Tillage systems and cover crops affecting soil phosphorus bioavailability in Brazilian Cerrado Oxisols

Marcos Rodrigues[a], Paul John Anthony Withers[b], Amin Soltangheisi[c], Vitor Vargas[d], Marquel Holzschuh[d], Paulo Sergio Pavinato[a,*]

[a] University of São Paulo, College of Agriculture "Luiz de Queiroz", Soil Science Department. Av. Pádua Dias 11, Box 9, Zip code 13418-900 Piracicaba, SP, Brazil.

[b] Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK.

[c] Center for Nuclear Energy in Agriculture (CENA)- University of Sao Paulo (USP)- Av. Centenário 303 - São Dimas, Zip Code 13400-970, Piracicaba – SP.

[d] SLC Agrícola, Rua Bernardo Pires, 128, 4º andar. Bairro Santana. Zip code 90.620.010. Porto Alegre, RS.

*Corresponding author. E-mail: pavinato@usp.br

Abstract

Crop rotation, cover crops introduction and no tillage adoption have improved tropical agriculture sustainability through improvements on soil conservation and water use efficiency. Soil fertility and topsoil phosphorus (P) accumulation is also altered by management, affecting P dynamics and its use for subsequent cash crops. Changes in soil P fractions promoted by no-tillage (NT) and conventional tillage (CT) in soybean/cotton crop systems with different cover crop rotations (fallow, maize as second crop, brachiaria and millet) were investigated in two long-term trials in Brazilian Oxisols (Ox-1 and Ox-2), and compared to soils under native Cerrado vegetation. Hedley's P fractionation was performed in soil samples taken from 0-5, 5-10 and 10-20 cm depth layers and P fractions grouped by their predicted lability. Long-term cultivation generated large amounts of legacy P in the soil (184-341 mg kg^{-1}) but only a small portion remained in labile fractions (11-16%), with a slight increase in non-labile P (<5%) and organic P (10-20%) concentrations under NT when compared to CT. Although the soil P remained mostly in less available fractions, the legacy P obtained by the difference between the soil P data from the agricultural land and the native area provided a useful approach for P accumulation estimative over the time, very close to the predicted P inputs/outputs accounting. Brachiaria recycled more P than other cover crops, increasing the labile P (5-20%) and all the organic P fractions (10-25%) over the time.

Keywords: phosphorus dynamics; Hedley P fractionation; long-term cultivation; brachiaria; millet.

1 Introduction

The expansion of agriculture in the tropical regions such as Brazilian Cerrado has led to a high P fertilizer demand due to the majority of P-fixing soils (Lopes and Guilherme, 2016; Roy et al., 2016). Moreover, soil P availability has increased slowly, as the vast majority of added fertilizer P is rapidly adsorbed onto the surface of Fe and Al (hydr)oxides (Novais et al., 2007; Rodrigues et al., 2016; Roy et al., 2016; Sousa and Lobato, 2004). The high P inputs for payment of the soil P fixation, although a commonly adopted strategy in tropical agriculture, will pressure national P demands (Withers et al., 2018) and may not be profitable to improve the P use efficiency in the future (Rowe et al., 2016; Withers et al., 2014), especially in the tropics (Roy et al., 2016).

Rotation systems with less soil disturbance have shown positive effects on soil P bioavailability (Rodrigues et al., 2016; Tiecher et al., 2012a, 2012b). Also, different plants have evolved biochemical, physiological, and structural mechanisms to exploit less labile soil P fractions, including mycorrhizal associations, root morphology adaptation and modifications in rhizosphere biochemistry through exudation of low molecular-weight organic acids, phosphatases, phytases and protons (Hinsinger et al., 2011; Lambers et al., 2015). Cover crops have positive effects on soil conservation by covering the soil surface from erosion processes (Calegari et al., 2013), but also contributes to soil P recycling by accumulating P in their roots and shoots (Boer et al., 2007; Tiecher et al., 2012a). This enhanced P bioavailability and storage in degradable organic P fractions (Soltangheisi et al., 2017; Teles et al., 2017; Tiecher et al., 2012a) may improve the yield of subsequent cash crops (Calegari et al., 2013; Carvalho et al., 2011). Moreover, continuous cropping increases root density, resulting in greater exudation of organic compounds, and organic P (Po) accumulation in the soil, which modulates P bioavailability in tropical soils such as Oxisols (Cross and Schlesinger, 1995). The Po fraction is less susceptible to strong adsorption on functional groups of Fe and Al (hydr)oxides compared to inorganic forms (Guppy et al., 2005; Hinsinger et al., 2011; Pavinato and Rosolem, 2008).

Soil P fractions have been successfully grouped and quantified (although not identified) by sequential chemical extractions, with the widely used Hedley's P fractionation procedure, by its ability to concomitantly determine Pi and Po fractions (Hedley et al., 1982). Despite several analytical limitations of this procedure (Condron and Newman, 2011), the conceptual P lability distribution predicted by the sequential reagents in the Hedley's P fractionation is considered useful for interpreting the soil P dynamics (Cross and Schlesinger, 1995), investigating the fate of native and applied P in agroecosystems (Negassa and Leinweber, 2009) and quantifying the P lability i.e., the P distribution in different fractions according to their potential to supply plants and the soil biota (Yang and Post, 2011).

In a previous study by the present authors (Rodrigues et al., 2016) it was observed a significant accumulation of all P fractions in the soil after decades of Cerrado cultivation due to high P fertilizer inputs (usually applied as twice as the offtakes). Although the observed P use efficiency was over 55%, these inputs still generating a significant amount of residual or legacy soil P (Haygarth et al., 2014; Withers et al., 2015). This legacy soil P is a relevant source of secondary P that can be utilised in a potential future scenario of P scarcity (Sattari et al., 2012; Withers et al.,2014; Rowe et al., 2016). In the soybean/cotton succession system typical of the Brazilian Cerrado, this legacy could supply P for decades of cultivation, buffering the impact of a sharp increase in the price of P fertilizer (Rodrigues et al., 2016, Withers et al., 2018). However, whether and how this sorbed P could be accessed by plants and how much of the residual P in tropical systems is potentially plant available is largely unknown.

The present study was established with the hypothesis that the introduction of no-tillage (NT) and cover crops on the soybean/cotton rotation increases P availability on the soil surface and potentially reduces P fixation into non-labile fractions. Changes in soil P fractions promoted by NT and conventional tillage (CT) in soybean/cotton crop systems with different cover crop rotations were investigated in two long-term trials in Brazilian Oxisols (Ox-1 and Ox-2), and compared to soils under native Cerrado vegetation.

2 Material and methods

2.1 Study sites description and experimental design

Two long-term field experiments established in the Brazilian Cerrado region, under soybean/cotton rotation system (with different tillage systems and cover crops during the winter season) were selected for the present study. Oxisol 1 (Ox-1) is located in Costa Rica, Mato Grosso do Sul (08º15'10" S; 03º12'41" W and alt. 790 m), with annual mean temperature of 24.8 ºC and mean precipitation of 1,950 mm. Both sites have soil classified as Typic Hapludox (Soil Survey Staff, 2014). Oxisol 2 (Ox-2) is located in Sapezal, Mato Grosso (13º56'33" S; 58º53'43" W and alt. 640 m), with annual mean temperature of 23.5 ºC and mean precipitation of 2,150 mm.

At Ox-1 site, the deforestation was in 1974 and soybean was grown under a conventional tillage system until 1993. At that time, NT and conventional tillage (CT) as main plots were established. In 2004, the cover crop treatments (fallow, millet, brachiaria and maize as second crop) were established after every soybean cultivation. The soybean was cultivated on the field trial at the even years, since 2004, until 2016 (sampling date). Cotton was cultivated in the odds seasons since 2005, until 2015 (five crop seasons until the sampling date).

At Ox-2 site, the deforestation occurred in 1997 and soybean was cultivated under CT for three consecutive seasons. In 2001, the CT and NT main plots were established. After the soybean harvesting in 2005, the same cover crop treatments used for Ox-1 were established and used after every soybean cultivation. The soybean was cultivated since 2006, until 2014. Cotton was cultivated in the even's seasons since 2006, until 2016 (six crop seasons until the sampling date).

The experimental design adopted at both sites was randomized blocks within a 2 x 4 split-plot distribution, with three replicates for each treatment. The tillage systems (CT and NT) were the main plots and the subplots consisted of the following four cover crops: fallow (no cover, with chemical weed control), millet (*Pennisetum glaucum* L.), brachiaria (*Brachiaria ruziziensis, syn. Urochloa ruziziensis)* and maize (*Zea mays* L.) as second crop. Each sub-plot had dimensions of 9.6 x 110 m and 8.3 x 107 m (width x length) for Ox-1 and Ox-2 respectively. The CT system used disk plough (twice annually) before the summer crop sowing, whereas in the NT system the soil was not disturbed (except for sowing operations), and the crop residues were chemically terminated (for weed control on the fallow, millet and brachiaria treatments, glyphosate was applied on the dosage of 3 L ha^{-1}; for maize the grains were harvested and the cover was only the straw) and left on the soil surface.

2.2 Soil sampling and chemical analysis

Composite samples (4 sub-samples in each plot) in the layers 0-10 and 10-20 cm from field trials and from native vegetation were collected for soil chemical and mineralogical characterization (Table 1). Samples were very well mixed and 500 g of each layer/location was brought to the lab for analysis. Accordingly, soil pH determined by $CaCl_2$, and organic matter content (SOM) determined by Walkley & Black method, were both increased by cultivation, especially in the top 0-10 cm layer, with also higher CEC in 0-10 cm than in 10-20 cm. As a general mineralogy evaluation, the amorphous/crystalline Fe ratio ($Fe_{Oxalate}$/Fe_{DCB}, Table 1) was lower than 0.05 (0.036 and 0.037 for Ox-1 and Ox-2 respectively), demonstrating that in both soils the crystalline oxides are predominant.

X-Ray diffraction (DRX) analysis of the clay fraction was performed according to the method described by Jackson (1969), but modified by saturating the clay fraction with 1 M KCl or 1 M $MgCl_2$ after Fe-oxide removal to identify the phyllosilicates. The following post-treatments were applied: K saturation at room temperature (25 °C); K saturation and heating up to 300 °C; K saturation and heating up to 500 °C; Mg saturation at room temperature (25 °C); Mg saturation with solvent ethylene glycol. Another sample without removing Fe was prepared to identify the Fe oxides present in the soils, followed by the oriented glass slide preparation before analysis. The diffractograms were collected in a Miniflex II Desktop X-Ray Diffractometer (RIGAKU), CuKα radiation, analysis interval from 10 to 50 °2Θ to the clay fraction without Fe samples and 5 to 30 °2Θ to the samples of clay+Fe. The results (Figures S1 and S2) clearly show the dominance of kaolinite as the only phyllosilicate, hematite and goethite as the Fe oxides and gibbsite as the Al hydroxide in both study sites. These minerals will be subsequently referred as Al and Fe oxides in the following sections.

For P fractionation analysis, the soil samples were collected after termination of the cover crops in August 2015 for Ox-2 site and in August 2016 for Ox-1 site. Samples were collected at 0-5, 5-10 and 10-20 cm depths using a handling trowel. Four trenches were opened in each sub-plot and were mixed to form a composite sample (around 500 g per composite sample) for each layer in each treatment. Additionally, for sampling in the adjacent Cerrado native areas, four sites around 30 m distance from each other and at least 30 m from the forest edge were delimited, and subsamples were collected from each site and mixed to obtain a composite sample. Samples were air dried and sieved (2 mm) prior to sequential P fractionation according to the method proposed by Hedley et al. (1982) with modifications by Condron et al. (1985).

The sequential extraction order and interpretation was based on the chemical extractants related P studies (Tiessen and Moir, 1993; Cross and Schlesinger, 1995; Gatiboni et al., 2007;

Condron and Newman, 2011). Soil P fractions were grouped according to their lability predicted by each extractant. Labile P included P_{AER}, Pi_{Bic} and Po_{Bic}; moderately labile P included $P_{Hid\text{-}0.1}$ (Pi and Po) and P_{HCl}, and non-labile P included $P_{Hid\text{-}0.5}$ (Pi and Po) and $P_{Residual}$.

In order to evaluate the cumulative legacy soil P present in the soil since deforestation, P fractionation was also performed on native Cerrado soil samples and the values subtracted from the cultivated plots. Thus, the net P values were evaluated in relation to their distribution in labile, moderately labile and non-labile P. The mean annual P fertilizer input since deforestation was estimated at 37.2 kg P ha^{-1} yr^{-1} in Ox-1 and 44.8 kg P ha^{-1} yr^{-1} in Ox-2, whereas the mean annual offtake of P by the cash crops across tilled plots were 15.2 and 13.1 kg P ha^{-1} yr^{-1} respectively. Annual inputs and outputs are described (Table S1). This balance left a net annual P surplus of 22.0 and 31.7 kg P ha^{-1} yr^{-1} respectively.

2.3 Statistical analysis

All the data on P fractions, P lability and legacy P pools were analysed to verify their normality (Shapiro-Wilk test) and homoscedasticity (Bartlett test). After checking these statistical assumptions, a two-way analysis of variance (ANOVA) was performed considering the randomized blocks within the split-plot design, where the soil cultivation systems (NT and CT) were considered as main plots and the cover crops (fallow, millet, brachiaria and maize) as subplots. When the treatment effect was significant by F-test ($p<0.05$), the means were compared by the Tukey's test ($p<0.05$). When the interaction effect was significant by F-test ($p<0.05$), means of cover crops were compared inside each tillage system by Tukey's test ($p<0.05$). Similar means comparisons were carried out to tillage effects inside each cover crop. All the statistical analyses were performed in the R Environment v.3.4 (R Core Team, 2017) and the split-plot design ANOVA and Tukey's test were performed using the ExpDes R package (Ferreira et al., 2013).

3 Results

3.1 Inorganic and organic P fractions

Observed values in P fractions within the labile, moderately labile and non-labile pools at 0-5 and 5-10 cm depths were similar between soil managements and/or cover crops, in both Ox-1 and Ox-2 (Tables S2 and S3). In order to simplify and clarify the effects of tillage systems and cover crops, these two layers were averaged to 0-10 cm and then analysed. The P_{AER} levels (Table 2) in the topsoil (0-10 cm) were affected by the interaction between tillage system and cover crop at both sites (Tables S2 and S3). At Ox-1, fallow under NT resulted in 44 and 50% higher P_{AER} compared to the CT and the average of other cover crops, respectively, whereas at Ox-2 it was brachiaria the cover crop which increased P_{AER} levels under NT. Under CT at Ox-2, maize maintained the highest P_{AER} level in the topsoil, which was 55, 54 and 33% higher than under fallow, millet, and brachiaria, respectively.

Tillage system and cover crop did not affect Pi_{Bic} and Po_{Bic} fractions in Ox-1 in either 0-10 and 10-20 cm layers (Table 2). At Ox-2, significant effects on Pi_{Bic} were observed only under brachiaria in NT system, which increased by 57 and 81% in 0-10 and 10-20 cm respectively, compared to other cover crops. Under CT, the positive effect of brachiaria was observed only in 10-20 cm layer, being significantly higher than fallow and millet, but not statistically different from maize. The Po_{Bic} concentrations were not affected by cover crops, but there was 47% more Po_{Bic} in CT than in NT in 10-20 cm layer at Ox-2.

At Ox-1, mod-labile $Pi_{Hid0.1}$ was reduced by 11, 13 and 21% under millet, maize and brachiaria, respectively, when compared to fallow in 0-10 cm (Table 3). There was an interaction between tillage × cover crop in $Pi_{Hid0.1}$ at Ox-2 (Table 3), whereas under NT brachiaria increased this fraction by 35% compared to the other cover crops, while under CT maize increased $Pi_{Hid0.1}$ by 28% relative to the other crops. At both sites, $Pi_{Hid-0.1}$ was not affected by either tillage systems or cover crops at 10-20 cm.

At Ox-1, brachiaria enhanced $Po_{Hid-0.1}$ by 28% compared to the other cover crops in the layer 0-10 cm, without any isolated effect of management, similarly 22% more $Po_{Hid-0.1}$ was detected in the layer 10-20 cm (Table 3). For Ox-2 brachiaria also changed this $Po_{Hid-0.1}$ fraction but only in the 10-20 cm layer and under NT system. Tillage system had a major effect on $Po_{Hid-0.1}$ levels at Ox2, with considerably higher values (+109%) under NT than under CT. Since the P_{HCl} fraction contributed the lowest (<1%) portion of total P at both sites (Table 3), and did not show any difference between either the cover crops or tillage systems, the results for this fraction were not statistically evaluated neither discussed.

Tillage systems x cover crops interaction did not affect $Pi_{Hid0.5}$, $Po_{Hid0.5}$ or residual P fractions at both sites and both depths, 0-10 and 10-20 cm (Table 4). At Ox-1, cover crops did not have any significant effect and the tillage system only slightly changed $Po_{Hid0.5}$ in the 0-10 cm, where NT increased $Po_{Hid0.5}$ by 29% compared to CT. Cover crop only affected $Pi_{Hid0.5}$ in Ox-2, with increments in this fraction of around 20 and 23%, respectively for maize and brachiaria in relation to fallow. Moreover, the $P_{Residual}$ was either not affected by the tillage systems and cover crops, for both sites evaluated (Table 4), ranging from 36 to 46% of the total P. About cover crop effect, brachiaria reduced slightly the proportion of $P_{Residual}$ (2% in site Ox-1 and 4% in site Ox-2), but only in 10-20 cm depth layer. This effect was not related to $P_{Residual}$ depletion but mostly due to the increment in other P fractions as consequence of brachiaria cultivation.

3.2 Phosphorus lability

Labile P (P_{AER} + Pi_{Bic} and Po_{Bic}) in top 0-10 cm was not significantly affected by tillage system or cover crop at site Ox-1 (Figure 1A). However, in the 10-20 cm layer, brachiaria enhanced labile P by 17% compared to fallow. At site Ox-2, interactions between tillage system × cover crop influenced labile P at both depths (Figure 2A). In 0-10 cm, labile P increased by 22-35% when brachiaria was cultivated under NT compared to other crops. In the CT system, only maize improved labile P. In the 10-20 cm layer, labile P with brachiaria and fallow were similar under NT, but maize, brachiaria and millet increased labile P by 59, 30, and 18%, respectively, when compared to fallow under CT.

A tillage system × cover crop interaction significantly influenced moderately labile P in the 0-10 cm, what was observed in both sites (Figures 1B, 2B). Under CT, fallow and maize increased mod-labile P compared to brachiaria and millet at site Ox-2 (Figure 2B), while no cover crop effect was observed under CT at site Ox-1 (Figure 1B). Under NT, millet, brachiaria and fallow showed more mod-labile P at Ox-1 (Figure 1B), whereas at Ox-2 fallow and brachiaria increased mod-labile P more than other cover crops (Figure 2B). Significant effects of brachiaria under NT were observed in the 10-20 cm layer at Ox-2, with 39-59% more mod-labile P than other cover crops. There was 41% more mod-labile P under NT than under CT (Figure 2B).

Non-labile P was not influenced by the tillage system nor by the cover crop species at Ox-1 (Figure 1C). Nevertheless, at Ox-2 some of the cover crops slightly increased non-labile P by 9-12% and 6-8% compared to fallow in the 0-10 and 10-20 cm layers, respectively (Figure 2C). A significant effect in total P content at Ox-1 was only observed in the topsoil (0-10 cm) (Figure 1D). Cover crops did not change total P, but NT under fallow and millet resulted in higher total P compared to CT system. At Ox-2, a small interaction between tillage system × cover crop was observed at both depths (Figure 2D). Overall, brachiaria under NT increased total P by around 12 and 19% in the layers 0-10 and 10-20 cm respectively, whereas under CT cover crop did not change total P levels in either soil layers.

3.3 Legacy phosphorus

The soil legacy P that had accumulated at both sites since deforestation was obtained by estimating the difference between total P in the agricultural treatments (tillage systems and cover crops) and the native Cerrado areas (Table S4). Labile, moderately labile and non-labile P pools were also calculated across the sampled 0-20 cm depth in cultivated areas and native vegetation. Considering the P fertilizer inputs and the annual offtakes in each site since deforestation, the expected total soil legacy P was around 924 and 527 kg ha^{-1} for Ox-1 and Ox-2 respectively (Table 5). The average legacy P determined by the P fractionation (0-20 cm soil depth) was 333.9 (±20) in Ox-1 (Figure 3A) and 182.9 (±15) mg kg^{-1} in Ox-2 (Figure 3B).

In order to verify the ability of the cover crops to prevent or enhance the legacy P, each separated pool was also analysed. Cover crops did not alter the lability of legacy P at Ox-1, as measured by the distribution of labile, moderately labile (Figure 3C) and non-labile (Figure 3E) pools. However, NT increased the non-labile portion of legacy P by 16% (Figure 3E). At Ox-2, brachiaria enhanced the legacy P from 44-51% under NT, whereas under CT both brachiaria and maize resulted in higher legacy P, 25 and 43% higher than fallow respectively (Figure 3B). Overall, both the labile and moderately labile net P balances were increased by brachiaria cultivation under NT in Ox-2 (Figure 3D). Nevertheless, the non-labile P was enhanced by 36-43% by cover crops in comparison to the fallow, with no differences between maize, millet and brachiaria (Figure 3F).

4 Discussion

4.1 Tillage and cover crop effects on P fractions and lability

The 0-5 and 5-10 cm results were averaged over 10 cm as they showed similar behaviour, similar to previous studies evaluating the effects of tillage system in soils (Rodrigues et al., 2016; Vieira et al., 2016). However, other studies evaluating the effect of cover crops on

P availability have reported higher P accumulation in the 0-5 cm depth, with small or no influence in deeper layers (Soltangheisi et al., 2017; Tiecher et al., 2012a, 2012b). There was a significant accumulation of SOM at the surface in those studies in response to the long-term deposition of organic residues, besides P fertilizer being broadcasted on the soil surface. In our study, SOM levels were not very different between the 0-5 and 5-10 cm layers (Table 1) and P fertilizer was applied in the sowing furrow (7-10 cm deep), suggesting that it was appropriate to consider the mean 0-10 cm for detection of fertilizer residual in the soil.

At both study sites, higher P_{AER} levels were observed in fallow under NT compared to CT, with a negative effect of cover crops under NT at Ox1 (Table 2). Resin removes just the amount of P readily-available to plants (Gatiboni et al., 2007; Hedley et al., 1982), and the soils were sampled immediately after cover crops termination, this reflects the recent uptake of P_{AER} by the cover crops, what was not detected in fallow under NT (long term effect). Thus, since the cover crops accumulate P into their tissues (roots and shoots) and the soil was sampled immediately after cover crops termination, the P recycling capacity of cover crops was underestimated here, i.e, the strong P adsorption and storage as non-labile P (Gatiboni et al., 2007; Rheinheimer et al., 2008) by P stocking in the soil as labile and moderately labile fractions (Boer et al., 2007; Calegari et al., 2013; Carvalho et al., 2014).

The use of brachiaria notably increased P_{AER} and Po_{Bic} at Ox-2 (Table 2). This crop has previously shown in intensification systems in Brazil that is able to improve soil P availability over the time (Almeida and Rosolem, 2016), because of its capacity to exudate organic acids or stimulate microbial enzyme activity around the roots (Jones, 1998; McLaughlin et al., 2011). The Po mineralization, by higher microbial and phosphatase enzyme activities also contribute to increase the labile P fraction (Louw-Gaume et al., 2010). This was observed here by decreasing Po_{Bic} fraction and, consequently, increasing labile Pi fractions (P_{AER} and Pi_{BIC}) in Ox-2, reinforcing that brachiaria is a useful cover crop for improving plant-available P in long-term cultivation systems, or potentially digging up P from deeper layers to the soil surface (Almeida et al., 2019).

As expected from many years of intensive cultivation with high fertilizer P inputs (surplus of 21.0 and 29.7 kg ha^{-1} yr^{-1} for Ox-1 and Ox-2 respectively), P_{AER} levels were above the agronomically-optimum critical level of 15 mg P kg^{-1} soil, adopted as the standard in Brazil's central region for the resin method (Sousa and Lobato, 2004). The increase in labile P (P_{AER} + Pi_{Bic} and Po_{Bic}) relative to the native area was equivalent to 80.4 and 59.0 kg ha^{-1} (considering labile P at 0-20 cm, related to the labile P in the native area), which means an annual addition of 0.91 and 1.64 mg kg^{-1} of P in the soil. Considering only P_{AER}, it was increased by an average of 0.48 and 1.03 mg kg^{-1} of P in the soil for Ox-1 and Ox-2, respectively. Hence the critical soil P level was achieved after approximately 24 years in the Ox-1, but after only 3.5 years at Ox-2 because of the higher rate of legacy labile P accumulation and the higher P_{AER} in the native vegetation related to a lower clay content in Ox-2 (Table 1).

The $Po_{Hid0.1}$ fraction can prove the ability of the brachiaria as cover crop in changing soil P dynamics, storing more organic P. However, this effect was more pronounced in site Ox-1, which explain the Po accumulation in this fraction, related to the organic P stored onto fulvic and humic acids adsorbed onto mineral and SOM surfaces (Linquist et al., 1997). In site Ox-2, Po accumulation was also detected, but only with brachiaria cultivation and when NT was adopted, evidencing the benefits promoted by the brachiaria introduction in NT rotation systems. Brachiaria is capable of recycling and storage P into its root system, relating this crop as a high potential to explore more soil P (Boddey et al., 1996; Almeida et al., 2019), leading to a higher plant P uptake and contributing to recycle this nutrient. Almeida and Rosolem (2016) reported a significant contribution of brachiaria in the labile P (P_{AER} + total P_{BIC}), indicating a relevant labile Po transformations into Pi, contributing to increase the plant P availability. Otherwise, Po_{Bic} and $Po_{Hid0.1}$ (labile and moderately labile organic P) showed similar trends to the ones observed by Tiecher et al. (2012a). Moreover, under CT system, this effect was also observed, but in deeper layers (10-20 cm), rather than restricted surface (0-10 cm) effects observed in NT.

Tillage effects were most pronounced for the $Po_{Hid0.5}$ fraction (non-labile organic P), with NT showing generally more organic P accumulation than CT at Ox1. At Ox-2, no effect of tillage system was observed in either the Pi or the Po extracted by 0.5 M NaOH. The residual P ($P_{Residual}$) was not affected by either tillage systems or cover crops in both study sites, similar to other reports (Rodrigues et al., 2016; Tiecher et al., 2012a, 2012b). The $P_{Residual}$ is considered an occluded P form with high P binding strength and low reversibility. In tropical soils, $P_{Residual}$ represents the majority of P present (Cherubin et al., 2016; Conte et al., 2003; Rodrigues et al., 2016; Tiecher et al., 2012b), and it is an important P fertilizer sink (Soltangheisi et al., 2017; Teles et al., 2017), leading to low PUE by crops. Although desirable, cover crops and tillage systems were not able to reduce the amount of P stored into this highly recalcitrant fraction ($P_{Residual}$).

4.2 Tillage and cover crops effects on legacy P bioavailability

Soil total P in the native Cerrado (Table 3S) in 0-5 cm was much higher than in the other layers, indicating the importance of plant P recycling in forests, especially in the tropics (Vincent et al., 2010). However, to estimate the legacy P and in order to compare the differences in the agricultural plots, this aspect was not taken in consideration in the present study and to avoid the tillage systems differences on P levels among the soil depths, only the average weighted 0-20 cm levels were taken into account.

Considering the average soil bulk density of 1.21 and 1.33 kg dm^{-3} for Ox-1 and Ox-2, respectively (Table 1), the total legacy P stock (in the 2000 dm^{-3} soil volume) calculated by the difference between the total P in the cultivated and native areas was 808.0 (±48.1) and 486.5

(±39.8) kg ha^{-1} in these respective sites, very close to the legacy P estimated by crop inputs and outputs (924 and 527 kg ha^{-1} for Ox-1 and Ox-2 respectively; Table 5 and Supplementary Table S1). The accumulated legacy P remaining in the soil amounted to 381.8 and 198.2 mg kg^{-1} for Ox-1 and Ox-2 respectively, which was 47.9 and 15.3 mg kg^{-1} higher than the legacy P predicted by the P fractionation for Ox-1 and Ox-2 respectively (which was 333.9 and 182.9 mg kg^{-1} respectively). This difference is probably related to the uncertainties involved in the first 20 and 4 years of cultivation for the Ox-1 and Ox-2, respectively, since the database was not available and estimations were according to the common rates adopted in the beginning of the soybean cultivation in the Cerrado region (Lopes and Guilherme, 2016; Sousa and Lobato, 2004). Also, new more productive varieties over the time may have interfered in this balance.

The Brazil's farmland in the central region has been managed at the expense of high agricultural inputs since the conversion from native Cerrado (Lopes and Guilherme, 2016), especially high P inputs to increase its availability, denominated as the soil 'P fixation tax' (Roy et al., 2016). This aspect is detached by the high P accumulation over the time (Table 5 and Figure 3A, 3B). Overall, a proportion of 48-51% and 47-57% of the legacy P was stored into non-labile P (Figure 3E, 3F) in the Ox-1 and Ox-2, respectively, reinforcing the high P input dependency (Roy et al., 2016), and consequently, the low P efficiency (Novais et al., 2007; Rodrigues et al., 2016; Withers et al., 2018) in tropical agricultural systems.

Despite the accumulation of the legacy P predominantly into the non-labile P fractions, the increase in labile and moderately labile legacy P promoted by cover crops in Ox-2 (Figure 3D), although modest, indicates the potential of these plants, especially brachiaria, in improving P supply for successive cash crops, consequently diminishing the P input rates (Rowe et al., 2016; Withers et al., 2014; Withers et al., 2018). This additional "source" of potentially available P can be estimated up to 50% (172 mg kg^{-1}, considering labile and moderately labile P) of the total legacy P in Ox-1. In Ox-2, this value ranged from 37% (61 mg kg^{-1}) under millet in CT, rising up to 60% (147 mg kg^{-1}) when brachiaria was cultivated under NT. It is well known the relevance of NT in enhancing P availability (Gatiboni et al., 2007; Rodrigues et al., 2016) and also the brachiaria effect increasing P lability, consequently preventing P fixation (Almeida and Rosolem, 2016), or even exploring less available P fractions (Merlin et al., 2016, Almeida et al., 2019).

Therefore, a large proportion of the legacy P may be considered as potentially bioavailable, being a supplementary P source for plants during periods of scarcity or when fertilizers may become prohibitively expensive. Based on the P pools affected by cover crops (Figure 3), we are suggesting that "bioavailable legacy P" could be measured as the amount of P fractions stored into labile (P_{AER} + Pi_{BIC} + Po_{BIC}) and moderately labile P pools ($Pi_{Hid0.1}$ + $Po_{Hid0.1}$ + P_{HCl}). Although positive effects of cover crops on non-labile Po were observed in the Ox-2, it is not considered as bioavailable in sustainable agricultural conditions. Although, how much and whether the moderately labile P can be used as a supplementary source of P by crops without yield penalties remains unclear and further investigations are required to support this affirmative.

5 Conclusions

A long cultivation period of up to 42 years generated a large amount of legacy P in the soil, but only a small portion of this legacy P remained in a labile form (11-16%), with a slight increase in non-labile P (<5%) under NT compared to CT. Although not all available, the legacy P obtained by the difference between the soil total P data from the agricultural land and the native area provided a useful approach for accumulation estimative over the time.

The use of brachiaria as a cover crop recycled more P to the soil than millet, maize or fallow, generating more total P and increasing labile P (5-20%) and all the organic P fractions

(10-25%) in the first 10 cm. As far as we could evaluate, moderately labile P may be accessed by some crops, although further investigations are necessary.

References

Almeida, D.S., Menezes-Blackburn, D., Zhang, H., Haygarth, P.M., Rosolem, C.A. 2019. Phosphorus availability and dynamics in soil affected by long-term ruzigrass cover crop. Geoderma. 337, 434–443. doi:10.1016/j.geoderma.2018.09.056

Almeida, D.S., Rosolem, C.A., 2016. Ruzigrass Grown in Rotation with Soybean Increases Soil Labile Phosphorus. Agron. J. 108, 2444. doi:10.2134/agronj2015.0478

Boddey, R., Rao, I.M., Thomas, R.J., 1996. Nutrient cycling and environmental impact of Brachiaria pastures, in: Miles, J., Maass, B.L., Do Valle, C.B. (Eds.), Brachiaria: Biology, Agronomy and Improvement. CIAT/EMBRAPA, Cali/Brasília, p.72–86.

Boer, C.A., Assis, R.L. de, Silva, G.P., Braz, A.J.B.P., Barroso, A.L. de L., Cargnelutti Filho, A., Pires, F.R., 2007. Ciclagem de nutrientes por plantas de cobertura na entressafra em um solo de cerrado. Pesqui. Agropecuária Bras. 42, 1269–1276.

Calegari, A., Tiecher, T., Hargrove, W.L., Ralisch, R., Tessier, D., de Tourdonnet, S., Guimarães, M. de F., Dos Santos, D.R., 2013. Long-term effect of different soil management systems and winter crops on soil acidity and vertical distribution of nutrients in a Brazilian Oxisol. Soil Tillage Res. 133, 32–39. doi:10.1016/j.still.2013.05.009

Carvalho, A.M. de, Bustamante, M.M. da C., Almondes, Z.A. do P., Figueiredo, C.C. de, 2014. Forms of phosphorus in an oxisol under different soil tillage systems and cover plants in rotation with maize. Rev. Bras. Ciência do Solo 38, 972–979. doi:10.1590/S0100-06832014000300029

Carvalho, A.M. de, Souza, L.L.P. de, Guimarães Júnior, R., Alves, P.C.A.C., Vivaldi, L.J., 2011. Cover plants with potential use for crop-livestock integrated systems in the Cerrado region. Pesqui. Agropecuária Bras. 46, 1200–1205. doi:10.1590/S0100-204X2011001000012

Cherubin, M.R., Franco, A.L.C., Cerri, C.E.P., Karlen, D.L., Pavinato, P.S., Rodrigues, M., Davies, C.A., Cerri, C.C., 2016. Phosphorus pools responses to land-use change for sugarcane expansion in weathered Brazilian soils. Geoderma 265, 27–38. doi:10.1016/j.geoderma.2015.11.017

Condron, L.M., Goh, K.M., Newman, R.H., 1985. Nature and distribution of soil phosphorus as revealed by a sequential extraction method followed by 31P nuclear magnetic resonance analysis. J. Soil Sci. 36, 199–207. doi:10.1111/j.1365-2389.1985.tb00324.x

Condron, L.M., Newman, S., 2011. Revisiting the fundamentals of phosphorus fractionation of sediments and soils. J. Soils Sediments 11, 830–840. doi:10.1007/s11368-011-0363-2

Conte, E., Anghinoni, I., Rheinheimer, D.S., 2003. Frações de fósforo acumuladas em Latossolo argiloso pela aplicação de fosfato no sistema plantio direto. Rev. Bras. Ciência do Solo 27, 893–900. doi:10.1590/S0100-06832003000500014

Cross, A.F., Schlesinger, W.H., 1995. A literature review and evaluation of the. Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. Geoderma 64, 197–214. doi:10.1016/0016-7061(94)00023-4

EMBRAPA, 1997. Manual de métodos de análise de solo. Centro Nacional de Pesquisa de Solos. 2.ed. rev. atual. – Rio de Janeiro, 212p. (EMBRAPA-CNPS. Documentos: 1)

Ferreira, E.B., Cavalcanti, P.P., Nogueira, D.A., 2013. Experimental Designs package.

Gatiboni, L.C., Kaminski, J., Rheinheimer, D. dos S., Flores, J.P.C., 2007. Biodisponibilidade de formas de fósforo acumuladas em solo sob sistema plantio direto. Rev. Bras. Ciência do Solo 31, 691–699. doi:10.1590/S0100-06832007000400010

Guppy, C.N., Menzies, N.W., Moody, P.W., Blamey, F.P.C., 2005. Competitive sorption reactions between phosphorus and organic matter in soil: A review. Aust. J. Soil Res. doi:10.1071/SR04049

Haygarth, P.M., Jarvie, H.P., Powers, S.M., Sharpley, A.N., Elser, J.J., Shen, J., Peterson, H.M., Chan, N.I., Howden, N.J.K., Burt, T., Worrall, F., Zhang, F., Liu, X., 2014. Sustainable phosphorus management and the need for a long-term perspective: The legacy hypothesis. Environ. Sci. Technol. doi:10.1021/es502852s

Hedley, M.J., Stewart, J.W.B., Chauhan, B.S., 1982. Changes in Inorganic and Organic Soil Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations. Soil Sci. Soc. Am. J. 46, 970. doi:10.2136/sssaj1982.03615995004600050017x

Hinsinger, P., Betencourt, E., Bernard, L., Brauman, A., Plassard, C., Shen, J., Tang, X., Zhang, F., 2011. P for Two, Sharing a Scarce Resource: Soil Phosphorus Acquisition in the Rhizosphere of Intercropped Species. Plant Physiol. 156.

Jackson, M.L., 1969. Soil Chemical Analysis - Advanced Course.

Jones, D.L., 1998. Organic acids in the rhizosphere - A critical review. Plant Soil. doi:10.1023/A:1004356007312

Lambers, H., Finnegan, P.M., Jost, R., Plaxton, W.C., Shane, M.W., Stitt, M., 2015. Phosphorus nutrition in Proteaceae and beyond. Nat. Plants 1, 15109. doi:10.1038/nplants.2015.109

Linquist, B.A., Singleton, P.W., Cassman, K.G., 1997. Inorganic and organic phosphorus dynamics during a build-up and decline of available phosphorus in an Ultisol. Soil Sci.

162, 254–264.

Lopes, A.S.S., Guilherme, L.R.G., 2016. A career perspective on soil management in the Cerrado region of Brazil, in: Advances in Agronomy. pp. 1–72. doi:10.1016/bs.agron.2015.12.004

Louw-Gaume, A.E., Rao, I.M., Gaume, A.J., Frossard, E., 2010. A comparative study on plant growth and root plasticity responses of two Brachiaria forage grasses grown in nutrient solution at low and high phosphorus supply. Plant Soil 328, 155–164. doi:10.1007/s11104-009-0093-z

McLaughlin, M.J., McBeath, T.M., Smernik, R., Stacey, S.P., Ajiboye, B., Guppy, C., 2011. The chemical nature of P accumulation in agricultural soils-implications for fertiliser management and design: An Australian perspective. Plant Soil 349, 69–87. doi:10.1007/s11104-011-0907-7

Mehra, O.P., Jackson, M.L. 1960. Iron oxide removal from soils and clays by a dithionite-citrate system buffered with sodium bicarbonate. In: CLays & clay mineral conference, 7., London. Proceedings. London, v.7, 1960. p.317-327.

Merlin, A., Rosolem, C.A., He, Z., 2016. Non-labile phosphorus acquisition by Brachiaria. J. Plant Nutr. 39, 1319–1327. doi:10.1080/01904167.2015.1109117

Negassa, W., Leinweber, P., 2009. How does the Hedley sequential phosphorus fractionation reflect impacts of land use and management on soil phosphorus : A review. J. Plant Nutr. Soil Sci. 305–325. doi:10.1002/jpln.200800223

Pavinato, A., 2009. Carbono e nutrientes no solo e a sustentabilidade do sistema soja-algodão no cerrado brasileiro (Tese). Universidade Federal do Rio Grande do Sul.

Pavinato, P.S., Rosolem, C.A., 2008. Disponibilidade de nutrientes no solo: decomposição e liberação de compostos orgânicos de resíduos vegetais. Rev. Bras. Ciência do Solo 32, 911–920. doi:10.1590/S0100-06832008000300001

R Core Team, 2017. R: A Language and Environment for Statistical Computing [Internet] [WWW Document]. Vienna, Austria. URL https://www.r-project.org/

Rheinheimer, D.S., Gatiboni, L.C., Kaminski, J., 2008. Fatores que afetam a disponibilidade do fósforo e o manejo da adubação fosfatada em solos sob sistema plantio direto. Ciência Rural 38, 576–586.

Rodrigues, M., Pavinato, P.S., Withers, P.J.A., Teles, A.P.B., Herrera, W.F.B., 2016. Legacy phosphorus and no tillage agriculture in tropical oxisols of the Brazilian savanna. Sci. Total Environ. 542, 1050–1061. doi:10.1016/j.scitotenv.2015.08.118

Rowe, H., Withers, P.J.A., Baas, P., Chan, N.I., Doody, D., Holiman, J., Jacobs, B., Li, H., MacDonald, G.K., McDowell, R., Sharpley, A.N., Shen, J., Taheri, W., Wallenstein, M., Weintraub, M.N., 2016. Integrating legacy soil phosphorus into sustainable nutrient management strategies for future food, bioenergy and water security. Nutr. Cycl. Agroecosystems 104, 393–412. doi:10.1007/s10705-015-9726-1

Roy, E.D., Richards, P.D., Martinelli, L.A., Coletta, L. Della, Rafaela, S., Lins, M., Vazquez, F.F., Willig, E., Spera, S.A., Vanwey, L.K., Porder, S., Lins, S.R.M., Vazquez, F.F., Willig, E., Spera, S.A., Vanwey, L.K., Porder, S., 2016. The phosphorus cost of agricultural intensification in the tropics. Nat. Plants 2, 2–7. doi:10.1038/nplants.2016.43

Sattari, S.Z., Bouwman, A.F., Giller, K.E., van Ittersum, M.K., 2012. From the Cover: Residual soil phosphorus as the missing piece in the global phosphorus crisis puzzle. Proc. Natl. Acad. Sci. 109, 6348–6353. doi:10.1073/pnas.1113675109

Schwertmann, U. 1964. Differenzierung der eisenoxide des bodens durch extraktion mit ammoniumoxalat-lösung. Z. Pflanzenernähr. Düng. Bodenkd, 105:194-202.

Soil Survey Staff, 2014. Keys to Soil Taxonomy, 12th ed. United States Department of Agriculture - Natural Resources Conservation Service, Washington, DC.

Soltangheisi, A., Rodrigues, M., Coelho, M.J.., Gasperini, A.M., Sartor, L.R., Pavinato, P.S., 2017. Soil phosphorus lability influenced by phosphate sources and cover crops under no-tillage. Soil Tillage Res.

Sousa, D., Lobato, E., 2004. Calagem e adubação para culturas anuais e semiperenes, 2nd ed, Cerrado: correção.

Teles, A.P.B., Rodrigues, M., Bejarano Herrera, W.F., Soltangheisi, A., Sartor, L.R., Withers, P.J.A., Pavinato, P.S., 2017. Do cover crops change the lability of phosphorus in a clayey subtropical soil under different phosphate fertilizers? Soil Use Manag. 33, 34–44. doi:10.1111/sum.12327

Tiecher, T., Santos, D.R., Calegari, A., 2012a. Soil organic phosphorus forms under different soil management systems and winter crops, in a long term experiment. Soil Tillage Res. 124, 57–67. doi:10.1016/j.still.2012.05.001

Tiecher, T., Santos, D.R., Kaminski, J., Calegari, A., 2012b. Forms of inorganic phosphorus in soil under different long term soil tillage systems and winter crops. Rev. Bras. Ciência do Solo 36, 271–282. doi:10.1590/S0100-06832012000100028

Tiessen, H., Moir, J.O., 1993. Characterization of available P by sequential extraction, in: Carter, M.R. (Ed.), Soil Sampling and Methods of Analysis. Canadian Society of Soil Science, Lewis Publications, Boca Raton, pp. 75–83.

Vieira, R.C.B., Fontoura, S.M.V., Bayer, C., Ernani, P.R., Anghinoni, I., de Moraes, R.P., 2016. Sampling layer for soil fertility evaluation in long-term no-tillage systems. Rev.

Bras. Cienc. do Solo 40, e0150143. doi:10.1590/18069657rbcs20150143

Vincent, A.G., Turner, B.L., Tanner, E.V.J., 2010. Soil organic phosphorus dynamics following perturbation of litter cycling in a tropical moist forest. Eur. J. Soil Sci. 61, 48–57. doi:10.1111/j.1365-2389.2009.01200.x

Withers, P.J.A., Rodrigues, M., Soltangheisi, A., Carvalho, T. S., Guilherme, L. R., Benites, V. D. M., Gatiboni, L. C., Sousa, D. M. G., Nunes, R. S. Rosolem, C. A., Andreote, F. D., Oliveira Jr, A., Coutinho, E. L. M., Pavinato, P. S., 2018. Transitions to sustainable management of phosphorus in Brazilian agriculture. Scientific reports, 8(1), 2537. doi: 10.1038/s41598-018-20887-z

Withers, P.J.A., Sylvester-Bradley, R., Jones, D.L., Healey, J.R., Talboys, P.J., 2014. Feed the crop not the soil: Rethinking phosphorus management in the food chain. Environ. Sci. Technol. 48, 6523–6530. doi:10.1021/es501670j

Withers, P.J.A., van Dijk, K.C., Neset, T.-S.S.S., Nesme, T., Oenema, O., Rubæk, G.H., Schoumans, O.F., Smit, B., Pellerin, S., 2015. Stewardship to tackle global phosphorus inefficiency: The case of Europe. Ambio 44, 193–206. doi:10.1007/s13280-014-0614-8

Yang, X., Post, W.M., 2011. Phosphorus transformations as a function of pedogenesis: A synthesis of soil phosphorus data using Hedley fractionation method. Biogeosciences 8, 2907–2916. doi:10.5194/bg-8-2907-2011

Table 1. Soil chemical and mineralogical characterization of the two locations (Ox-1 and Ox-2) in cultivated (trials) and native vegetation areas.

Study site		Time of cultivation (Trial establishment)	Soil layer	CEC[1]	pH CaCl$_2$	SOC[2]	DCB[3] Fe	DCB[3] Al	Oxalate[4] Fe	Oxalate[4] Al	Granulometry[\$] Sand	Granulometry[\$] Silt	Granulometry[\$] Clay
			cm	mmol$_c$ kg^{-1}			g kg^{-1}						
Ox-1	Cultivated	42 years (22 years)	0-10	91.8	5.0	25.9	48.8	10.7	1.78	6.73	101	243	656
			10-20	67.8	4.7	20.8							
	Native	-	0-10	68.8	4.8	26.2							
			10-20	52.9	4.6	20.0							
Ox-2	Cultivated	18 years (14 years)	0-10	93.7	4.9	23.9	48.1	13.1	1.76	7.29	185	363	452
			10-20	77.9	4.8	19.5							
	Native	-	0-10	78.7	3.8	26.3							
			10-20	97.2	4.0	18.4							

[1] CEC: cation exchange capacity; [2] SOC: soil organic carbon; [3] DCB: dithionite, citrate and bicarbonate, extracted according to Mehra and Jackson (1960); [4] Oxalate: acidic ammonium oxalate extraction according Schwertmann (1964). DCB, Oxalate and Granulometry evaluated only from native vegetation soil samples (mean 0-20 cm). [\$]Analysed and presented by Pavinato (2009).

Table 2. Labile P fractions in two Brazilian Cerrado Oxisols (Ox-1 and Ox-2) after decades of no-tillage (NT) and conventional tillage (CT) managements and cover crops rotation in the soybean/cotton cash crop production.

Cover Crops	Ox-1						Ox-2					
	0 – 10 cm			10 – 20 cm			0 – 10 cm			10 – 20 cm		
	NT	CT	Mean	NT	CT	Mean	NT	CT	Mean	NT	CT	Mean
	Inorganic P extracted by anion exchange resin (P_{AER}, mg kg^{-1})											
Fallow	35.1 Aa	24.4 Ba	29.7	20.1	18.7	19.4 ns	37.1 Aa	27.7 Bb	32.4	31.8	21.5	26.7 ns
Millet	20.6 Ab	24.4 Aa	22.5	22.2	19.4	20.8	26.8 Ab	27.8 Ab	27.3	23.7	21.7	22.7
Brachiaria	24.7 Ab	24.0 Aa	24.4	27.7	22.0	24.9	43.4 Aa	32.1 Bb	37.8	34.1	22.4	28.3
Maize	25.0 Ab	27.8 Aa	26.4	15.8	20.9	18.3	37.7 Aa	42.8 Aa	40.3	20.3	28.5	24.4
Mean	26.4	25.1		21.4 ns	20.3		36.2	32.6		27.5 ns	23.5	
	Inorganic P extracted by 0.5 M $NaHCO_3$ (Pi_{Bic}, mg kg^{-1})											
Fallow	27.2	21.7	24.4 ns	14.5	16.5	15.5 ns	28.9 Ab	25.5 Aa	27.2	38.5 Ab	19.5 Bb	29.0
Millet	16.3	19.4	17.8	19.8	13.5	16.7	25.6 Ab	22.2 Aa	23.9	34.1 Ab	16.3 Bb	25.2
Brachiaria	18.0	16.7	17.3	15.6	17.4	16.5	41.0 Aa	24.7 Ba	32.8	62.7 Aa	33.0 Ba	47.9
Maize	19.1	23.0	21.1	10.0	20.9	15.5	23.9 Bb	31.0 Aa	27.4	30.9 Ab	31.6 Aa	31.3
Mean	20.2 ns	20.1		15.0 ns	17.1		29.9	25.9		41.5	25.1	
	Organic P extracted by 0.5 M $NaHCO_3$ (Po_{Bic}, mg kg^{-1})											
Fallow	22.4	23.1	22.7 ns	20.8	14.7	17.7 ns	42.3	41.9	42.1 ns	47.4	38.1	42.8
Millet	20.5	23.2	21.9	17.7	20.3	19.0	45.1	45.5	45.3	36.9	55.7	46.3
Brachiaria	21.7	26.0	23.8	17.5	23.4	20.4	47.3	47.7	47.5	27.8	47.3	37.6
Maize	21.9	27.7	24.8	17.4	19.0	18.2	44.6	46.9	45.7	28.3	65.5	46.9
Mean	21.6 ns	25.0		18.3 ns	19.3		44.8 ns	45.5		35.1 B	51.6 A	

For each soil and within each depth and P fraction, means followed by the same capital letter in line and small letter in column were not significantly different by Tukey's test ($p<0.05$).
ns: not significant; NT, no-tillage system; CT, conventional tillage system.

Table 3. Moderately labile P fractions in two Brazilian Cerrado Oxisols (Ox-1 and Ox-2) after decades of no-tillage (NT) and conventional tillage (CT) soil managements and cover crops rotation in the soybean/cotton cash crop production.

Cover Crops	Ox-1						Ox-2					
	0 – 10 cm			10 – 20 cm			0 – 10 cm			10 – 20 cm		
	NT	CT	Mean	NT	CT	Mean	NT	CT	Mean	NT	CT	Mean
	Inorganic P extracted by 0.1 M NaOH ($Pi_{Hid0.1}$, mg kg^{-1})											
Fallow	219.9	217.9	218.9 a	130.4	149.8	140.1 ns	83.7 Ab	88.0 Aab	85.8	74.1	67.8	71.0 ns
Millet	213.6	177.1	195.3 ab	135.2	106.1	120.6	76.6 Ab	70.8 Ab	73.7	74.5	70.3	72.4
Brachiaria	163.5	182.4	172.9 b	152.8	117.7	135.2	104.5 Aa	69.7 Bb	87.1	90.9	79.8	85.3
Maize	190.2	191.4	190.8 ab	128.4	137.2	132.8	71.8 Bb	97.2 Aa	84.5	76.0	84.9	80.5
Mean	196.8 ns	192.2		136.7 ns	127.7		84.1	81.4		78.9 ns	75.7	
	Organic P extracted by 0.1 M NaOH ($Po_{Hid0.1}$, mg kg^{-1})											
Fallow	142.7	100.5	121.6 b	104.6	95.8	100.2 b	60.8	47.3	54.1 a	29.6 Ab	24.4 Aa	27.0
Millet	136.8	128.0	132.4 b	107.9	119.9	113.9 b	34.3	45.1	39.7 b	23.4 Ab	17.4 Aa	20.4
Brachiaria	173.2	155.8	164.5 a	107.3	151.1	129.2 a	46.8	39.8	43.2 ab	65.6 Aa	31.4 Ba	48.5
Maize	120.2	141.6	130.9 b	91.5	117.4	104.5 b	53.9	43.8	48.9 ab	35.9 Ab	21.2 Ba	28.6
Mean	143.2 ns	131.5		102.8 ns	121.1		48.9 ns	44.0		38.6	23.6	
	Inorganic P extracted by 1 M HCl (P_{HCl}, mg kg^{-1})											
Fallow	2.2	1.4	1.8 #	1.4	0.9	1.1 #	1.0	1.0	1.0 #	0.9	0.7	0.8 #
Millet	1.6	1.7	1.7	1.0	1.0	1.0	1.4	1.0	1.2	1.0	0.8	0.9
Brachiaria	1.6	1.6	1.6	1.0	1.3	1.2	2.0	1.2	1.6	1.2	1.1	1.2
Maize	2.0	1.5	1.8	1.0	1.2	1.1	1.4	1.3	1.3	1.0	0.8	0.9
Mean	1.8 #	1.6		1.1 #	1.1		1.4 #	1.1		1.1 #	0.9	

For each soil and within each depth and P fraction, means followed by the same capital letter in line and small letter in column were not significantly different by Tukey's test ($p<0.05$).

#, not statically evaluated; ns: not significant; NT: no-tillage system; CT: conventional tillage system.

Table 4. Non-labile P fractions in two Brazilian Cerrado Oxisols (Ox-1 and Ox-2) after decades of no-tillage (NT) and conventional tillage (CT) soil managements and cover crops rotation in the soybean/cotton cash crop production.

Cover Crops	Ox-1						Ox-2					
	0 – 10 cm			10 – 20 cm			0 – 10 cm			10 – 20 cm		
	NT	CT	Mean	NT	CT	Mean	NT	CT	Mean	NT	CT	Mean
	Inorganic P extracted by 0.5 M NaOH ($Pi_{Hid0.5}$, mg kg^{-1})											
Fallow	49.2	46.9	48.0 ns	45.2	42.5	43.9 ns	68.5	64.6	66.6 b	60.4	62.1	61.2 ns
Millet	47.1	47.5	47.3	44.7	45.9	45.3	71.3	71.2	71.3 b	61.8	71.1	66.5
Brachiaria	43.8	51.6	47.7	46.2	48.5	47.3	83.5	80.3	81.9 a	66.7	67.7	67.2
Maize	49.9	48.5	49.2	47.1	40.8	44.0	75.1	84.5	79.8 a	59.2	73.8	66.5
Mean	47.5 ns	48.6		45.8 ns	44.4		74.6 ns	75.1		62.0 ns	68.7	
	Organic P extracted by 0.5 M NaOH ($Po_{Hid0.5}$, mg kg^{-1})											
Fallow	91.7	61.5	76.6 ns	44.9	33.8	39.3 ns	21.8	34.0	27.9 ns	30.8	32.7	31.8 ns
Millet	75.5	62.5	69.0	37.2	35.1	36.1	51.3	36.4	43.9	37.2	27.3	32.2
Brachiaria	78.5	58.3	68.4	32.8	47.1	39.9	29.8	39.1	34.5	52.4	34.0	43.2
Maize	76.6	67.2	71.9	45.2	42.0	43.6	35.5	29.5	32.5	42.5	25.8	34.2
Mean	80.6 A	62.3 B		40.0 ns	39.5		34.6 ns	34.7		40.7 ns	30.0	
	Residual P (mg kg^{-1})											
Fallow	313.8	300.9	307.3 ns	293.8	270.4	282.1 ns	240.9	249.4	245 ns	227.4	251.5	239.5 ns
Millet	325.3	299.7	312.5	291.4	288.4	289.9	258.3	262.6	260	232.0	254.5	243.2
Brachiaria	313.4	301.3	307.4	298.4	270.1	284.2	260.6	264.5	262	236.3	261.1	248.7
Maize	318.1	314.8	316.4	296.7	301.1	298.9	249.3	270.0	259	247.1	251.5	249.3
Mean	317.6 ns	304.2		295.0 ns	282.5		252.3 ns	261.6		235.7 ns	254.7	

For each soil and within each depth and P fraction, means followed by the same capital letter in line and small letter in column were not significantly different by Tukey's test ($p<0.05$).

ns: not significant; NT: no-tillage system; CT: conventional tillage system.

Table 5. Estimated soil Legacy P considering the P balance (P inputs – P outputs) in two long-term field trials in the Brazilian Cerrado (Ox-1 and Ox-2).

Study Site	Total P inputs	Total P outputs	Net P (Legacy)	Years since deforestation (trial establishment)
	------------------------ kg ha^{-1} ------------------------			
Ox-1	1560.6	636.7	923.9	42 (22)#
Ox-2	806.3	279.2	527.1	18 (14)#

#: relative to the period of soil sampling (2016 for Ox-1 and 2015 for Ox-2) and the deforestation time. Values inside the brackets denote the time (years) after tillage differentiation.

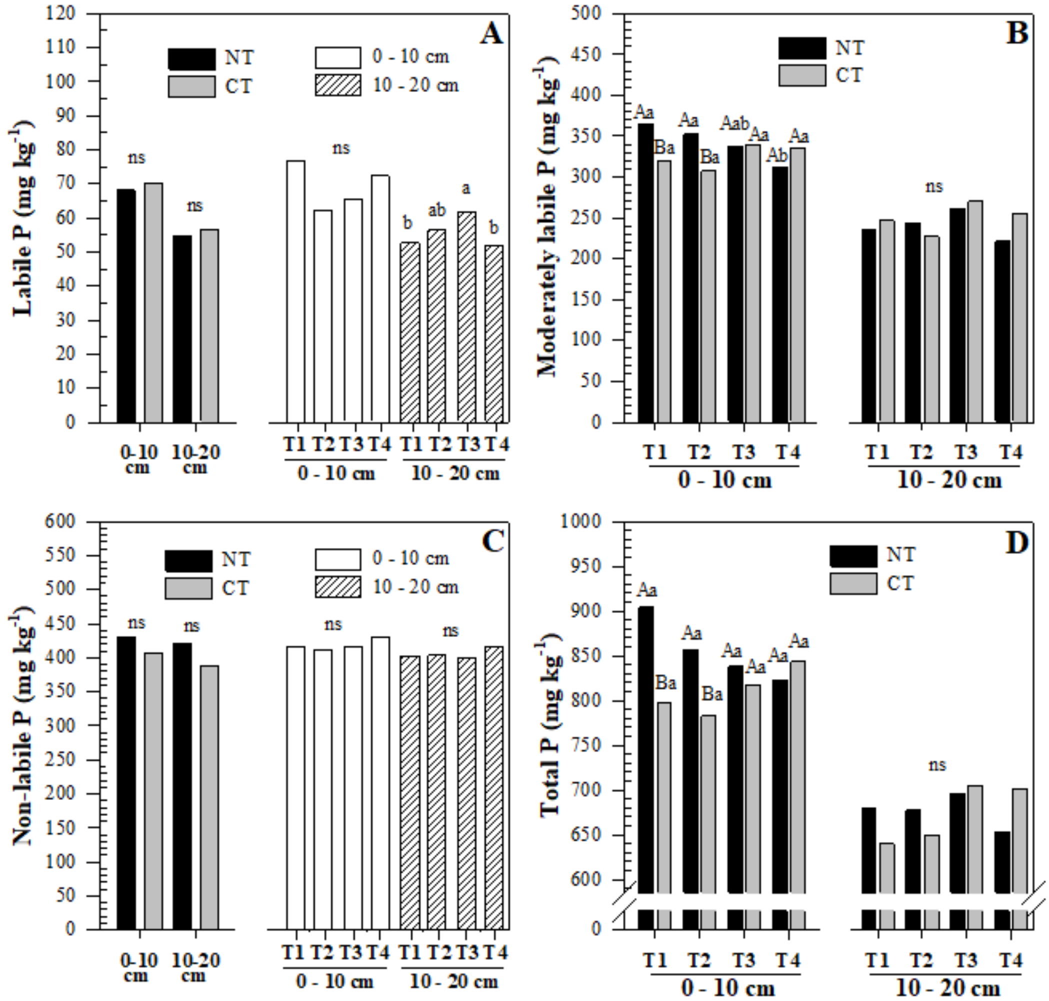

Figure 1. Soil phosphorus lability after long-term effects of tillage systems and cover crops in a Brazilian Cerrado Oxisol 1 (Ox-1). A) Labile P; B) Moderately labile P; C) Non-labile P; D) Total P. Within each depth and P fraction, means followed by the same capital letter (tillage systems) and small letter (cover crops) were not significantly different at $p<0.05$ by Tukey test. ns: not significant differences observed (n=3). NT: no-tillage; CT: conventional tillage; T1: fallow; T2: millet; T3: brachiaria; T4: maize.

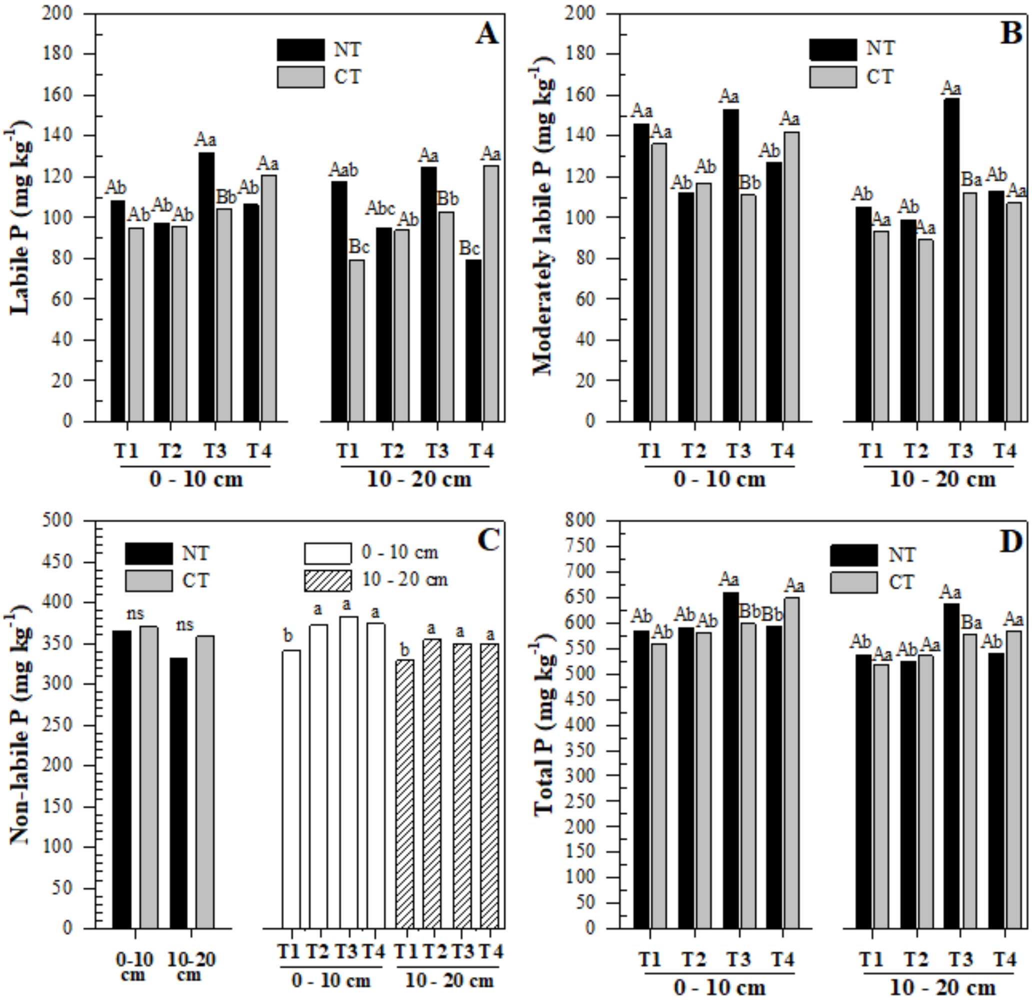

Figure 2. Soil phosphorus lability after long-term effects of tillage systems and cover crops in a Brazilian Cerrado Oxisol 2 (Ox-2). A) Labile P; B) Moderately labile P; C) Non-labile P; D) Total P. Within each depth and P fraction, means followed by the same capital letter (tillage systems) and small letter (cover crops) were not significantly different at $p<0.05$ by Tukey test. ns: not significant differences observed (n=3). NT: no-tillage; CT: conventional tillage; T1: fallow; T2: millet; T3: brachiaria; T4: maize.

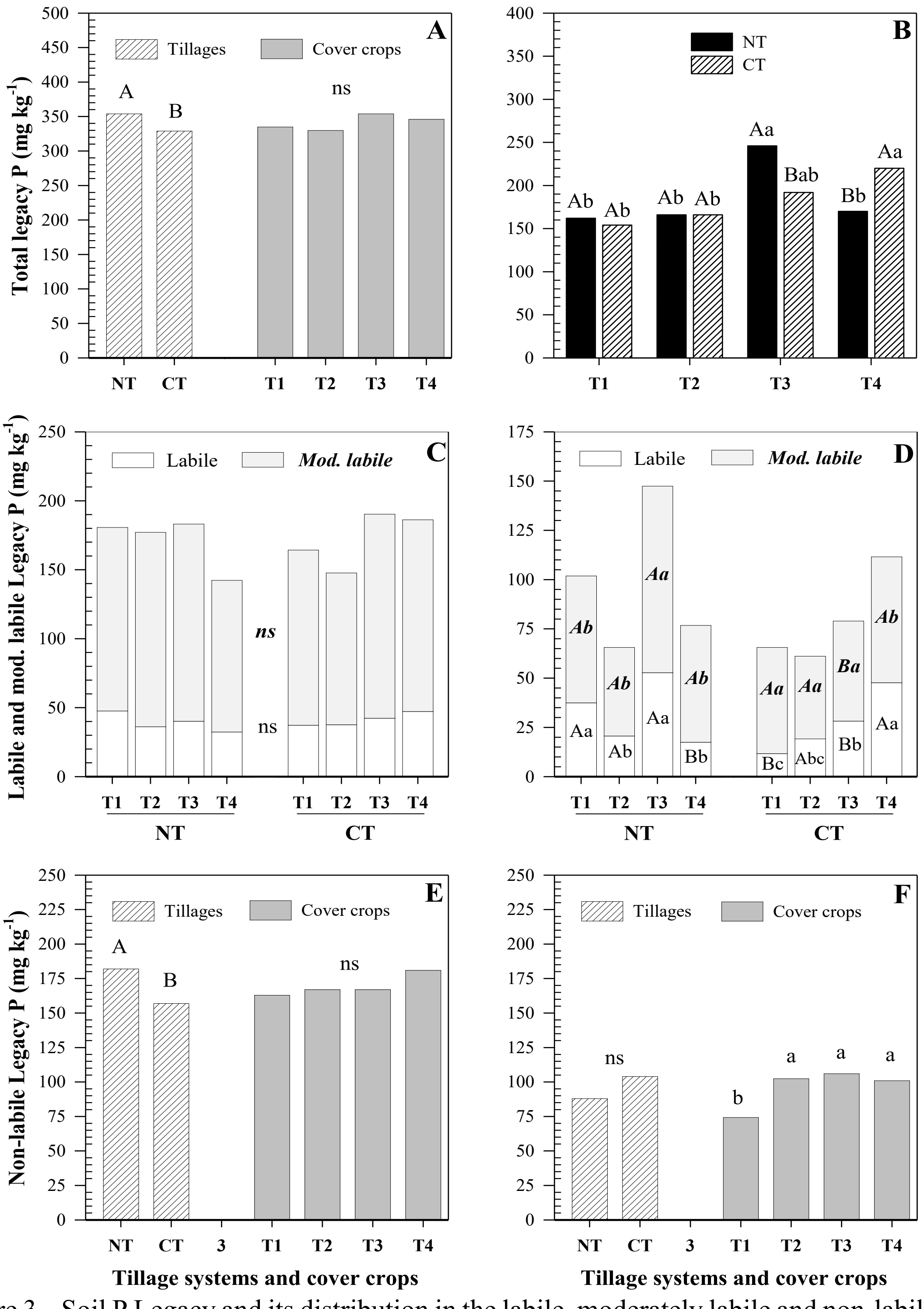

Figure 3. Soil P Legacy and its distribution in the labile, moderately labile and non-labile pools in soil top 20 cm of two Brazilian Oxisols after decades of tillage systems and cover crops cultivation. A) Total legacy P in the Ox-1; B) Total legacy P in the Ox-2; C) Labile and moderately labile Legacy P pools in the Ox-1; D) Labile and moderately labile Legacy P pools in the Ox-2; E) Non-labile P pool in the Ox-1; F) Non-labile P pool in the Ox-2. Within each depth and P fraction, means followed by the same capital letter (tillage systems) and small letter (cover crops) were not significantly different at $p<0.05$ by Tukey test. ns: not significant differences observed (n=3). NT: no-tillage; CT: conventional tillage; T1: fallow; T2: millet; T3: brachiaria; T4: maize.

Table S1. Summary of P inputs and outputs at Ox-1 and Ox-2 field trials.

Ox-1

Obs	Year Count	Crop Season	Cultivated Crop	P Input ($kg\ ha^{-1}$)	P Output ($kg\ ha^{-1}$)
Time before trial, conventional tillage (CT)	1	1974/75	Soybean	39.3	12.36
	2	1975/76	Soybean	39.3	12.36
	3	1976	Soybean	39.3	12.36
	4	1977	Soybean	39.3	12.36
	5	1978	Soybean	39.3	12.36
	6	1979	Soybean	39.3	12.36
	7	1980	Soybean	39.3	12.36
	8	1981	Soybean	39.3	12.36
	9	1982	Soybean	39.3	12.36
	10	1983	Soybean	39.3	12.36
	11	1984	Soybean	39.3	12.36
	12	1985	Soybean	39.3	12.36
	13	1986	Soybean	39.3	12.36
	14	1987	Soybean	39.3	12.36
	15	1988	Soybean	39.3	12.36
	16	1989	Soybean	39.3	12.36
	17	1990	Soybean	39.3	12.36
	18	1991	Soybean	39.3	12.36
	19	1992	Soybean	39.3	12.36
	20	1993	Soybean	39.3	12.36
Tillage differentiation	21	1994/95	Maize	39.2	27.08
	22	1995	Soybean	22.7	16.42
	23	1996	Maize	41.9	26.76
	24	1997	Soybean	25.9	14.49
	25	1998	Cotton	48.0	14.16
	26	1999	Soybean	23.4	14.89
	27	2000	Maize	37.3	30.58
	28	2001	Cotton	52.5	16.96
	29	2002	Soybean	23.8	15.99
	30	2003	Maize	44.5	32.36
	31	2004	Cotton	56.6	16.30
Cover crops effects (average P inputs and outputs for fallow, brachiaria, millet and maize)	32	2005/06	Soybean	15.7	11.36
	33	2006	Cotton	39.1	18.43
	34	2007	Soybean	15.7	14.04
	35	2008	Cotton	47.4	15.04
	36	2009	Soybean	15.9	13.16
	37	2010	Cotton	60.7	16.48
	38	2011	Soybean	15.7	15.24
	39	2012	Cotton	56.8	16.21
	40	2013	Soybean	17.5	14.02
	41	2014	Cotton	59.0	15.88
	42	2015	Soybean	15.3	13.56
Total				**Inputs**	**Outputs**
				1560.6	**636.7**

Ox-2

Obs	Year Count	Crop Season	Cultivated Crop	P Input ($kg\ ha^{-1}$)	P Output ($kg\ ha^{-1}$)
Time before field trial	1	1997/98	Soybean	52.40	14.56
	2	1998	Cotton	54.36	15.21
	3	1999	Soybean	50.69	14.56
Tillage differentiation	4	2000/01	Soybean	33.01	13.74
	5	2001	Cotton	53.05	12.62
	6	2002	Soybean	30.56	14.97
	7	2003	Cotton	56.59	16.28
	8	2004	Soybean	30.56	15.74
Cover crops effects (average P inputs and outputs for fallow, brachiaria, millet and maize)	9	2005/06	Cotton	62.88	15.47
	10	2006	Soybean	30.56	21.79
	11	2007	Cotton	62.88	13.95
	12	2008	Soybean	22.00	20.21
	13	2009	Cotton	55.89	14.65
	14	2010	Soybean	32.75	15.57
	15	2011	Cotton	55.89	14.53
	16	2012	Soybean	34.93	14.52
	17	2013	Soybean	43.66	15.13
	18	2014	Soybean	43.66	15.71
Total				**Inputs**	**Outputs**
				806.3	**279.2**

Table S2. Summary of the analysis of variance (ANOVA) for phosphorus fractions after decades of tillage systems and cover crops cultivation in a Brazilian Cerrado Oxisol 1 (Ox-1).

Factor	**Hedley phosphorus fractions**											
	P_{AER}	**Pi_{Bic}**	**Po_{Bic}**	**$Pi_{Hid0.1}$**	**$Po_{Hid0.1}$**	**P_{HCl}**	**$Pi_{Hid0.5}$**	**$Po_{Hid0.5}$**	**$P_{Residual}$**	**Pi_{Total}**	**Po_{Total}**	**P_{Total}**
	0 – 5 cm											
Tillage (a)	ns	ns	*	ns	ns	-	ns	*	ns	ns	ns	ns
Crops (b)	*	ns	*	*	*	-	ns	*	ns	*	*	*
a*b	*	ns	ns	ns	*	-	ns	*	ns	ns	*	*
CV_a (%)	9.80	34.30	5.12	7.77	10.99	-	6.70	7.11	10.73	3.64	7.29	1.68
CV_b (%)	11.40	22.02	10.42	13.99	18.87	-	11.48	11.00	6.02	5.82	11.63	3.78
$\bar{y}$	5.498 / 7.444	29.485 / 12.149	1.658 / 6.861	234.65 / 760.27	214.2 / 631.5	-	9.175 / 27.218	31.69 / 75.85	1105.73 / 347.79	467.5 / 1191.4	299.6 / 763.1	194.8 / 987.5
	5-10 cm											
Tillage (a)	*	ns	ns	ns	ns	-	ns	ns	ns	ns	*	*
Crops (b)	ns	ns	ns	*	ns	-	ns	*	ns	*	ns	*
a*b	ns	ns	ns	*	ns	-	ns	*	ns	ns	ns	ns
CV_a (%)	9.80	14.57	12.79	9.40	23.01	-	8.10	18.95	6.27	5.95	15.04	0.56
CV_b (%)	27.64	41.27	18.90	7.13	16.76	-	8.11	13.21	5.88	6.45	12.33	6.91
$\bar{y}$	7.204 / 58.035	12.726 / 102.178	7.536 / 16.457	324.9 / 187.1	1060.7 / 562.3	-	16.882 / 16.932	146.03 / 71.02	382.29 / 335.99	1308.6 / 1538.2	1163.5 / 782.6	22.0 / 3331
	10-20 cm											
Tillage (a)	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	*	ns
Crops (b)	ns	ns	ns	ns	*	-	ns	ns	ns	ns	ns	ns
a*b	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	*	ns
CV_a (%)	9.37	40.33	3.48	9.31	12.57	-	9.93	16.36	3.78	3.93	6.19	4.10
CV_b (%)	24.97	59.22	16.08	17.37	15.32	-	7.73	24.71	7.94	7.61	11.86	7.79
$\bar{y}$	3.821 / 27.120	41.808 / 90.160	0.430 / 9.174	151.58 / 527.39	198.01 / 294.25	-	20.083 / 12.180	42.298 / 96.502	119.22 / 526.28	392.89 / 1473.13	1163.5 / 782.6	392.89 / 1473.13
	0-10 cm #											
Tillage (a)	ns	ns	ns	ns	ns	-	ns	*	ns	ns	ns	*
Crops (b)	*	ns	ns	*	*	-	ns	ns	ns	*	*	ns
a*b	*	ns	ns	ns	ns	-	ns	ns	ns	ns	*	*
CV_a (%)	8.47	22.09	7.27	8.46	15.65	-	4.33	9.96	2.77	3.11	9.89	0.64
CV_b (%)	16.27	26.40	9.92	9.17	14.55	-	7.43	7.76	3.92	4.63	10.20	4.16
$\bar{y}$	4.753 / 17.554	19.823 / 28.325	2.867 / 5.340	270.83 / 318.17	462.21 / 399.51	-	4.341 / 12.762	50.70 / 30.79	74.21 / 148.30	350.50 / 775.80	527.4 / 560.4	194.8 / 987.5

* significant different detected at $p < 0.05$. ns: not significant difference observed.
# obtained by average values from 0-5 and 5-10 cm results.
$\bar{y}$ general mean. - not evaluated.

Table S3. Summary of the analysis of variance (ANOVA) for phosphorus fractions after decades of tillage systems and cover crops cultivation in a Brazilian Cerrado Oxisol 2 (Ox-2).

Factor	**Hedley phosphorus fractions**											
	P_{AER}	**Pi_{Bic}**	**Po_{Bic}**	**$Pi_{Hid0.1}$**	**$Po_{Hid0.1}$**	**P_{HCl}**	**$Pi_{Hid0.5}$**	**$Po_{Hid0.5}$**	**$P_{Residual}$**	**Pi_{Total}**	**Po_{Total}**	**P_{Total}**
	0 – 5 cm											
Tillage (a)	ns	ns	ns	ns	*	-	*	ns	ns	ns	*	ns
Crops (b)	*	ns	ns	ns	*	-	*	*	*	*	ns	ns
a*b	*	ns	ns	ns	ns	-	*	*	ns	*	ns	*
CV_a (%)	22.82	8.18	18.68	7.80	15.55	-	0.54	23.62	4.00	2.56	7.02	5.75
CV_b (%)	13.52	17.51	10.76	19.16	32.19	-	5.36	16.16	3.51	4.33	13.23	5.94
$\bar{y}$	58.584 / 20.578	4.474 / 20.486	74.863 / 24.831	41.13 / 248.04	50.1 / 214.7	-	0.18 / 17.34	72.12 / 33.76	109.78 / 84.77	152.81 / 437.19	80.6 / 285.9	1208.0 / 1292.1
	5-10 cm											
Tillage (a)	ns	ns	ns	*	*	-	ns	ns	ns	ns	ns	*
Crops (b)	*	*	ns	*	ns	-	*	ns	ns	*	ns	*
a*b	*	*	ns	*	*	-	*	*	ns	*	*	ns
CV_a (%)	14.74	35.32	13.78	7.61	19.01	-	19.42	50.36	9.85	5.88	15.75	0.56
CV_b (%)	14.32	18.84	11.37	18.82	21.60	-	10.16	21.40	5.40	5.09	9.43	6.91
$\bar{y}$	27.113 / 25.578	111.31 / 31.65	36.78 / 25.04	40.25 / 246.25	36.78 / 25.04	-	195.965 / 53.642	283.053 / 51.112	615.51 / 184.92	774.5 / 581.1	386.32 / 138.51	22.0 / 3331
	10-20 cm											
Tillage (a)	ns	*	ns	ns	*	-	ns	ns	ns	ns	ns	ns
Crops (b)	ns	*	*	ns	*	-	ns	ns	ns	*	*	ns
a*b	ns	*	*	ns	*	-	ns	ns	ns	*	ns	ns
CV_a (%)	23.21	15.90	23.75	28.33	19.63	-	11.40	28.57	8.15	9.87	5.90	6.72
CV_b (%)	21.50	18.14	12.62	16.16	18.69	-	10.05	22.66	4.92	5.32	12.09	4.91
$\bar{y}$	35.100 / 30.106	28.11 / 36.57	106.09 / 29.97	479.86 / 156.07	37.32 / 33.85	-	55.569 / 43.107	102.04 / 64.17	398.79 / 145.44	1952.6 / 566.9	41.96 / 176.43	1400.3 / 747.0
	0-10 cm #											
Tillage (a)	ns	ns	*	ns	ns	-	ns	ns	ns	ns	ns	ns
Crops (b)	*	*	ns	ns	*	-	*	ns	ns	*	ns	*
a*b	*	*	ns	*	ns	-	ns	ns	ns	*	ns	*
CV_a (%)	10.71	21.38	15.03	2.61	16.47	-	9.27	33.76	6.33	4.20	10.66	3.82
CV_b (%)	11.65	14.21	7.34	10.81	16.64	-	5.73	14.34	2.82	3.12	7.92	3.45
$\bar{y}$	13.587 / 16.096	35.489 / 15.674	46.094 / 10.978	4.65 / 80.07	58.492 / 59.750	-	48.167 / 18.425	137.065 / 24.722	264.13 / 52.56	402.60 / 222.70	181.10 / 100.16	528.00 / 430.80

* significant different detected at $p < 0.05$. ns: not significant difference observed.
# obtained by average values from 0-5 and 5-10 cm results.
$\bar{y}$ general mean.

- not evaluated.

Table S4. Hedley's P fractions (mg kg^{-1}) in Brazilian Cerrado native soils (Ox-1 and Ox-2).

Soil	P AER	Pi Bic	Po Bic	Pi Hid0.1	Po Hid0.1	P HCl	Pi Hid0.5	Po Hid0.5	P Residual	P Total
	---------- mg kg^{-1} ----------									
	0 – 5 cm									
Ox-1	3.9 (±0.2)	4.0 (±0.3)	15.6 (±2.3)	118.2 (±21)	98.8 (±17)	1.0 (±0.6)	75.9 (±7.1)	48.5 (±7.8)	147.4 (±10)	513.3 (±39)
	5 – 10 cm									
Ox-1	3.2 (±0.2)	2.5 (±0.4)	16.1 (±1.2)	106.5 (±8.6)	90.9 (±9.9)	0.7 (±0.1)	62.0 (±6.9)	42.2 (±7.6)	135.6 (±15)	459.6 (±17)
	10 – 20 cm									
Ox-1	3.1 (±0.1)	2.2 (±0.4)	16.7 (±2.2)	74.4 (±10)	29.9 (±8.4)	0.9 (±0.4)	66.3 (±3.9)	25.0 (±7.2)	135.0 (±7.6)	353.6 (±11)
	0 – 20 cm (weighted mean)									
Ox-1	3.3 (±0.1)	2.7 (±0.4)	16.3 (±1.9)	93.4 (±13)	62.4 (±11)	0.8 (±0.3)	67.7 (±5.5)	35.2 (±7.5)	138.2 (±10)	420.0 (±20)
	0 – 5 cm									
Ox- 2	15.7 (±4.3)	23.0 (±3.3)	51.7 (±4.1)	56.5 (±6.3)	6.9 (±2.6)	0.4 (±0.1)	82.2 (±8.9)	26.3 (±6.6)	178.7 (±2.7)	441.3 (±11)
	5 – 10 cm									
Ox- 2	11.0 (±3.8)	13.6 (±1.1)	62.2 (±10)	55.2 (±9.7)	5.5 (±2.8)	0.4 (±0.1)	68.4 (±9.8)	8.8 (±2.1)	180.9 (±3.1)	406.0 (±13)
	10 – 20 cm									
Ox- 2	9.7 (±2)	9.7 (±0.8)	42.8 (±2.1)	51.8 (±4.6)	6.8 (±1.6)	0.4 (±0.2)	60.4 (±7.9)	12.3 (±4.9)	175.3 (±11)	369.3 (±17)
	0 – 20 cm (weighted mean)									
Ox- 2	11.5 (±3)	14.0 (±1.5)	49.9 (±4.8)	53.8 (±6.3)	6.5 (±2.1)	0.4 (±0.1)	67.8 (±8.6)	14.9 (±4.6)	177.5 (±7.4)	396.5 (±15)

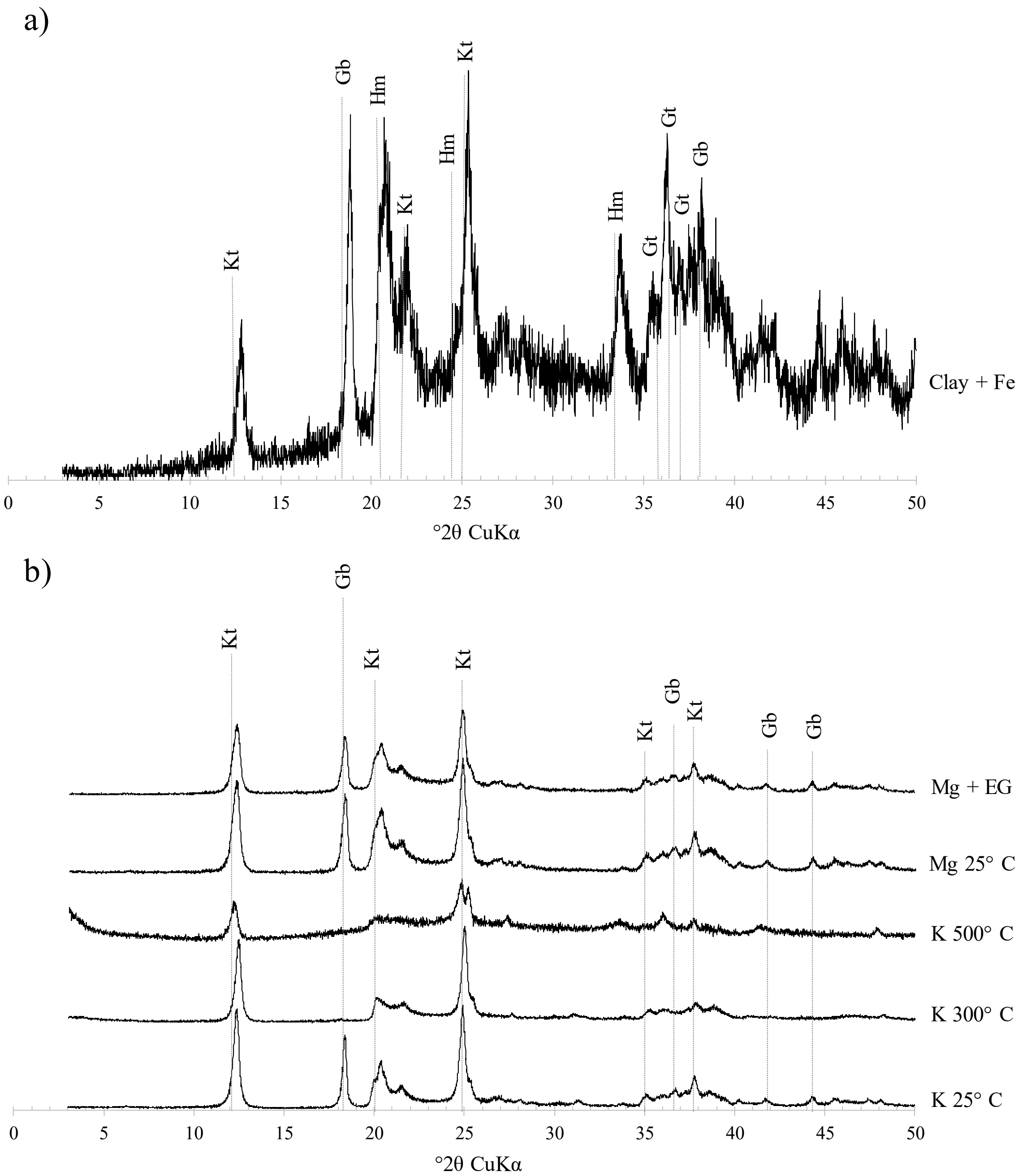

Figure S1. X-ray diffraction of Ox-1 soil clay fraction: (a) Sample prepared without Fe removal and (b) clay with Fe removed (K^+ saturation at room temperature, K 25℃; sample saturated with K^+ and heated up to 300℃, K 300℃; sample saturated with K^+ and heated up to 500℃, K 500℃; Mg^{2+} saturation at room temperature, Mg 25℃; sample saturated with Mg^{2+} and solved with ethylene glycol, Mg + EG), oriented blades. *Identified minerals: gibbsite (Gb), goethite (Gt), hematite (Hm) and kaolinite (Kt).*

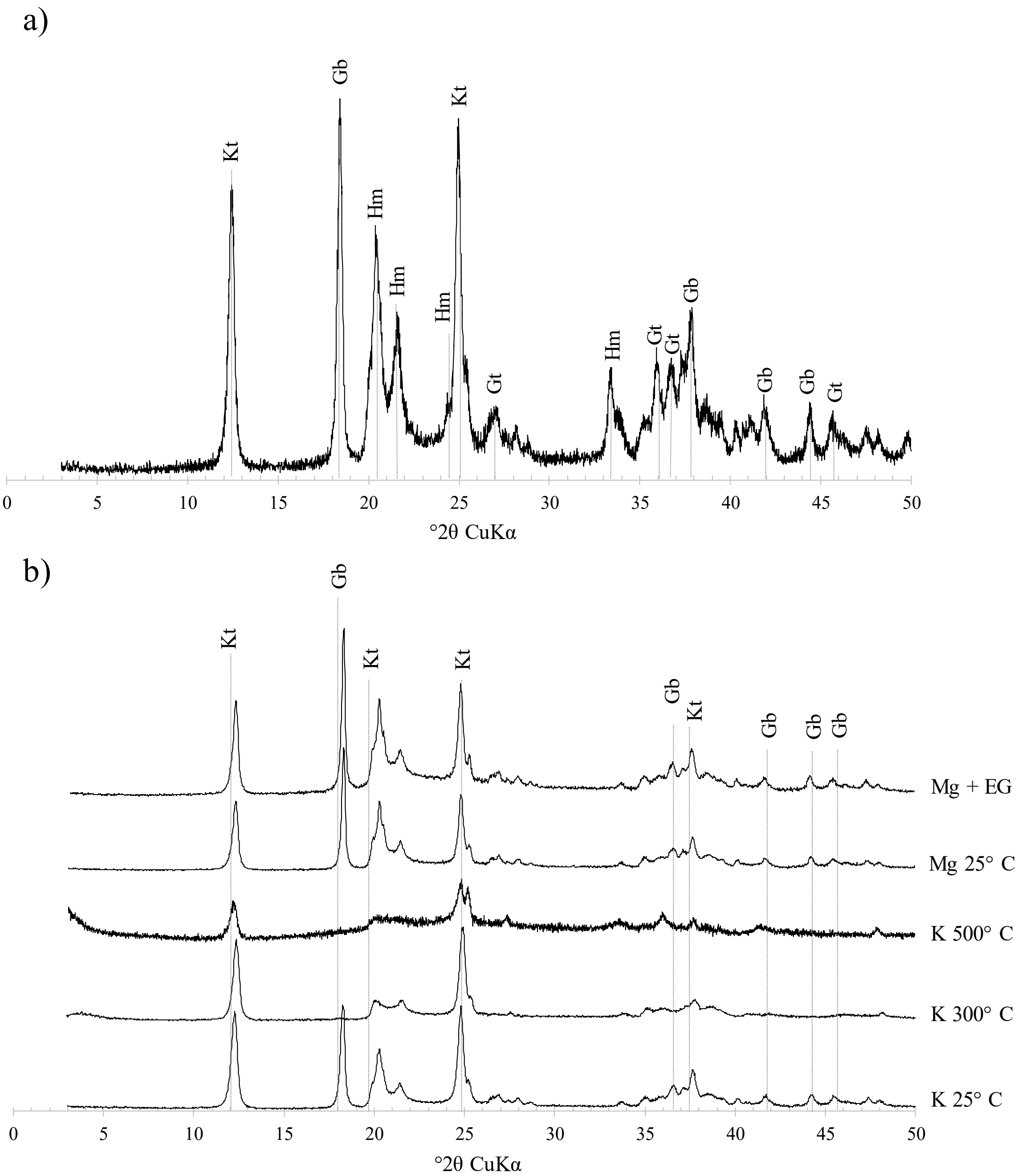

Figure S2. X-ray diffraction of Ox-2 soil clay fraction: (a) Sample prepared without Fe removal and (b) clay with Fe removed (K^+ saturation at room temperature, K 25℃; sample saturated with K^+ and heated up to 300℃, K 300℃; sample saturated with K^+ and heated up to 500℃, K 500℃; Mg^{2+} saturation at room temperature, Mg 25℃; sample saturated with Mg^{2+} and solved with ethylene glycol, Mg + EG), oriented blades. *Identified minerals: gibbsite (Gb), goethite (Gt), hematite (Hm) and kaolinite (Kt)*