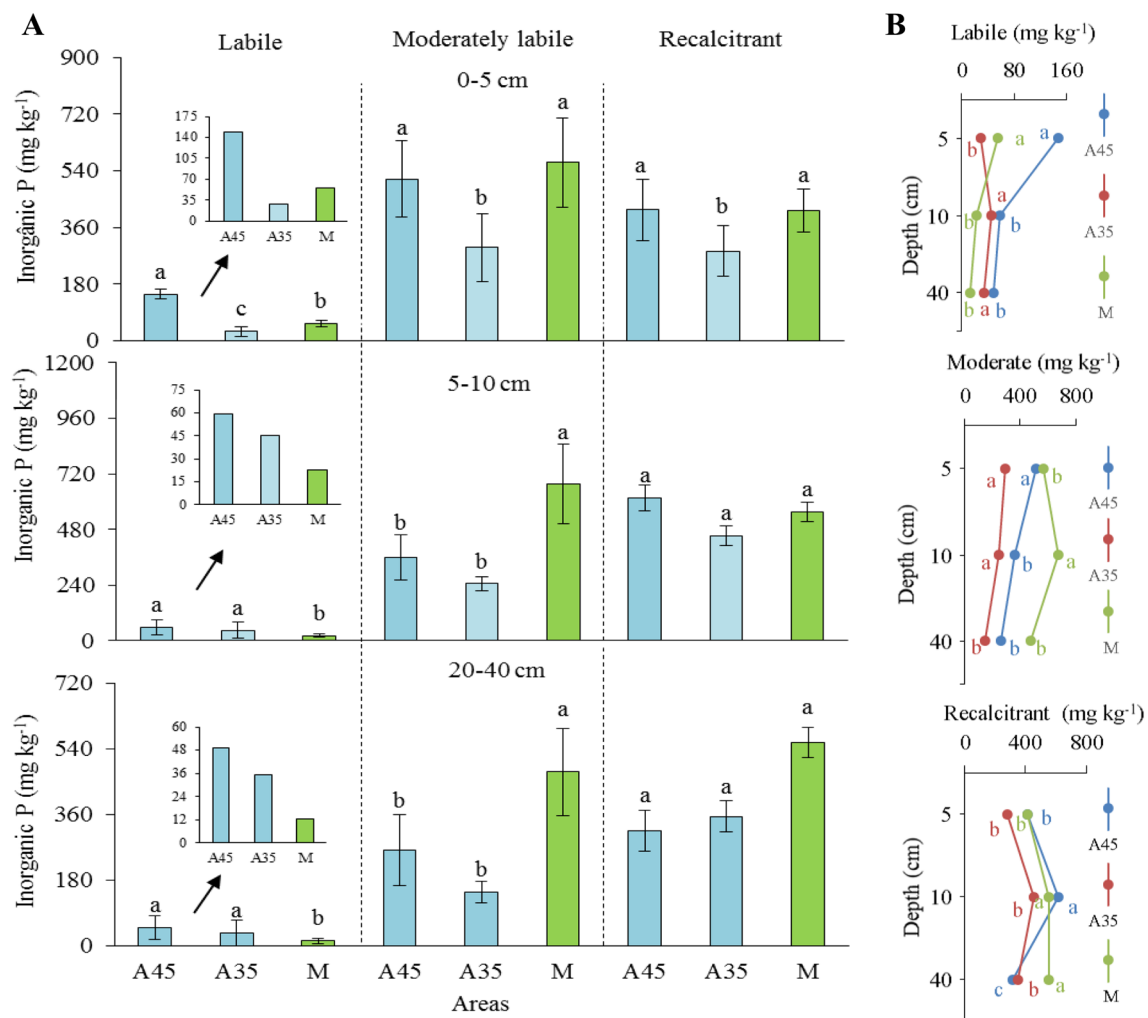

Fig. 2 Organic and inorganic phosphorus content of soil in areas cultivated with yerba mate and subjected to swine manure application for 45 (A45) and 35 (A35) years versus a native reference forest (M) in southern Brazil. Averages followed by the same letters in the comparisons are considered similar by the Tukey test at 5% significance. Each value represents the average, and the error bar represents the standard deviation of the average ($n = 4$)

higher in the superficial layer (0–5 cm) and in the subsurface layer (20–40 cm). The higher accumulations of inorganic P in these layers may be indicative that: (1) in the superficial layer it caused the partial mineralization of the organic fraction forming inorganic P (Mackay et al. 2017; Turrion et al. 2018; Ye et al. 2015) and (2) in the subsurface layer it caused the accumulation of inorganic P translocated from the upper layers (Giroto et al. 2013; Aronsson et al. 2014; Lourenzi et al. 2014).

Inorganic P fractions

The higher P inorganic fraction in the soil surface layer (0–5 cm) after 45 years of SM application was partly due to the higher accumulation of the highly labile Pi fraction

Fig. 3 Fractions of inorganic phosphorus in soil cultivated with yerba mate subjected to SM application for 45 (A45) and 35 (A35) years versus a native reference forest (M) in southern Brazil. Averages followed by the same letters in the comparisons of areas (**a**) and aver- ages followed by the same letters when comparing the three depths inside the same area (**b**) are considered similar by the Tukey test at 5% significance. Each value represents the average, and the error bar in A represents the standard deviation of the average ($n=4$)

(NaHCO$_3$ 0.5 mol L^{-1}) (Fig. 3a). Statistical analysis indicated significant difference ($p<0.05$) in the accumulation of this fraction in A45 when compared with A35 and M. This increase in A45 was 406% and 168% greater than that in A35 and M, respectively. Similar results were obtained by Tiecher et al. (2017) and Boitt et al. (2018) in agricultural systems subjected to SM for eight and fifteen years, respectively. A significant proportion of total P content of SM occurs in highly labile forms (Abioye et al. 2010; Li et al. 2014). According to Oliveira Filho et al. (2019), approximately 50% of the total P content of SM is in highly water soluble forms. Thus, the application of SM in agriculture contributes positively to an increase in the labile fraction of inorganic P in the soil. Moreover, analyzing the effect of the depth factor on labile inorganic P content (Fig. 3b), we observed that only A45 showed

significant difference ($p<0.05$), with reduced content with increasing sample depth.

According to Dechmi et al. (2013) and Boitt et al. (2018), the accumulation of the most labile inorganic fraction of P in soil after the application of SM may mean the risk of contamination of the groundwater by excess of P. This contamination occurs basically by the leaching of inorganic phosphate to the deeper layers of the soil and is dependent on the volume of drainage water that passes through the soil profile after the application of SM (Lourenzi et al. 2015). According to Aronsson et al. (2014), only three monthly applications of SM (13 kg P ha^{-1}) in soil cultivated with spring cereals promoted the accumulation of 0.16 kg ha^{-1} of soluble P in the leaching water. Logically, the effect of groundwater contamination should be greater as SM application time increases (Yang et al. 2017).

Thirty-five years of SM application was not sufficient for labile Pi to reach the same levels as that under natural conditions in the 0–5 cm layer. Statistically, the labile inorganic P content in A35 and M in this soil layer is similar ($p = 0.2430$). Much of the labile Pi added to the soil surface via SM during the 35 years was probably absorbed by the plants (Santin et al. 2017) or interacted with the mineral matrix of the soil, limiting the available P fractions (Withers et al. 2018). However, continued application (45 years), probably saturated the P adsorption sites of the soil (iron and aluminum oxides), reducing adsorption and promoting the permanence of phosphate in its more available form, supporting the proposal of Frank et al. (2018).

In the layers of 5–10 and 20–40 cm, statistical analysis results showed significant differences in labile inorganic P content between areas submitted to SM application (A45 e A35) and M, with the DS application areas showing higher accumulations of this fraction of P. In the 5–10 cm layer, the A45 area showed labile inorganic P values 2.6 times higher than in the reference. In the A35 area this accumulation was approximately 2 times greater. In the 20–40 cm layer, the accumulation of the most labile inorganic fraction of P was 4 times (A45) and 2.7 times (A35) higher than the value observed in the same layer of the reference area. These characteristics indicate a possible vertical movement of the most labile inorganic fraction of P in the soil, as proposed by Lourenzi et al. (2014) and Aronsson et al. (2014). In addition, the high content of N in SM promotes greater mobility and leaching of the phosphate ion in the soil profile (Yan et al. 2016), increasing the risk of contamination of groundwater by excess of P.

In general, in both the surface and subsurface layers, the fractions of moderately labile and recalcitrant inorganic P decreased following 35 years of SM application, but returned to values close to natural conditions after 45 years of SM application. Statistical analysis showed a significant difference for the most stable inorganic fractions of P between A35 and M, proving the tendency to return to the initial levels found in M of these fractions in the soil with the continued application of SM. The depth factor (Fig. 3b) seems to have little effect on the accumulation of moderately labile and recalcitrant inorganic P fractions, with little statistical variation due to the increase in sampling depth. These fractions require a longer period of time to form because they are associated with P forms bound to high stability secondary minerals, which serve as buffers to maintain adequate levels of labile Pi in the soil solution (Oliveira Filho et al. 2017; Weihrauch and Opp 2018). Certainly, the buffering effect was more evident in the moderately labile P fraction (extracted with H_2SO_4) compared to the more recalcitrant P fraction (extracted with NaOH 0.1 mol L^{-1}). The former was consumed more quickly by becoming Pi labile, resulting in its content being lost with successive crops (Rossi et al. 2016).

Organic P fractions and residual P

The organic fraction of highly labile P (obtained with NaHCO$_3$ 0.5 mol L^{-1} extraction) represents a source of organic P that mineralizes quickly and is available in a short period of time (Weihrauch and Opp 2018). In this fraction, is included the P of the microbial biomass, which represents the immediate source of labile P of the plants after the death of the microorganisms (Zhang et al. 2018). In the soil surface layer (0–5 cm) of the studied areas, statistical analysis indicated significant difference for labile organic P content between A45 and A35 ($p < 0.05$), between A35 and M ($p < 0.0001$) and between A45 and M ($p < 0.05$). This fraction was higher in the area with 35 years of SM application (134.8 mg kg^{-1}) and lower in the area with 45 years of SM application (46.1 mg kg^{-1}) and in the natural forest (23.9 mg kg^{-1}) (Fig. 4a). Therefore, the accumulation of organic labile P following SM application (35 years) allows this fraction to be mineralized for inorganic P-labile. This phenomenon explains the greater accumulation of labile inorganic P in A45 versus A35 observed in Fig. 3. For this fraction, the depth factor showed a significant difference ($p < 0.05$) only for area A35, with content reduction with increasing sampling depth (Fig. 4b).

According to Zhao et al. (2019), the accumulation of the labile organic P fraction in the soil surface layer is due to reduced activity of the phosphomonoesterase enzyme, which has the function of catalyzing the transformation of organic P to inorganic P. It is probable that with increasing time of SM application, the soil microbial demand for the formation of stable organic matter (humic substances) promoted the mineralization of the labile organic P associated with the labile fractions of organic matter in the soil. This promoted the increase of the fraction of P of high lability in the superficial layer after 45 years of the SM application (Tian et al. 2016; Wang et al. 2016).

In the subsurface soil layer, SM application had no effect on labile organic P content after 35 ($p = 0.2245$) and 45 years ($p = 0.3998$). The effects of SM application on labile organic P were more evident in the surface soil layer. For the more stable organic fractions (moderately labile and recalcitrant), the area with no intervention consistently had greater or equal P content in the surface soil layer when compared to the soils where SM was applied. In this case, the depth factor was significant only for the recalcitrant organic P fraction in A35 ($p < 0.05$), reducing its content with increasing sampling depth (Fig. 4b). According to our results, we found that the soil organic fractions of P varied in less intensity when compared to the inorganic fractions of the nutrient as a function of different land use systems.
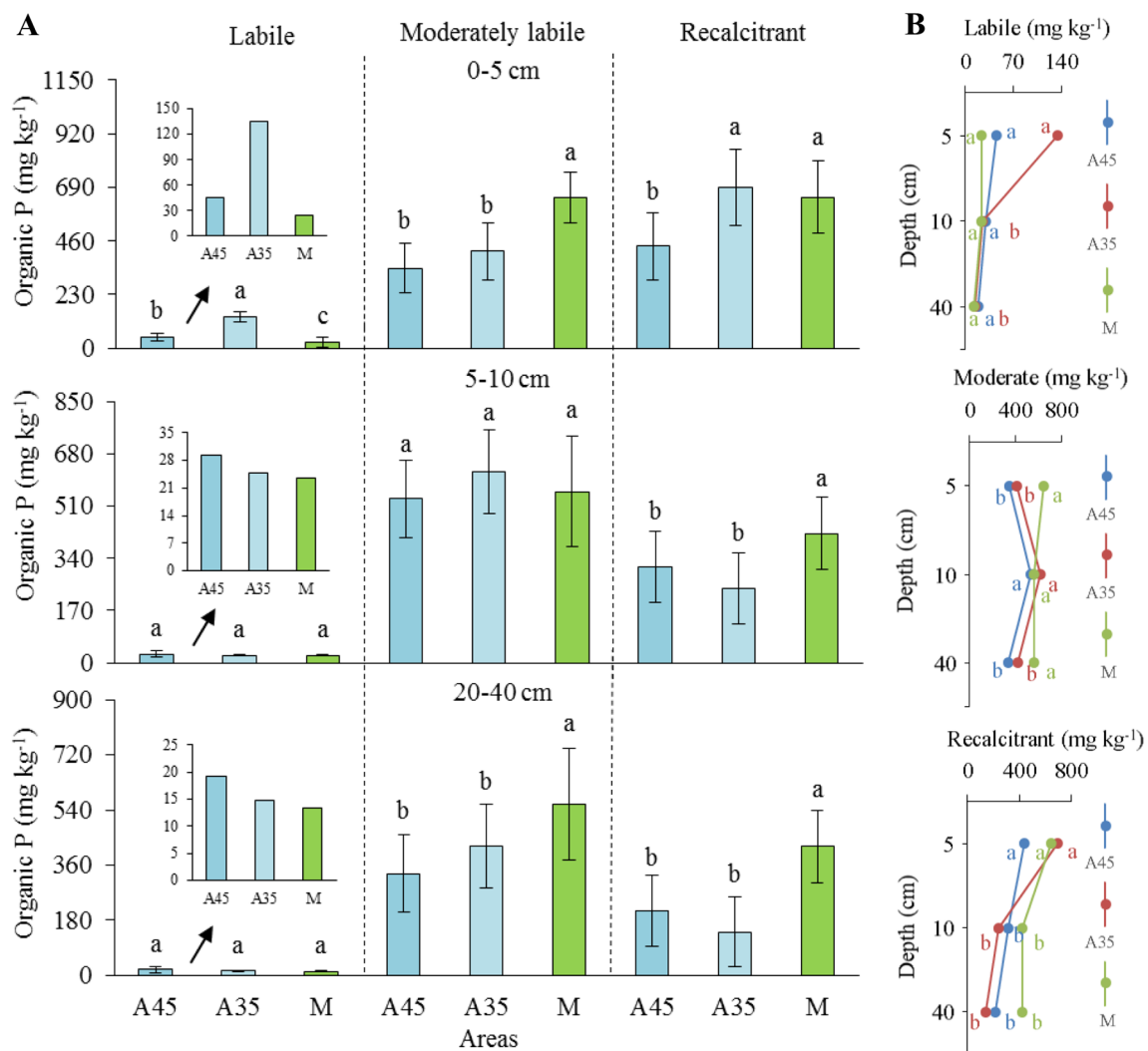
Fig. 4 Fractions of organic phosphorus in soil cultivated with yerba mate subjected to SM application for 45 (A45) and 35 (A35) years versus a native reference forest (M) in southern Brazil. Averages followed by the same letters in the comparisons of areas (**a**) and aver- ages followed by the same letters when comparing the three depths inside the same area (**b**) are considered similar by the Tukey test at 5% significance. Each value represents the average, and the error bar in A represents the standard deviation of the average ($n=4$)

The crop caused the more stable P organic fractions (moderately labile and recalcitrant) to decline in most layers of the soil, except for the 5–10 cm layer in moderately labile fraction. Consequently, successive SM application was not sufficient to replace these fractions to initial levels. These fractions represent forms of P associated with organic matter stabilized in the soil, which form part of recalcitrant humic structures (Spain et al. 2018). These structures may be lost in long periods of cultivation (Rossi et al. 2016). Similarly, this phenomenon explains the higher residual P content in the forest area when compared to the cultivated areas with SM application in the 0–5 and 5–10 cm soil layers (Fig. 5a).

In the 20–40 cm layer, however, no statistical differences were observed in the content of the residual P fraction among the three evaluated areas ($p=0.3543$), indicating that in this depth, the content of this fraction did not change under the effects of the vegetation removal to the crop and the SM application over time. For the depth factor, however, the residual P content in M showed a significant difference with increasing depth (from 5 to 40 cm), reducing the content of this fraction and statistically equaling the other areas (Fig. 5b).

The residual P fraction is formed by highly resistant organic and inorganic P forms, which cannot be extracted with conventional approaches. In general, these forms represent phosphates that are strongly associated with organic matter stabilized in the soil, as well as inorganic phosphates forming part of the mineral structure of the soil, which requires great energy for extraction (Spain et al. 2018; Weihrauch and Opp 2018; Lemming et al. 2019). Like the
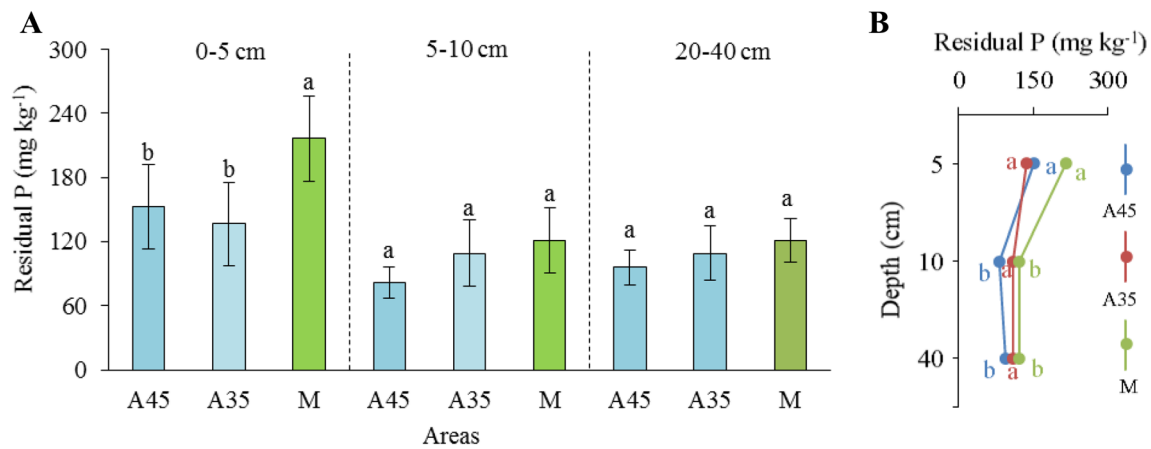
Fig. 5 Residual phosphorus in soil cultivated with yerba mate and subjected to SM application for 45 (A45) and 35 years (A35) versus the native reference forest (M) in southern Brazil. Averages followed by the same letters in the comparisons of areas (**a**) and averages fol-lowed by the same letters when comparing the three depths inside the same area (**b**) are considered similar by the Tukey test at 5% signifi-cance. Each value represents the average, and the error bar in A rep-resents the standard deviation of the average ($n = 4$)

other more stable fractions in the superficial soil layers, this fraction was lost from the soil under cultivation, with 45 and 35 years of SM application not being sufficient for the new formation of this P fraction. However, it is possible that the recurrent addition of labile P through SM application on the soil surface inhibits the formation of stabilized organic mat-ter containing P, due to the increase of SOM mineralization (priming effect) leading to the release of inorganic P (Liu et al. 2017; Ma et al. 2013).

Soil P adsorption

As observed in Fig. 3, forty-five years of SM application promotes the accumulation of the more labile Pi fraction. This results in reducing the adsorption sites of the phos-phate with the mineral matrix of the soil. This phenomenon increased the values of remaining P and reduced the effec-tive adsorption of ion phosphate with the mineral matrix of P in the soil (Fig. 6a, b). Remaining P values are inversely proportional to soil P adsorption. Thus, high remaining P values indicate reduced P adsorption capacity by the soil mineral matrix. Schmieder et al. (2018) estimated that 76% of soil inorganic P in soils subjected to SM application for 40 years was associated with iron and aluminum oxide sur-faces, promoting the saturation of these sites. After satu-ration, Boitt et al. (2018) estimated a loss of 6–38% P to the groundwater from the total P applied via SM. This loss might lead to high risk of environmental contamination by the vertical movement of phosphate ions in the soil profile.

　　Our results clearly showed that P adsorption was lower (higher remaining P value) in all layers of A45 compared to the other areas. Thus, inorganic P might shift deeper into the
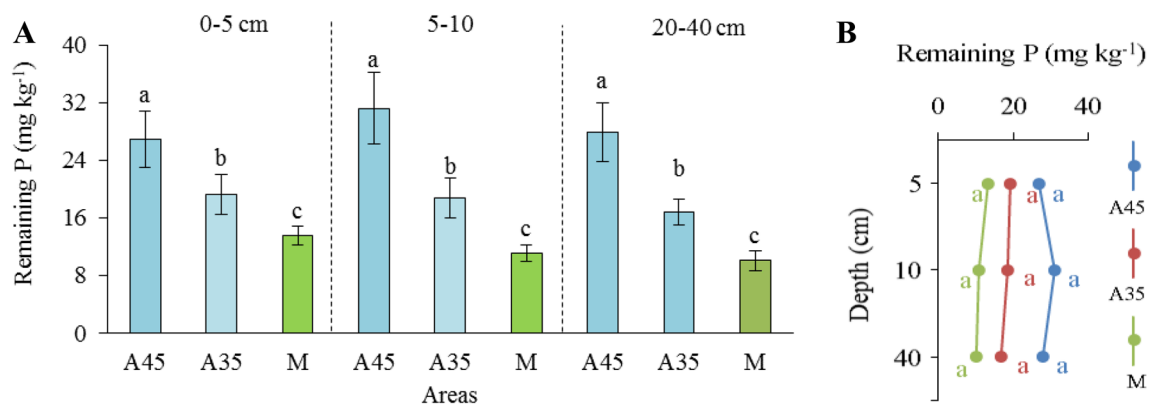
Fig. 6 Remaining phosphorus in soil cultivated with yerba mate and subjected to SM application for 45 (A45) and 35 years (A35) versus the native reference forest (M) in southern Brazil. Averages followed by the same letters in the comparisons of areas (**a**) and averages fol-lowed by the same letters when comparing the three depths inside the same area (**b**) are considered similar by the Tukey test at 5% signifi-cance. Each value represents the average, and the error bar in A rep-resents the standard deviation of the average ($n = 4$)

soil layer, even though it is considered to have low mobility in soils (da Rosa Couto et al. 2017). Of note, the area without anthropogenic intervention had the lowest levels of labile inorganic P and contributed to higher adsorption values (low remaining P), both in the surface and subsurface soil layers, indicating the inversely proportional relationship between labile inorganic P content and P adsorption potential by the soil mineral matrix.

Conclusions

Sequential extraction of P content in soil is important as it provides detailed information on the dynamics of the various forms (organic and inorganic) of P accumulation in soil. As presented in this work, the quantification of the total soil P content alone does not provide evidence of the potential for soil contamination by P excess. Currently, there is a great concern about the eutrophication of surface and groundwater by the excess of labile inorganic P, with great potential for mobility and solubility. Our results confirm that in the areas submitted to the application of the SM, a considerable increase in this fraction was observed. Basically, the more labile inorganic P fraction (extracted with $NaHCO_3$ 0.5 mol L^{-1}) increased. We observed the accumulation of the most labile inorganic P fraction in the deepest layers of the profile in the area submitted to 45 years of SM application. This may be indicative of the vertical movement of P in the soil profile. The accumulation of this fraction promoted the saturation of P adsorption sites in the mineral matrix of the soil, as indicated by the higher values of remaining P observed in the cultivated areas with SM application. The increase in the labile inorganic fraction, which was associated with reduced adsorption, might present a risk of environmental contamination to groundwater, due to the accumulation of P in the environment.

References

Abioye S, Ice D, Akinremi O, Nyachoti M, Flaten D (2010) Characterizing fecal and manure phosphorus from pigs fed phytase supplemented diets. J Agr Sci 2(4):3–12

Alvarez VVH, Fonseca DM (1990) Definição de doses de fósforo para determinação da capacidade máxima de adsorção de fosfatos e para ensaios em casa de vegetação. Rev Bras Ciênc Solo 14:49–55

Arnhold E (2013) Package in the R environment for analysis of variance and complementary analyses. Braz J Vet Res Anim Sci 50(6):488–492

Aronsson H, Liu J, Ekre E, Torstensson G, Salomon E (2014) Effects of pig and dairy slurry application on N and P leaching from crop rotations with spring cereals and forage leys. Nutr Cycl Agroecosyst 98:281–293. https://doi.org/10.1007/s10705-014-9611-3

Beutler SJ, Pereira MG, Loss A, Perin A, Anjos LHC (2015) Humic substances and phosphorus fractions in areas with crop-livestock integration, pasture and natural Cerrado vegetation in Goiás Brazil. Trop Subtrop Agroecosys 18:11–25

Bezerra RPM, Loss A, Pereira MG, Perin A (2015) Phosphorus fractions and correlation with soil attributes under no-tillage and crop-livestock integration systems in Cerrado of Goias. Semina Ciên Agrár 36(2):1287–1306. https://doi.org/10.5433/1679-0359.2015v36n3p1287

Boitt G, Schmitt DE, Gatiboni LC, Wakelin SA, Black A, Sacomori W, Cassol PC, Condron LM (2018) Fate of phosphorus applied to soil in pig slurry under cropping in Southern Brazil. Geoderma 321:164–172. https://doi.org/10.1016/j.geoderma.2018.02.010

Bom FJT, McLaren TI, Doolette AL, Magid J, Frossard E, Oberson A, Jensen LS (2019) Influence of long-term phosphorus fertilisation history on the availability and chemical nature of soil phosphorus. Geoderma 355:1–12. https://doi.org/10.1016/j.geoderma.2019.113909

Bosch-Serra AD, Ortiz C, Yague MR, Boixadera J (2015) Strategies to optimize nitrogen efficiency when fertilizing with pig slurries in dryland agricultural systems. Eur J Agron 67:27–36. https://doi.org/10.1016/j.eja.2015.03.003

Bowman RA (1989) A sequential extraction procedure with concentrated sulfuric acid and dilute base for soil organic phosphorus. Soil Sci Soc Am J 53:362–366. https://doi.org/10.2136/sssaj1989.03615995005300020008x

Chang SC, Jackson ML (1957) Fractionation of soil phosphorus. Soil Sci 84:133–144

da Rosa Couto R, Ferreira PAA, Ceretta CA, Lourenzi CR, Facco DB, Tassinari A, Piccin R, De Conti L, Gatiboni LC, Schapanski D, Brunetto G (2017) Phosphorus fractions in soil with a long history of organic waste and mineral fertilizer addition. Bragantia 76(1):155–166

Dean LA (1937) Distribution of the forms of soil phosphorus. Soil Sci Soc Am Proc 2:223–227

Dechmi F, Isidoro D, Stambouli T (2013) A phosphorus index for use in intensive irrigated areas. Soil Use Manag 29(1):64–75. https://doi.org/10.1111/j.1475-2743.2012.00448.x

De Conti L, Ceretta CA, Ferreira PAA, Lorensini F, Lourenzi CR, Vidal RF, Tassinari A, Brunetto G (2015) Effects of pig slurry application and crops on phosphorus content in soil and the chemical species in solution. R Bras Ci Solo 39:774–787. https://doi.org/10.1590/01000683rbcs20140452

Donagema KD, Campos DVB, Calderano SB, Teixeira WG, Viana JHM (2011) Manual de métodos de análise de solos. Embrapa Solos, Rio de Janeiro

FAO (2013) Tackling climate change though livestock: a global assessment of emissions and mitigation opportunites. https://www.fao.org/3/i3437e.pdf. Accessed 18 Apr 2017

FAO (2017) Agricultural Outlook 2017–2026. https://dx.doi.org/10.1787/agr_outlook-2017-en. Accessed 25 Sep 2018

FAO (2018) Food and Agriculture Organization of the United Nations. Meat Market Review. https://www.fao.org/3/I9286EN/i9286en.pdf. Accessed 22 June 2018

Frank S, Bergstrom L, Riddle M, Gustafsson JP, Klysubun W, Zehetner F, Condron L, Kirchmann H (2018) Phosphorus speciation in a long-term manure-amended soil profile – Evidence from wet chemical extraction, ^{31}P-NMR and P K-edge XANES spectroscopy. Geoderma 322:19–27. https://doi.org/10.1016/j.geoderma.2018.01.026

Girotto E, Ceretta CA, Lourenzi CR, Lorensini F, Tiecher TL, Vieira RCB, Trentin G, Basso CJ, Miotto A, Brunetto G (2013) Nutrient transfers by leaching in a no-tillage system through soil treated with repeated pig slurry applications. Nutr Cycl Agroecosyst 95:115–131. https://doi.org/10.1007/s10705-013-9552-2

González Jiménez JL, Healy MG, Daly K (2019) Effects of fertiliser on phosphorus pools in soils with contrasting organic matter content:

a fractionation and path analysis study. Geoderma 338:128–135. https://doi.org/10.1016/j.geoderma.2018.11.049

Guardini R, Comin JJ, Schmitt DE, Tiecher T, Brender MA, Santos DR, Mezzari CP, Oliveira BS, Gatibone LC, Bruneto G (2012) Accumulation of phosphorus fractions in Typic Hapludalf soil after long-term application of pig slurry and deep pig litter in a no-tillage system. Nutr Cycl Agroecosys 93:215–225. https://doi.org/10.1007/s10705-012-9511-3

Guareschi RF, Pereira MG, Perin A (2015) Adsorption of P and forms of iron in no-tillage areas in the Cerrado biome. Acta Sci Agron 31(1):109–116. https://doi.org/10.4025/actasciagron.v37i1.17686

Guareschi RF, Pereira MG, Perin A (2016) Phosphorus fractions in an agricultural chronosequence under tillage regimes in the Cerrado area in Goiás Brazil. Semina Ciên Agrár 37(2):611–623. https://doi.org/10.5433/1679-0359.2016v37n2p611

Hedley MJ, Stewart JWB, Chauhan BS (1982) Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci Soc Am J 46:970–976. https://doi.org/10.2136/sssaj1982.03615995004600050017x

IBGE (2016) Produção da Extração Vegetal e da Silvicultura. https://biblioteca.ibge.gov.br/ visualização /periodicos/74/pevs_2016_v31.pdf. Accessed 15 Sep 2018.

IPARDES (2019) Caderno Estatístico: município de Presidente Castelo Branco. https://www.ipardes.gov.br/cadernos/MontaCadPdf1.php?Municipio=87180. Accessed 16 Oct 2019.

Lemming C, Oberson A, Magid J, Bruun S, Scheutz C, Frossard E, Jensen LS (2019) Residual phosphorus availability after long-term soil application of organic waste. Agr Ecosyst Environ 270:65–75. https://doi.org/10.1016/j.agee.2018.10.009

Li G, Li H, Leffelaar PA, Shen J, Zhang F (2014) Characterization of phosphorus in animal manures collected from three (dairy, swine, and broiler) farms in China. PLoS ONE 9:1–8. https://doi.org/10.1371/journal.pone.0102698

Liu XJA, Sun J, Mau RL, Finley BK, Compson ZG, Van GN, Brown JR, Schwartz E, Dijkstra P, Hungate BA (2017) Labile carbon input determines the direction and magnitude of the priming effect. Appl Soil Ecol 109:7–13. https://doi.org/10.1016/j.apsoil.2016.10.002

Lourenzi CR, Ceretta CA, Cerini JB, Ferreira PAA, Lorensini F, Girotto E, Tiecher TL, Schapanski DE, Brunetto G (2014) Available content, surface runoff and leaching of phosphorus forms in a Typic Hapludalf treated with organic and mineral nutrient sources. R Bras Ci Solo 38:544–556. https://doi.org/10.1590/S0100-06832014000200019

Lourenzi CR, Ceretta CA, Tiecher TL, Lorensini F, Cancian A, Stefanello L, Girotto E, Vieira RCB, Ferreira PAA, Brunetto G (2015) Forms of phosphorus transfer in runoff under no-tillage in a soil treated with successive swine effluents applications. Environ Monit Assess 187(209):1–16. https://doi.org/10.1007/s10661-015-4437-2

MAPA (2018) Estatística de Comércio Exterior do Agronegócio Brasileiro-AGROSTAT. https://indicadores.agricultura.gov.br/agrostat/index.htm. Accessed 15 Sep 2018.

Mackay JE, Macdonald LM, Smernik RJ, Cavagnaro TR (2017) Organic amendments as phosphorus fertilisers: Chemical analyses, biological process and plant P uptake. Soil Biol Biochem 107:50–59. https://doi.org/10.1016/j.soilbio.2016.12.008

Ma X, Ambus P, Wang S, Wang Y, Wang C (2013) Priming of soil carbon decomposition in two inner Mongolia grassland soils following sheep dung addition: a study using ^{13}C natural abundance approach. PLoS ONE 8(11):1–9. https://doi.org/10.1371/journal.pone.0078578

Menezes JFS, Silva MP, Caetano JO, Cantão VCG, Benites MB (2017) Maize yield after long-term application of pig slurry. Rev Bras Eng Agrí 21:686–690. https://doi.org/10.1590/1807-1929/agriambi.v21n10p686-690

Missong A, Holzmann S, Bol R, Nischwitz V, Puhlmann H, von Wilpert K, Siemens J, Klumpp E (2018) Leaching of natural colloids from forest topsoils and their relevance for phosphorus mobility. Sci Total Environ 634:305–315

Moreno-Garcia B, Guillen M, Quilez D (2017) Response of paddy rice to fertilization with pig slurry in northeast Spain: strategies to optimize nitrogen use efficiency. Field Crop Res 208:44–54. https://doi.org/10.1016/j.fcr.2017.01.023

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36

Oliveira Filho JS, Pereira MG, Aquino BF, Viana TVA (2017) Phosphorus forms and adsorption in a typic Quartzipsamment cultivated with sugarcane harvested without burning. Rev Caatinga 30:343–352. https://doi.org/10.1590/1983-21252017v30n209rc

Oliveira Filho JS, Viana TVA, Azevedo BM, Sousa GG, Pereira MG (2019) Semina: Ciên. Agrár. 40:2017–2120. https://doi.org/10.5433/1679-0359.2019v40n5Supl1p2107

Penha HGV, Menezes JFS, Silva CA, Lopes G, Carvalho CA, Ramos SJ, Guilherme LRG (2015) Nutrient accumulation and availability and crop yields following long-term application of pig slurry in a Brazilian Cerrado soil. Nutr Cycl Agroecosys 101:259–269. https://doi.org/10.1007/s10705-015-9677-6

R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rigo AZ, Corrêa JC, Mafra AL, Hentz P, Grohskopf MA, Gatiboni LC, Bedendo G (2019) Rev Bras Cienc Solo 43:1–15. https://doi.org/10.1590/18069657rbcs20180130

Rosset SR, Guareschi RF, Pinto LASR, Pereira MG, Lana MC (2016) Phosphorus fractions and correlation with soil attributes in a chronosequence of agricultural under no-tillage. Semina Ciên Agrár 37(6):3915–3926. https://doi.org/10.5433/1679-0359.2016v37n6p3915

Rossi CQ, Pereira MG, Gazolla PR, Perin A, Gonzalez AP (2016) Organic phosphorus fractions in the soil in chronosequence of sugarcane crops managed with pre-harvest burning in the cerrado biome of the state of Goias, Brazil. Biosci J 32:436–445. https://doi.org/10.14393/BJ-v32n2a2016-22245

Santin D, Benedetti LB, Barros NF, Fontes LL, Almeida IC, Neves JCL, Wendling I (2017) Harvest handling and phosphate nutrition in yerba-mate (Ilex paraguariensis) culture during production stage. Ciência Florestal 27:783–797. https://doi.org/10.5902/1980509828629

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumbreras JF, Coelho MR, Almeida JA, Araujo Filho JC, Oliveira JB, Cunha TJF (2018) Sistema Brasileiro de Classificação de Solos, 5th edn. Embrapa, Brasília

Spain AV, Tibbett M, Ridd M, McLaren TI (2018) Phosphorus dynamics in a tropical forest soil restored after strip mining. Plant Soil 427:105–123. https://doi.org/10.1007/s11104-018-3668-8

Sun M, Jaisi DP (2018) Distribution of inositol phosphates in animal feed grains and excreta: distinctions among isomers and phosphate oxygen isotope compositions. Plant Soil 430:291–305. https://doi.org/10.1007/s11104-018-3723-5

Tian J, Wei K, Condron LM, Chen Z, Xu Z, Chen L (2016) Impact of land use and nutrient addition on phosphatase activities and their relationships with organic phosphorus turnover in semi-arid grassland soils. Biol Fertil Soils 52:675–683. https://doi.org/10.1007/s00374-016-1110-z

Tiecher T, Zafar M, Mallmann FJK, Bortoluzzi EC, Bender MA, Ciotti LH, Santos DR (2014) Animal manure phosphorus characterization by sequential chemical fractionation, release kinetics and 31P-NMR analysis. Rev Bras Ciênc Solo 38:1506–1514. https://doi.org/10.1590/S0100-06832014000500016

Tiecher T, Tiecher TL, Mallmann FJK, Zafar M, Ceretta CA, Lourenzi CR, Brunetto G, Gatiboni LC, Santos DR (2017) Chemical, biological, and biochemical parameters of the soil P cycle after long-term pig slurry application in no-tillage system. Rev Bras Ciênc Solo 41:1–16. https://doi.org/10.1590/18069657rbcs20170037

Turrión MB, Bueis T, Lafuente F, López O, San José E, Eleftheriadis A, Mulas R (2018) Effects on soil phosphorus dynamics of municipal solid waste compost addition to a burnt and unburnt forest soil. Sci Total Environ 642:374–382. https://doi.org/10.1016/j.scitotenv.2018.06.051

Wang J, Wu Y, Zhou J, Bing H, Sun H (2016) Carbon demand drives microbial mineralization of organic phosphorus during the early stage of soil development. Biol Fertil Soils 52:825–839. https://doi.org/10.1007/s00374-016-1123-7

Weihrauch C, Opp C (2018) Ecologically relevant phosphorus pools in soils and their dynamics: the story so far. Geoderma 325:183–194. https://doi.org/10.1016/j.geoderma.2018.02.047

Wienhold BJ, Miller PS (2004) Phosphorus fractionation in manure from swine fed traditional and low-phytate corn diets. J Environ Qual 33:389–393. https://doi.org/10.2134/jeq2004.3890

Withers PJA, Rodrigues M, Soltangheisi A, Carvalho TS, Guilherme LRG, Benites VM, Gatiboni LC, Sousa DMG, Nunes RS, Rosolem CA, Andreote FD, Oliveira A Jr, Coutinho ELM, Pavinato PS (2018) Transitions to sustainable management of phosphorus in Brazilian agriculture. Sci Rep 8:2537. https://doi.org/10.1038/s41598-018-20887-z

Yang Y, Zhang H, Qian X, Duan J, Wang G (2017) Excessive application of pig manure increases the risk of P loss in calcic cinnamon soil in China. Sci Total Environ 609:102–108. https://doi.org/10.1016/j.scitotenv.2017.07.149

Yan X, Wei Z, Hong Q, Lu Z, Wu J (2017) Phosphorus fractions and sorption characteristics in a subtropical paddy soil as influenced by fertilizer sources. Geoderma 295:80–85. https://doi.org/10.1016/j.geoderma.2017.02.012

Yan Z, Chen S, Li J, Alva A, Chen Q (2016) Manure and nitrogen application enhances soil phosphorus mobility in calcareous soil in greenhouses. J Environ Manag 18:26–35. https://doi.org/10.1016/j.jenvman.2016.05.081

Ye D, Li T, Yu H, Chen G, Zheng XZZ (2015) P accumulation of *Polygonum hydropiper*, soil P fractions and phosphatase activity as affected by swine manure. Appl Soil Ecol 86:10–18. https://doi.org/10.1016/j.apsoil.2014.10.002

Zhang L, Ding X, Peng Y, George TS, Feng G (2018) Closing the loop on phosphorus loss from intensive agricultural soil: a microbial immobilization solution? Front. Microbiol 9:104. https://doi.org/10.3389/fmicb.2018.00104

Zederer DP, Talkner U (2018) Organic P in temperate forest mineral soils as affected by humus from and mineralogical characteristics and its relationship to the foliar P content of European beech. Geoderma 325:162–171. https://doi.org/10.1016/j.geoderma.2018.03.033