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Long-term land use and tillage influence on phosphorus species in Brazilian Oxisols: A multi-technique assessment by chemical P fractionation, ^{31}P NMR and P K-edge XANES spectroscopies

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ABSTRACT

Phosphorus (P) interacts with soil constituents in many ways, forming compounds of distinct stabilities in a number of chemical inorganic and organic forms. The identification of these P compounds in tropical agro-ecosystems is especially relevant to understanding potential P availability in these common P-fixing soils, providing information to improve P use efficiency. In this study, we investigated P partitioning and speciation in Brazilian Cerrado Oxisols under different management systems using a combination of sequential chemical P fractionation (SPF), P K-edge XANES and ^{31}P NMR spectroscopy. Topsoil (0–10 cm) samples were obtained from soils under three management regimes, including native vegetation (Cerrado), no-tillage (NT), and conventional tillage (CT) agriculture. Four representative agricultural sites in the Brazilian Cerrado were selected: Ox-1 (Costa Rica-MS), Ox-2 (Sapezal-MT), Ox-3 (Cristalina-GO) and Ox-4 (Tasso Fragoso-MA). In all sites, the soils are classified as Typic Hapludox, varying in cultivation time, soil clay content and oxide types. Long-term cultivation in NT and CT led to large P accumulation in the soil. Chemical P fractionation indicated that labile P was the smallest pool at all sites. P K-edge XANES showed that P is mostly sorbed to the Fe and Al hydr(oxides), goethite, hematite and gibbsite. It also showed that phytate was the predominant organic P species, depleting in sequence from native vegetation to NT and CT management systems. ^{31}P NMR showed that monoester P was present in all soils and management systems, whereas diester-P was found only in soils under native vegetation. The combined use of SPF, XANES and ^{31}P NMR data was necessary to fully understand inorganic and organic P chemistry and reactivity in these tropical agricultural croplands.

1. Introduction

The environmental and agronomic aspects of phosphorus (P) use in agricultural systems are of concern throughout the world (MacDonald et al., 2011), as P is an essential plant nutrient often required in regular amounts to sustain crop production (Novais et al., 2007; Novais and Smyth, 1999). However, phosphate rock from which inorganic fertilisers are manufactured is a non-renewable resource that may become scarcer over time (Cordell et al., 2009; Elser et al., 2014), and excess use of P in agriculture leads to unnecessary soil P accumulation and increased P losses to water bodies, causing endemic and widespread eutrophication (Elser and Bennett, 2011; Withers et al., 2014). Resolving these competing economical, resource and environmental issues requires improvements in P use efficiency, especially in tropical regions, like Brazil, where future P fertiliser demands are forecast to increase significantly following agricultural intensification (Roy et al., 2016; Withers et al., 2018).

In tropical soils, such as the Brazilian Savanna (Cerrado), the high sorbing capacity and strong retention of P contribute greatly to its low bioavailability. High inputs of inorganic P fertilisers are needed on a

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regular basis for high productivity, leading to complex chemical forms of P in soils (Rodrigues et al., 2016). Successive and potentially excessive P inputs over time have resulted in a buildup of residual P in many soils globally (Condron et al., 2013), termed 'legacy P'. Exploring this legacy P by implementing best management practices to increase its bioavailability has received much attention recently in the drive towards more sustainable agricultural production (Condron et al., 2013; Menezes-Blackburn et al., 2018; Rowe et al., 2016; Sattari et al., 2012). However, advanced soil and nutrient management to prevent crop P deficiencies and guarantee high P use efficiency in these tropical environments requires a detailed understanding of soil P speciation to allow more accurate prediction of P bioavailability and mobility in soils (Negassa and Leinweber, 2009).

The Hedley P fractionation scheme allows to quantify and categorize soil P into chemically and biologically different pools, from inorganic to organic, and from labile to non-labile P according to strength of the extractant (Hedley et al., 1982). This technique can evaluate soil P dynamics among different natural (Soltangheisi et al., 2019) and agroecosystems (Condron and Newman, 2011), and help to predict P lability for plant uptake (Cross and Schlesinger, 1995; Soltangheisi et al., 2019). However, soil P fractionation techniques are operationally defined by the strength of the extractants which does not allow determination of different P species influencing P bioavailability (Negassa and Leinweber, 2009).

X-ray Absorption Near Edge Structure (XANES) spectroscopy (P K-edge) has been widely used to characterize the inorganic P forms in soils more precisely (Abdala et al., 2018; Hesterberg, 2010; Hesterberg et al., 1999; Koch et al., 2018; Kruse et al., 2015). It has been particularly deployed to identify Ca phosphates (e.g., apatites, brushite, di-calcium, tri-calcium and octocalcium phosphates), Fe-P compounds (e.g., strengite), and Al-P compounds (e.g., variscite) as well as P bound to Al- and Fe- (hydr)oxide minerals in soils. However, P K-edge XANES has limited application to distinguish P associated with organic molecules, here referred to organic P, due to the lack of more distinctive features in the XANES spectrum of organic P compounds in soils relative to their inorganic counterparts. When it comes to speciation of organic P, phytic acid (myo-inositol hexakisphosphate, IHP) is often considered in P K-edge XANES data analysis (Liu et al., 2015; Prietzel et al., 2013). However, due to the difficulties in separating out organic P (Po) spectra features to those of P sorbed to Fe and Al minerals (pre-edge peak, white line, post-edge shoulder and features), it is necessary to combine P K-edge XANES spectroscopy with complementary techniques that are more sensitive to Po chemistry (Kruse et al., 2015), such as ^{31}P nuclear magnetic resonance (NMR).

Solution ^{31}P NMR spectroscopy is by far the most widely used spectroscopic technique for the speciation of soil organic P as it provides the most detailed and accurate information in most circumstances (Cade-Menun, 2015; Doolette and Smernik, 2011; Liu et al., 2015, 2013; Negassa et al., 2010). The use of ^{31}P NMR offers the possibility to concentrate the sample before analysis, increasing the detection limits of Po species characterization (Cade-Menun, 2005). Understanding the cycling of organic P pools and species leads to more efficient use of inorganic fertilisers in agroecosystems through the use of legacy P, which organic P is an important part of it, and helps us to improve agricultural P efficiencies in these systems. However, a downside of the technique is that it provides limited information on inorganic P (Pi) speciation and requires an extraction step before analysis (Doolette and Smernik, 2011; Negassa et al., 2010). Cade-Menun et al. (2010) successfully used ^{31}P NMR to identify specific inorganic and organic P forms and characterize their distribution patterns within soil depths in a long-term tillage study using P amendments. Stutter et al. (2015) and Solomon et al. (2002) investigated the effect of land use changes on the accumulation and changes of organic P species in soils. Stutter et al. (2015) showed that the accumulation of different soil P species in temperate soils is related to soil properties which is being controlled by a number of abiotic and biotic related processes. Solomon et al. (2002) observed that the proportions of diester-P decreased while the proportions of orthophosphate monoesters increased with land use change from natural forest to cultivated fields in tropical soils. However, only a few studies have investigated the effect of tillage systems (Redel et al., 2011) and P fertiliser use (Ahlgren et al., 2013; Guggenberger et al., 1996) on the stratification of P forms in soil profiles employing ^{31}P NMR, which was the aim of our study.

In environmental or industrial samples, where P may exist in a wider range of forms under low concentrations, chemical fractionation has been combined with XANES (Cade-Menun et al., 2015; Kruse and Leinweber, 2008) and ^{31}P NMR (Cade-Menun et al., 2015; McDowell et al., 2006) for a more accurate P characterisation. However, to date, there have been only a handful of published studies combining all three techniques to characterize P in cultivated soil samples (Kruse et al., 2015). The aim of this study was to evaluate the changes in soil P species promoted by land use change from conversion of native Cerrado (Savanna) into conventional and no-till cropping systems, by the combined use of sequential chemical P fractionation (Hedley) along with P K-edge XANES and ^{31}P NMR spectroscopies. We hypothesized that: (a) soil use and management change soil P availability and its structural composition; and (b) no-tillage can maintain more P in less strongly sorbed forms which are more plant available.

2. Material and methods

2.1. Study sites and soil characterization

Soils from four long-term field trials established in the Brazilian Cerrado region were evaluated in this study. All four soils are classified as Typic Hapludox (Soil Survey Staff, 2014), but vary in clay, Fe and Al contents. Moreover, the conversion time from the native Cerrado to agricultural land diverged among the sites. Previous characterization of legacy P in these soils after conversion to agriculture up to 2012 had highlighted that a large portion of fertiliser P inputs is stored in non-labile forms in the soil (Rodrigues et al., 2016). This study evaluated the period of cultivation up to 2016 and includes more specific analysis of P species to inform management strategies for improving P use efficiency in Brazilian oxisols.

The climate across the four sites is relatively similar and classified as Aw – tropical, according to Köppen's classification system (Alvares et al., 2013). The average annual precipitation (last 30 years) was 1930, 2150, 1600, and 1500 mm for Ox-1 (Costa Rica, Mato Grosso do Sul, 08º15'10"S / 53º12'41"W), Ox-2 (Sapezal, Mato Grosso, 13º56'33"S / 58º53'43"W), Ox-3 (Luziânia, Goiás, 16º15'02"S / 47º37'02"W) and Ox-4 (Tasso Fragoso, Maranhão, 8º30'57"S / 46º03'47"W), respectively. The rainfall distribution is characterized by a well-defined rainy season from October to April and five months of dry season (with less than 80 mm occasional rain) from May to September.

The experiments were established at different times, providing an opportunity to study the long-term effect of land use and cultivation time on soil P accumulation, speciation and partitioning. Also, in all experimental sites, the no-tillage (NT) and conventional tillage (CT) systems were compared with each other as well as with adjacent native vegetation (NV - Cerrado), allowing us to investigate the effect of tillage systems and land-use change on P dynamics and species. At Ox-1 and Ox-3 sites, the Cerrado was deforested back in 1974 and 1977, respectively, whereas Ox-2 and Ox-4 experienced shorter cultivation times, with deforestation in 1997 and 1990, respectively. Soybean (*Glycine max* L.) and maize (*Zea mays* L.) were grown at Ox-1 and Ox-3 under continuous CT for 20 and 15 years, respectively, before the beginning of the experiment in 1994 and 1992, respectively. At Ox-2, soybean and cotton (*Gossypium hirsutum* L.) were grown under CT, and occasionally under NT, until the experiment establishment in 2001. At Ox-4, soybean was grown under CT from 1990 to 1995. Thereafter, crop rotations of soybean and maize had been alternated until the experiment was established in 2001. At establishment, two different tillage systems (NT and CT) were compared at all Ox sites, which adopted a summer rotation with cotton and soybean (the latter followed by millet, *Pennisetum glaucum* L., as cover crop in the winter/dry season), except for site Ox-3 where a cotton/soybean/maize rotation was adopted. The mean annual P fertilizer addition over the experimental period was 30.5, 46.4, 35.0, and 46.4 kg P ha^{-1} at Ox-1, Ox-2, Ox-3, and Ox-4, respectively. Mean annual offtake of P across tilled plots was 19.4, 13.4, 23.5, and 13.6 kg P ha^{-1}, respectively. This leaves a net annual P surplus of 11.1, 33.0, 11.5, and 32.8 kg P ha^{-1}, respectively, for the cultivated plots at each site. More details about experimental plot sizes and management can be found in Rodrigues et al. (2016).

Topsoil samples (0–10 cm) were collected under different land-uses (NT, CT and NV) at 22, 15, 24 and 15 years after the adoption of distinct management systems in Ox-1, Ox-2, Ox-3 and Ox-4, respectively. Four subsamples were collected in each plot to generate a representative composite sample. For NV, three plots inside the Cerrado were established at least 30 m distant from the border and at least 50 m between them. Similar to the agricultural plots, four subsamples were collected in each plot and homogeneized to obtain a representative composite sample. Then, the soil samples were air-dried, grounded, sieved (2 mm grid) and stored prior to the laboratory analyses. Samples were analyzed for particle size distribution and chemical composition (Table 1).

2.2. *Soil chemical P fractionation*

The air-dried samples were subjected to a sequential chemical P fractionation according to the Hedley procedure (Hedley et al., 1982) with modifications (Condron et al., 1985). The main advantage of this method is its capability to determine both inorganic (Pi) and organic (Po) fractions concomitantly (Condron and Newman, 2011). Although the sequential P fractionation extractants give some uncertainty and may overlap in terms of the P forms extracted across variable agroecosystems (Condron and Newman, 2011), the method nevertheless provides useful insights into the lability of soil P. Detailed methodology and changes in P fractions have been previously reported by Rodrigues et al., (2020, 2016).

2.3. *Phosphorus by K-edge XANES spectroscopy analysis*

The reference standards for P-XANES analysis were either obtained from commercial suppliers (amorphous Ca-phosphate, Na-phytate, tricalcium phosphate, octacalcium phosphate, deoxyribonucleic acid, lecithin), from third persons (gibbsite, kaolinite and hematite were obtained from Dr. Audrey Gamble from the Sparks' lab at the University of Delaware) or prepared in the lab by sorbing phosphate as a K_2HPO_4 salt to mineral analogues of commonly found clay minerals and oxides in soils. Goethite was synthesized according to Abdala et al. (2015). The preparation of the standards with the sorption of P in clay minerals and oxides was carried out by equilibrating each of the solids separately with Milli-Q DI water on a 1:20 (w:v) ratio at pH 5.5 ± 0.2. The suspensions were shaken end-over-end at 60 rpm for 7 days, having the pH adjusted with 1.0, 0.1 and 0.01 mol L^{-1} solutions of KOH or HCl every other day (3 times a day). After pH stabilization, the standards were centrifuged and reacted with a 0.8 mol L^{-1} P solution (as KH_2PO_4, Sigma Aldrich), having the pH adjusted to 5.5 ± 0.2 over a 7-day reaction time. At the end of this period, the samples were centrifuged and the solutions stored for colorimetric P analysis (Murphy and Riley, 1962). In order to eliminate any residual of soluble P, the samples were washed three times with 0.01 mol L^{-1} KCl (1:20 w:v ratio) for 15 min, horizontally shaken at 150 rpm, centrifuged and dried (45 °C) for 72 h.

A composite sample representing soils from each land-use and from each study site was chosen to perform the P-XANES analysis. Air-dried soil samples were finely ground in an automated ball mill, followed by milling in an agate mortar until a 100 mesh screen sieve (0.149 mm). This is an important step to avoid background noise in the spectra. The replicate equivalent to the median result of the total P determined by the

Table 1

Soil chemical and particle size characterization (0–10 cm) of the four study sites under tillage management systems (NT and CT) and native vegetation (NV).

Study Sites	Tillage System	pH-$CaCl_2$[a]	Clay	DCB Fe[b]	DCB Al	Org. C[c]	Mehlich-1 P	Total P[d]
					—g kg^{-1}—	—mg kg^{-1}—		
Ox-1	NT	6.0	656	48.9	11.2	23.2	10.8	739
	CT	5.5				19.2	12.9	714
	NV	4.6				20.7	2.7	477
Ox-2	NT	5.0	452	48.1	13.1	20.2	11.0	632
	CT	4.8				17.5	6.0	602
	NV	3.8				19.0	1.8	440
Ox-3	NT	5.6	653	26.6	12.1	18.4	6.8	734
	CT	5.2				15.7	7.8	747
	NV	4.0				18.9	0.9	419
Ox-4	NT	4.9	255	19.8	5.8	12.7	25.8	438
	CT	4.7				10.2	14.5	348
	NV	3.8				15.7	1.1	225

NT: No Tillage; CT: Conventional Tillage.

[a] Determined after equilibrium with 0.01 mol L^{-1} $CaCl_2$ solution (1:2.5 soil:solution ratio).

[b] DCB: dithionite, citrate and bicarbonate extraction, according to the method described by Mehra and Jackson (1958).

[c] According to the Walkey-Black method.

[d] Extracted by the sulfuric digestion method (Olsen and Sommers, 1982) with colorimetric determination using the molybdate-blue method (Murphy and Riley, 1962).

sequential chemical fractionation was selected. Phosphorus K-edge XANES analyses were conducted at the Soft X-Ray beamline of the Brazilian Synchrotron Light Laboratory (LNLS), in Campinas, Brazil (Craievich and Rodrigues, 1998). The operating conditions of the storage ring at the LNLS during data collection was 1.37 GeV with a current range of 110–250 mA.

For analysis, fine-milled soil samples were uniformly spread on double-sided carbon tape to form a thin layer, mounted on a stainless steel sample holder and inserted into an experimental chamber that operates at vacuum conditions (~10^{-7} mbar). P-XANES data were obtained by fluorescence mode using a Si-drift detector. Detector dead time was monitored throughout data collection to not exceed 8%, which was achieved by moving in and out the detector in the experimental chamber. A reference energy (E^0) value of 2150.7 eV (corresponding to the second derivative of a calcium phosphate standard used for energy calibration at beamline) was used for energy calibration and scans were collected in energy ranging from 2120 to 2300 eV. The data were collected with varying step sizes of 1.0 eV from 2120 to 2145 eV, 0.2 eV from 2145.2 to 2180 eV, 1.0 eV from 2181 to 2220 eV and 3.0 eV from 2223 to 2300 eV, and an accumulation time of 1.0 s per data point. Each spectrum was obtained by averaging 8–15 individual scans for the soil samples and at least three scans for the reference standards.

Background data were subtracted by fitting a first-order polynomial to the pre-edge region from $E^0 - 30$ to $E^0 - 10$ eV and the spectrum was normalized over the reference energy of 2151 eV by fitting a second-order polynomial over the post-edge region from $E^0 + 25$ to $E^0 + 140$ eV. The phosphate species included in the fit were the ones that best represented the phosphate species found in the studied soils and were chosen based on the pre-edge, white line maximum, first post-edge crest and second post-edge crest (eV) similarities between the standards and the soil samples.

Linear combination fitting (LCF) analysis of P-XANES data was performed using the ATHENA program in the DEMETER software package (Ravel and Newville, 2005) over the $E^0 - 10$ to $E^0 + 30$ eV energy range. Fitting was done iteratively, using up to five different reference spectra at a run. Reference spectra that yielded fit as negative or represented values lower than the XANES sensitivity (5%, Beauchemin et al., 2003; Werner and Prietzel, 2015) in the fit were eliminated. LCF was performed using all possible binary, ternary and quaternary combinations of the last five selected reference spectra (after fitting with all the reference compounds, the higher percentage ones were selected for a final LCF with the most probable five species). The E^0 was allowed to vary and the weights of all P standards used were forced to sum one. The goodness-of-fit was judged by the chi-squared and R values, and P standards yielding the best fit (at the maximum quantity relative to the quaternary combinations) were determined as the most likely P species present in the investigated soil samples.

2.4. ^{31}P nuclear magnetic resonance (^{31}P NMR) spectroscopy

Solution ^{31}P NMR spectroscopy was performed using a modified version of the Cade-Menun and Preston (1996) procedure. For each soil type and land use, a duplicate air-dried soil sample (3 g) was subjected to P extraction with 25 mL of combined 0.25 mol L^{-1} NaOH and 0.05 mol L^{-1} Na_2EDTA, shaking at 250 rpm for 6 h at room temperature (~20 °C). We used a smaller soil to solution ratio (1:8) than commonly used methods (1:20), but it provides better quality NMR spectra without compromising efficiency of P species detection (Moata et al., 2015). The extracts were then centrifuged (45 min, 5000 rpm) and filtered using Whatman n.42 filter paper. A 1.0 mL aliquot was diluted for total P measurement by ICP-OES and the remaining filtrate was freeze-dried (36–48 h). The lyophilized extracts were re-dissolved in 0.6 mL deuterium oxide (D_2O), 0.6 mL of 10 mol L^{-1} NaOH and 0.4 mL of the NaOH-EDTA extracting solution (1.0 M NaOH and 0.1 M EDTA), vortexed, stand for 15 min and then vortexed again (Cade-Menun and Liu, 2014). The extracts were centrifuged (30 min, 5000 rpm) to avoid remaining particles that might contribute to line broadening and transferred to 5 mm NMR probes.

The ^{31}P NMR spectra were acquired on a Bruker Advance 400 MHz NMR spectrometer (7.5 T, 161.9 MHz), equipped with a 5-mm broadband probe. The NMR instrumental parameters were a 90° pulse, 0.68 s acquisition time, 4.321 s pulse delay, controlled temperature at 20 °C, without proton decoupling. For each sample, an amount of 3000–3500 scans (~4–5 h running time) were collected to achieve a reasonable spectral resolution (higher signal:noise ratio). The chemical shift (ppm) was referenced to an external 85% H_3PO_4 standard via the lock signal. Three parameters were observed to define the peaks: peak height and line width to determine the relative contribution of the species, and the chemical shift to identify them, by comparisons to data from the literature (Cade-Menun, 2015; Cade-Menun et al., 2010; Doolette et al., 2009; Turner et al., 2003b). Integration of peak areas on spectra was processed with a line broadening of 3 Hz using a Bruker Topspin 2.0 software and MestReNova v.6.0. Quantification of P species was performed by spectra deconvolution analysis, providing the relative P concentration in the NaOH-EDTA extracts, based on the total NMR signal area for each species. If specific identification could not be made, they were grouped into compounds or compound classes (Cade-Menun et al., 2010; Doolette et al., 2009).

3. Results and discussion

Ox-1 and Ox-3 have high clay contents (650 g kg^{-1}), while Ox-2 has slightly less clay content (450 g kg^{-1}) and Ox-4 has a high proportion of sand (700 g kg^{-1}) with only 250 g kg^{-1} of clay. Ox-1 and Ox-2 had higher contents of crystalline Fe and Al (via DCB analysis) related to Ox-3 and Ox-4. Also, Ox-4 had much lower crystalline Al content compared to the other three soils. Organic C content were not substantially changed over the years of cultivation since there were no further differences between the cultivated soils (NT and CT) and NV soil. Levels of available (Mehlich-1) and total P were substantially altered by land use change from NV to cropland in all study sites to provide suitable comparisons of P species.

3.1. Chemical P fractions

In NV, total P ranged from 225 (Ox-4) to 478 (Ox-1) mg kg^{-1} (Fig. 1A). In cultivated areas, total P was much higher than NV in all sites, ranging from 349 (CT-Ox4) to 748 (CT-Ox3) mg kg^{-1}. This reflects the high annual P inputs required to overcome P fixation in Cerrado soils (Lopes and Guilherme, 2016; Rodrigues et al., 2016; Withers et al., 2018). In general, total P levels were very similar between NT and CT systems at all sites because the overall P balance was similar for both tillage systems, i.e., no relevant differences were observed in the annual P balance ascribed to P off takes between NT and CT.

Overall, inorganic P was the dominant fraction ($\geq$ 70% of total P) at all study sites regardless of land-use although significantly less dominant in soils under native vegetation (NV - Cerrado) than in agricultural use (Figs. 1A and 1B). The land use change from NV to agriculture (both NT and CT) resulted in a significant increase in both organic and inorganic P fractions, but there was no difference between tillage systems. Abdala et al. (2015) reported similar behavior in soils receiving long-term manure applications (20–40 years) in southern Brazil, with more accumulation in inorganic forms (~80% of the total P), regardless of management system (NT or CT). These results suggest that inorganic P forms in tropical and subtropical agricultural soils are influenced more by inherent soil properties than by the soil management system, P application timing and P source, even when organic amendments are applied. In spite of our expectation to see higher Po accumulation under long-term NT (Rheinheimer et al., 2002; Tiecher et al., 2012), we observed that 67–83% and 69–85% of total P accumulated in inorganic forms under CT and NT, respectively, without any significant difference between them.

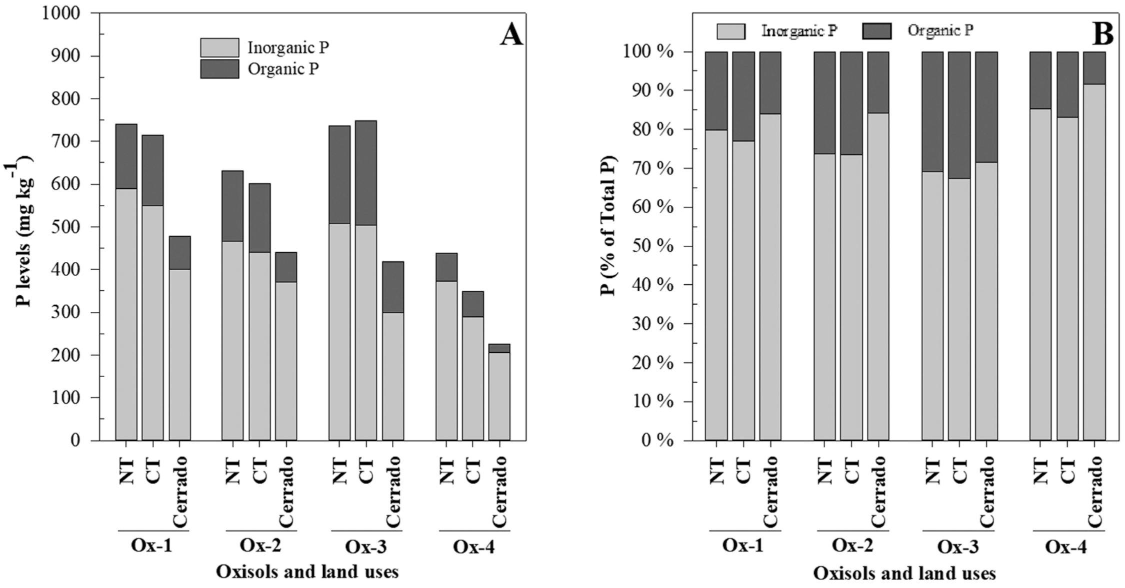

Fig. 1. Inorganic and organic P levels (A) and their relative contribution in the total P (B) of 0–10 cm depth of four tropical soils (Ox-1; Ox-2; Ox-3 and Ox-4) under distinct land uses. NT: no tillage; CT: conventional tillage; Cerrado: native vegetation (NV).

Organic P ranged widely from 8% to 33% of the total P (Fig. 1B), with the lowest values in Ox-4 due to its low clay content (Table 1). The weak stability of the P organo-complexes under lower levels of clay content makes organic P more susceptible to degradation in sandy soils in this regional climate, restricting its accumulation. Although dominated by inorganic P forms, organic P has been considered a potential sink of labile P in tropical soils (Novais et al., 2007; Novais and Smyth, 1999; Soltangheisi et al., 2019). The increase in organic P after Cerrado conversion to agriculture, regardless of tillage system, in all study sites, is a potentially positive step towards increased P availability in tropical soils (Dodd and Sharpley, 2016; Rodrigues et al., 2016). The cover crops that followed the summer crops in the rotations at all sites undoubtedly contributed additional C (Table 1), especially in the surface layer under NT, which consequently increased the organic P in this layer (Fig. 1), as found by Rodrigues et al. (2020).

Large differences in inorganic labile P were observed between

Table 2
Relative distribution of P according to Hedley soil chemical fractionation in topsoil (0–10 cm) samples of long-term field trials in Brazilian Cerrado Oxisols.

Land Use	P fractions (%, relative to total P)											
	P_{AER}	Pi_{BIC}	$Pi_{HID-0.1}$	P_{HCl}	$Pi_{HID-0.5}$	$P_{Residual}$	Inorganic P	Po_{BIC}	$Po_{HID-0.1}$	$Po_{HID-0.5}$	Organic P	Total P
	Ox-1											
NT	3.5	4.3	14.5	0.7	18.2	38.4	79.7	4.8	9.9	5.5	20.2	100.0
	(± 0.3)	(± 0.1)	(± 1.4)	(± 0.1)	(± 1)	(± 0.7)	(± 3.5)	(± 1.4)	(± 3.8)	(± 0.6)	(± 5.9)	(± 1.6)
CT	3.8	4.5	17.3	0.6	16.2	34.6	76.9	4.9	10.2	8.0	23.1	100.0
	(± 0.3)	(± 0.2)	(± 2.1)	(± 0.1)	(± 1.2)	(± 2.1)	(± 6.1)	(± 0.7)	(± 0.7)	(± 2.7)	(± 4)	(± 3.5)
NV	1.1	2.8	11.1	0.4	20.6	47.8	83.9	4.8	8.6	2.7	16.1	100.0
	(± 0.3)	(± 0.6)	(± 0.8)	(± 0.2)	(± 5)	(± 4.2)	(± 11.1)	(± 1.6)	(± 1.9)	(± 0.2)	(± 3.7)	(± 6.7)
	Ox-2											
NT	4.2	7.6	11.6	0.6	15.6	34.0	73.6	5.0	12.8	8.5	26.3	100.0
	(± 0.9)	(± 0.6)	(± 1.3)	(± 0.2)	(± 3.6)	(± 0.9)	(± 7.6)	(± 1)	(± 0.9)	(± 0.6)	(± 2.5)	(± 3.2)
CT	2.7	4.5	15.3	0.5	13.7	36.7	73.4	5.7	12.4	8.4	26.5	100.0
	(± 0.4)	(± 0.7)	(± 1.6)	(± 0.1)	(± 1.6)	(± 6.5)	(± 10.9)	(± 1.2)	(± 3.6)	(± 0.5)	(± 5.3)	(± 5.1)
NV	2.0	4.3	13.5	0.4	14.9	48.9	84.0	6.6	6.3	3.1	16.0	100.0
	(± 0.2)	(± 0.3)	(± 2.1)	(± 0.1)	(± 2)	(± 5.2)	(± 9.9)	(± 1.5)	(± 2.8)	(± 0.4)	(± 4.7)	(± 1.1)
	Ox-3											
NT	2.4	5.4	11.0	0.5	13.8	36.1	69.1	2.0	18.7	10.2	30.9	100.0
	(± 0.3)	(± 0.3)	(± 3.3)	(± 0)	(± 2)	(± 5.7)	(± 11.7)	(± 1)	(± 2.8)	(± 2.3)	(± 6.1)	(± 2.2)
CT	2.7	4.6	12.3	0.4	15.5	31.9	67.4	4.4	21.9	6.3	32.6	100.0
	(± 0.4)	(± 0.4)	(± 3.3)	(± 0)	(± 4.1)	(± 4.4)	(± 12.6)	(± 1.8)	(± 2.7)	(± 0.5)	(± 4.9)	(± 0.3)
NV	0.9	3.9	7.1	0.3	14.6	44.9	71.6	3.9	16.6	7.8	28.3	100.0
	(± 0.1)	(± 0.5)	(± 0.5)	(± 0.1)	(± 2.2)	(± 1.2)	(± 4.6)	(± 0.7)	(± 0.9)	(± 0.3)	(± 2)	(± 3.1)
	Ox-4											
NT	4.7	9.2	23.1	1.4	20.0	26.9	85.3	5.5	5.5	3.9	14.9	100.0
	(± 0.2)	(± 2.4)	(± 1.5)	(± 0.3)	(± 4.4)	(± 0.7)	(± 9.5)	(± 1.1)	(± 0.3)	(± 0.5)	(± 1.9)	(± 4.6)
CT	3.9	7.8	19.3	1.3	18.4	32.5	83.1	3.2	5.8	7.8	16.8	100.0
	(± 0.7)	(± 0.5)	(± 1.1)	(± 0.6)	(± 2.4)	(± 2)	(± 7.2)	(± 0.7)	(± 1.1)	(± 2.7)	(± 4.5)	(± 3.7)
NV	2.6	4.4	15.1	1.7	23.9	44.0	91.6	3.6	2.1	2.5	8.2	100.0
	(± 0.2)	(± 1.1)	(± 1.3)	(± 0.4)	(± 2.9)	(± 4)	(± 9.9)	(± 1.2)	(± 0.6)	(± 0.6)	(± 2.4)	(± 4)

NT: No Tillage; CT: Conventional Tillage; NV: Native Vegetation (Cerrado). Values in parenthesis are the standard deviation (n = 3). P_{AER}: P extracted with anion exchange resin; Pi_{BIC} and Po_{BIC}: inorganic and organic P extracted with $NaHCO_3$ 0.5 mol L^{-1}, respectively; $Pi_{HID-0.1}$ and $Po_{HID-0.1}$: inorganic and organic P extracted with NaOH 0.1 mol L^{-1}, respectively; P_{HCl}: P extracted with HCl 1.0 mol L^{-1}; $Pi_{HID-0.5}$ and $Po_{HID-0.5}$: inorganic and organic P extracted with NaOH 0.5 mol L^{-1}, respectively; $P_{Residual}$: P extracted with concentrated H_2SO_4, 30% H_2O_2, and saturated magnesium chloride.

cultivated soils and the NV as a consequence of fertiliser additions, with up to a three-fold increase at Ox-1 (Table 2). The relative amount of inorganic labile P (P_{AER} and Pi_{BIC}) was not significantly affected by management systems, except for Ox-2 and to a lesser extent in Ox-4, where we detected more labile P in both fractions under NT compared to CT (Table 2). At these two latter sites, there was a much larger net annual P input (Rodrigues et al., 2016). Significant effects of cultivation on labile Po (Po_{BIC}) were generally absent, except at Ox-3, where more Po_{BIC} was detected under CT related to NT (Table 2). Further details on the distribution of labile Pi and Po fractions with depth in the soil profile are given in Rodrigues et al. (2020, 2016).

The P_{HCl} fraction, representing Ca-phosphates (Hedley et al., 1982), was not detected in considerable amounts (0.3–1.7%) at any site (Table 2) since these tropical soils have no appreciable calcium in the parent material. Additionally, soil pH was less than 6.5 at all sites (Table 1), being a favorable condition for solubilization of any Ca-phosphates that may have formed after inorganic P fertiliser addition (Andersson et al., 2016). Phosphorus extracted with NaOH (both 0.1 and 0.5 mol L^{-1}), representing inorganic and organic P sorbed to Fe and Al (hydr)oxides (Condron et al., 1985), constituted 60–82% of the total P (Table 2), with similar proportions of Fe-P + Al-P forms in both CT and NT systems at all sites. The relative proportions of P forms associated with Fe-P + Al-P reduced significantly with land use change from NV to cropland (NT and CT) at all sites. The most recalcitrant P fraction (occluded P) determined by the final digestion ($P_{Residual}$) did not differ between tillage systems (NT and CT), with only slightly higher proportions in the NV (Table 2). These results confirm previous work showing that applied P fertiliser in Brazil's Oxislos becomes, in large part, associated with the Fe- and Al-P forms extracted by NaOH and of low or very low bioavailability (Novais et al., 2007; Novais and Smyth, 1999).

3.2. P-XANES spectroscopy

Prior to linear combination fitting (LCF) of our P-XANES data, reference spectra for P sorbed to mineral analogues (as described in session 2.3) were selected to provide identifiable spectral characteristics to allow interpretation of each soil sample spectrum. The observed characteristics are related to the presence of a pre-edge, edge or white line position and 1st and 2nd post-edge crests (Calvin, 2013; Werner and Prietzel, 2015). The corresponding energies for the pre-edge, white line, and 1st and 2nd post-edge crests of the reference spectra for the mineral analogues used in LC analysis are presented in Table 3.

The P-XANES data of the soil samples were performed in three steps. To provide the first hint of chemical P species in the soils, the most improbable reference Ca-P species (brushite, dicalcium phosphate, tricalcium phosphate, octacalcium phosphate and amorphous Ca-P) were used in the LCF with the P sorbed onto goethite (common Fe oxide in tropical soils). The best fit (lower R-factor and chi^2 values) was used to select of the best Ca-P standard to follow the LCF. In the second step, the "best" selected Ca-P was analyzed with the reference organic P species (IHP, Lecithin and DNA) and P sorbed onto amorphous Fe and Al minerals. The "best" fitting (maximum of ternary combinations) standard was selected and added for the 3rd step, which included one Ca-P standard, the selected organic P standard and the P sorbed onto Fe and Al minerals (P-sorbed onto amorphous Fe and Al, goethite, hematite, gibbsite, and kaolinite) standards.

Linear combination analysis was performed according to Abdala et al. (2018). Overall, P-XANES spectra of the soil samples showed white line maxima sitting around 2151.8 ± 0.2 eV which indicates that P was mostly bound to the crystalline Fe-minerals (Table 3), regardless of the management systems or sites evaluated. The 1st post edge crest in all land uses and in all sites varied from 2157.6 to 2158.2 eV, indicating the dominance of Fe/Al P phases, or eventually, organic P phases (represented by phytic acid). The 2nd post edge region ranged from 2167.8 to 2168.5 eV and did not have any specific trend regarding different soil management systems.

The LCF fitting of the samples was satisfactory, denoted by small values of R^2, ranging from 0.001 to 0.01 (Table 4), a statistical parameter which infers good agreement between measured and the predicted spectral features in the samples. The same was observed for the reduced-chi^2 values, which were very small, ranging from 0.001 to 0.01 (Table 4). The XANES analysis confirms the anticipated dominance of Fe minerals in sorbing P suggested by the chemical fractionation but additionally identifies goethite and hematite as the major Fe-P minerals in all soils, accounting for as much as 83% of the total P (CT – Ox-4; Table 4). A smaller proportion of P was sorbed onto gibbsite, accounting for as much as 14% (CT – Ox-3), which did not vary widely among cultivated soils (NT and CT) and NV, irrespective of the sites studied. Koch et al. (2018) also observed that P associated with Fe- and Al-(hydr) oxides was the dominant P form in temperate Cambisol soils in Germany. These Fe-P + Al-P fractions corresponded to 75–86%, 80–86%, 75–80% and 81–92% of the total P in Ox-1, Ox-2, Ox-3 and Ox-4, respectively (Table 4). The dominance of P associated to Fe- and Al-(hydr)oxides is explained by the high content of secondary Fe and/or Al minerals in our weathered tropical soils (Pavinato, 2009). Lower proportions of these P forms were reported by Koch et al. (2018), with values of Fe-P + Al-P ranging from 50% to 71% of total P reflecting less pronounced weathering.

Organic phosphates appeared only as phytate and ranged from 8% to 25% of total P (Table 4). Abdala et al. (2018) similarly reported small abundance of organic P detected by LCF analysis (12–18%) even in manure-amended soils. Moreover, LCF analysis indicated the depletion of organic P due to conversion to agriculture, especially under CT, from

Table 3
Phosphorus K-edge XANES spectral features and corresponding energies of reference spectra of P sorbed to mineral analogues used in the Linear Combination analysis.

Reference Standards	Pre-edge Energy (eV)	White line maximum	Post-edge shoulder	1st post-edge crest	2nd post-edge crest
Myo-inositol hexakiphosphate (phytic acid)	-	2151.5	-	2158.6	2166.8
DNA	-	2151.3	-	-	2165.7
Lecithin	-	2151.4	-	-	2166.9
Am-Ca phosphate	-	2151.4	2153.2 – 2157.3	2161.4	2168.0
Brushite	-	2151.6	2153.5 – 2158.0	2161.5	2168.8
Tri-calcium phosphate	-	2151.3	2153.5 – 2158.0	2161.0	2168.5
Octa-calcium phosphate	-	2151.3	2153.3 – 2157.5	2161.5	2168.0
P sorbed to Ferrihydrite*	2145.0 – 2148.8	2152.0	-	-	2168.0
P sorbed to Am-Al hydroxide*	-	2152.0	-	2160.6	2169.2
P sorbed to Goethite*	2145.4 – 2148.7	2152.0	-	2158.8	2168.5
P sorbed to Hematite*	2144.0 – 2148.3	2152.0	-	2159.6	2167.8
P sorbed to Gibbsite*	-	2152.0	-	2160.4	2168.8
P sorbed to Kaolinite*	-	2152.0	-	2157.5	2168.8

[*] Standards of P sorbed in different minerals analogues (sorption according to Section 2.3).

Table 4
Relative proportions of the P chemical species determined by linear combination fitting (LCF) analysis of P K-edge XANES data from the topsoil (0–10 cm) of four tropical soils (Ox-1; Ox-2; Ox-3 and Ox-4) under different land uses.

Relative % of P species (from the standards used to combinations)												
	P sorbed onto Fe oxides			P sorbed onto Al (hydr) oxides		Kaolinite-P	Ca-P		Organic P		R factor	Reduced Square chi
Land Use	Goethite	Hematite	Am-Fe[a]	Gibbsite-P	Am- yAl[b]		Apatite	Other Ca-P[c]	DNA	Phytate		
Ox-1												
NT	21 (± 8)	49 (± 7)	-	9 (± 3)	-	-	-	-	-	21 (± 2)	0.003	0.005
CT	30 (± 5)	49 (± 4)	-	7 (± 2)	-	-	-	-	-	14 (± 1)	0.002	0.002
NV	33 (± 4)	36 (± 4)	-	7 (± 3)	-	-	-	-	-	24 (± 7)	0.004	0.009
Ox-2												
NT	22 (± 8)	47 (± 6)	-	12 (± 5)	-	-	-	-	-	19 (± 2)	0.003	0.006
CT	25 (± 5)	50 (± 4)	-	11 (± 6)	-	-	-	-	-	14 (± 2)	0.004	0.009
NV	20 (± 8)	51 (± 9)	-	9 (± 5)	-	-	-	-	-	20 (± 3)	0.006	0.010
Ox-3												
NT	39 (± 6)	26 (± 5)	-	10 (± 1)	-	-	-	-	-	25 (± 2)	0.004	0.005
CT	41 (± 4)	25 (± 3)	-	14 (± 2)	-	-	-	-	-	20 (± 1)	0.001	0.001
NV	33 (± 4)	34 (± 4)	-	9 (± 1)	-	-	-	-	-	24 (± 1)	0.010	0.010
Ox-4												
NT	51 (± 6)	28 (± 5)	-	8 (± 2)	-	-	-	-	-	13 (± 1)	0.002	0.005
CT	50 (± 2)	33 (± 1)	-	9 (± 2)	-	-	-	-	-	8 (± 2)	0.010	0.010
NV	37 (± 1)	34 (± 1)	-	8 (± 1)	-	-	-	-	-	21 (± 2)	0.007	0.010

NT: no tillage; CT: conventional tillage; NV: native vegetation (Cerrado). Values in parenthesis indicate the predicted uncertainties according to LC analysis.

[a] Refers to P sorbed to the mineral analogue of Ferrihydrite

[b] Refers to P sorbed to the mineral analogue of non-crystalline Al hydroxide (non x-Al, as γ-alumina)

[c] Other calcium phosphates (brushite, dicalcium phosphate, tricalcium phosphate and beta octacalcium phosphate)

20% to 24% under the NV to 8–20% under CT for all sites, with a more pronounced depletion in Ox-4 site, a site where depletion in phytate was also observed for NT. This depletion in organic P could be due to the oxidation of carbon stocks in the soils when native soils are regularly cultivated, especially under the high temperatures experienced in the Cerrado.

However, the results of LCF for organic P contradict the outcomes of chemical P fractionation since much more organic P content was detected by the latter in cultivated soils (NT and CT) compared to NV (Table 2), suggesting that either the LCF may underestimate the organic P fractions in soils due to a lack of spectra clarity in soils with low Po content (Beauchemin et al., 2003) or that chemical P fractionation may overestimate organic P since inorganic polyphosphates that do not react with molybdate are labelled as organic P (McLaughlin et al., 2011; Turner et al., 2006).

In summary, the relative differences in P-XANES spectra among the four sites were mostly linked to soil pedogenesis rather than P input history and/or soil management (Koch et al., 2018), although some differences were detected between cultivated (NT and CT) and native soils (NV), including higher goethite-P in the cultivated soils at sites Ox-2, Ox-3, and Ox-4 compared to NV. A similar trend of Fe-P accumulation in fertilized and unfertilized treatments was reported by Liu et al. (2015, 2013).

3.3. ^{31}P NMR spectroscopy

The amounts of P detected by ^{31}P NMR spectroscopy after extraction with NaOH+EDTA (standard procedure) are shown in Table 5. The extraction efficiency, the relative proportion of P detected by NMR in total P obtained from chemical fractionation, was not high, ranging from only 12–32%. This can be related to the high P fixation capacity of our soils (Oxisols), which was also identified by chemical fractionation, where around 27–49% of total P was accumulated as residual P after NaOH extractions (Table 2), and in the P-XANES results where a dominance of Fe- and Al-(hydr)oxides (goethite, hematite and gibbsite) was observed (Table 4). The low efficiency of our extraction is evident when we compare these results with other studies like Koch et al. (2018), who extracted 51–77% of total P in temperate German soils, or Liu et al. (2013) who extracted 36–50% of total P in Canadian soils using the same procedure.

Table 5
Total P levels extracted by NaOH+EDTA and extraction efficiency of the NMR procedure from the topsoil (0–10 cm) of four tropical soils (Ox-1; Ox-2; Ox-3 and Ox-4) under different land uses.

Study Sites	Tillage System	NaOH+EDTA P mg kg^{-1}	NaOH+EDTA Extraction efficiency (% of total P)
Ox-1	NT	220.5 (± 17.2)	29.8
	CT	203.0 (± 16.0)	28.4
	NV	68.8 (± 2.9)	14.4
Ox-2	NT	125.4 (± 12.3)	19.8
	CT	134.8 (± 25.6)	22.4
	NV	54.2 (± 5.9)	12.3
Ox-3	NT	191.8 (± 35.6)	26.1
	CT	220.8 (± 29.5)	29.6
	NV	68.8 (± 2.9)	16.4
Ox-4	NT	139.9 (± 21.1)	32.0
	CT	86.9 (± 16.8)	25.0
	NV	35.4 (± 2.9)	15.7

NT: No Tillage; CT: Conventional Tillage; NV: Native Vegetation (Cerrado). Values in parenthesis are the standard deviation (n = 3).

Even though the extraction efficiency was very low, organic P species were identified predominantly as orthophosphate monoesters (mono-P) and diesters (diester-P) at all sites (Table 6). However, it was not possible to identify all the peaks in mono and diester regions due to the very low amounts of these compounds and the detection limit of NMR. Orthophosphate diesters were only detected in soils under NV and in much smaller quantities compared to monoesters, which is due to their fast hydrolytic degradation during extraction and measurement (Turner et al., 2003c) and their higher susceptibility to microbial degradation (Turner et al., 2003a). Accordingly, the absence of diesters in agricultural soils was previously reported by Cade-Menun (2005), while Condron et al. (1990) stated that they may be detected in agricultural soils in relatively small quantities (4–9%). The inorganic species were related to orthophosphate (P-ortho) and pyrophosphates. The observed species were composed, in large part, by inorganic-orthophosphate (observed in the 6 ppm spectral region), and small amounts of monoester-P (spectral

Table 6
Contents of different P species (mg kg^{-1}) in four Brazilian Oxisols with long-term land use change obtained from ^{31}P NMR analysis.

Study Sites	Land use	P species by ^{31}P NMR (mg kg^{-1})					
		Organic P			Inorganic P		
		Monoesters (5.2–3.0)	Diesters (2.0 to −2.5)	Total Po	Ortho-P (5.9 – 6.1)	Pyro-P (~ −4)	Total Pi
Ox1	NT	10.4 (± 0.8)	-	10.4	202.4 (± 15.8)	7.7 (± 0.6)	210.1
	CT	6.3 (± 0.5)	-	6.3	182.7 (± 14.4)	14.0 (± 1.1)	196.7
	NV	6.0 (± 0.3)	3.6 (± 0.2)	9.6	56.5 (± 2.4)	2.7 (± 0.1)	59.2
Ox2	NT	11 (± 1.1)	-	11.0	103.6 (± 10.2)	10.8 (± 1.1)	114.4
	CT	3.5 (± 0.7)	-	3.5	123.9 (± 23.5)	7.4 (± 1.4)	131.3
	NV	11.7 (± 1.3)	2.5 (± 0.3)	14.1	37.2 (± 4.1)	2.8 (± 0.3)	40.1
Ox3	NT	2.9 (± 0.5)	-	2.9	176.1 (± 32.7)	12.9 (± 2.4)	188.9
	CT	4.4 (± 0.6)	-	4.4	200.0 (± 26.7)	16.3 (± 2.2)	216.4
	NV	6.1 (± 0.3)	8.7 (± 0.4)	14.8	49.7 (± 2.1)	4.3 (± 0.2)	54.0
Ox4	NT	9.1 (± 1.4)	-	9.1	126.2 (± 19)	4.6 (± 0.7)	130.8
	CT	6.8 (± 1.3)	-	6.8	76.7 (± 14.8)	3.4 (± 0.7)	80.1
	NV	5.0 (± 0.4)	-	5.0	28.5 (± 2.3)	1.9 (± 0.2)	30.4

NT: No Tillage; CT: Conventional Tillage; NV: Native Vegetation (Cerrado). Values in parenthesis are the standard deviation.

region from 5.2 to 3.0 ppm), diester-P (spectral region between 2.5 and 2.0 ppm) and the inorganic pyrophosphates (around 4.0 ppm).

The relative distribution of the P-species determined by ^{31}P NMR is presented in Fig. 2A. In general, inorganic P species were dominant at all sites, ranging from 75% to 92% of total P extracted. Under NV, a higher contribution (~22–26%) of organic P species was observed, compared to the mean of the cultivated areas in Ox-2 and Ox-3 (6.0% and 3.5%, respectively), and CT in Ox-1 (3.0%), confirming the outcomes of P K-edge XANES analysis (Table 4). Considering organic P species, differences between tillage systems (NT and CT) were also detected at two sites, being 67% and 233% higher under NT relative to CT in Ox-1 and Ox-2, respectively.

Phosphorus species determined by ^{31}P NMR were converted into real P content in the soil (Fig. 2B) by considering the soil total P (Table 1) and the solution ^{31}P NMR extraction efficiency (Table 5).

In general, organic P totals determined by ^{31}P NMR were smaller than the values obtained from chemical fractionation and XANES, equivalent to, on average, less than 10% of total P. This result was not expected since this technique should be very precise in measuring soil organic P species (Cade-Menun, 2005). P K-edge XANES detected 14–25% of organic P (phytate) in total P, except in Ox-4, the site with lower clay and less organic matter content, whilst chemical fractionation detected 14.9–32.6% of total P as organic P, with also smaller values in Ox-4.

4. Conclusions

Long-term P inputs following conversion from native Cerrado forest clearly generated a substantial P accumulation in the topsoil (legacy P) under NT and CT management regimes at four Oxisol soils of varying clay content in Brazil as determined by sequential chemical P fractionation. This method was useful to identify labile P fractions, which constituted the lowest proportion of total P in our study, and to highlight the dominance of inorganic Fe/Al oxyhydroxide P forms. Additional analyses by P K-edge XANES further showed that the major part of the total P at all four sites was sorbed on the Fe- and Al-(hydr)oxides, including goethite, hematite and gibbsite. Among organic P species, only phytate was identified in our soils by this technique, with larger amounts in soils under native vegetation than in cultivated soils, as might be expected. ^{31}P NMR analysis identified recalcitrant monoester-P as the major form of organic P across all soils and management systems, whereas diester-P was found only in the soils under native vegetation.

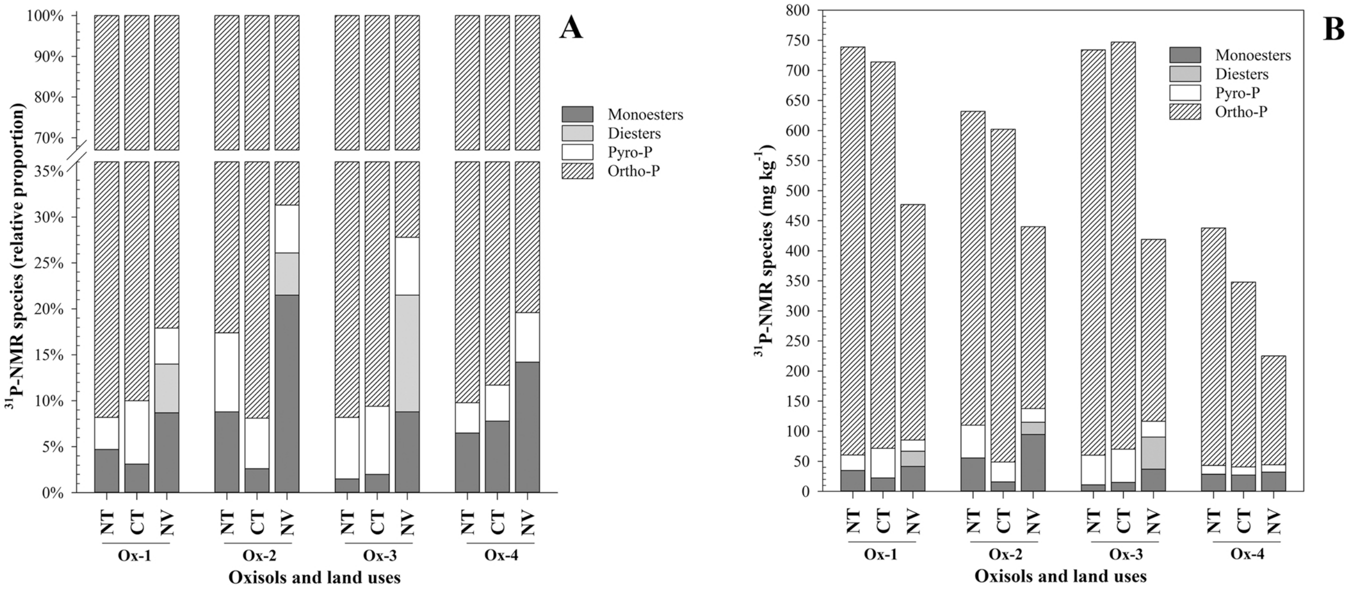

Fig. 2. Relative distribution (%) of P species (^{31}P NMR) (A) and P species levels estimated by ^{31}P NMR in relation to the total P (mg kg^{-1}) in the topsoil (0–10 cm) of four tropical soils (Ox-1; Ox-2; Ox-3 and Ox-4) with long-term land use change and distinct tillage systems. NT: no tillage; CT: conventional tillage; NV: native vegetation (Cerrado).

Orthophosphate-P was the dominant form of the inorganic P species, but a significant amount of pyrophosphate was also detected in all the samples.

The combined use of chemical fractionation, XANES and NMR provided a clearer understanding of the inorganic and organic P forms in tropical soils than the use of one method in isolation. A combination of at least chemical fractionation followed by NMR is appropriate when the sample matrix is predominantly organic P, and a combination of at least or chemical fractionation followed by XANES is recommended when inorganic P predominates. Our results suggest that progress towards greater P use efficiency in farming systems on Brazilian Oxisols depends on innovative agronomic techniques to release the large amounts of legacy soil P bound to Fe and Al, and to a lesser extent on monoester-P.

Declaration of Competing Interest

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Data availability

Data will be made available on request.

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