Assessing organic phosphorus status of Cerrado oxisols (Brazil) using ^{31}P-NMR spectroscopy and phosphomonoesterase activity measurement

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[1]*Laboratoire d'Ecologie des Sols Tropicaux, IRD, 32 avenue H. Varagnat, 93 143 Bondy Cedex, France (e-mail: lardyl@yahoo.fr);* [2]*IRD/Embrapa Cerrados, CP 7091, 71619-970 Brasilia-DF, Brazil;* [3]*UMR Sol et Environnement, INRA-ENSAM, 2 place Pierre Viala, 34060 Montpellier Cedex, France.*
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Chapuis-Lardy, L., Brossard, M. and Quiquampoix, H. 2001. **Assessing organic phosphorus status of Cerrado oxisols (Brazil) using ^{31}P-NMR spectroscopy and phosphomonoesterase activity measurement**. Can. J. Soil Sci. **81**: 591–601. Plant production in the Brazilian savannas, also known as the Cerrados, is limited mainly by low P availability in soils. Little is known about the P status in the Cerrados region, despite its increasing significance for the country's agriculture.

^{31}P-NMR spectroscopy of alkali extracts and **phosphomonoesterase (PME)** activity measurements were used to study forms of P and their stability in oxisols of natural and pasture systems. **Total P content (Pt)** in topsoils ranged from 301 to 456 mg kg^{-1} and **organic P content (Po)** from 84 to 194 mg kg^{-1} with the highest values under natural vegetation. The estimation of forms of soil P with different lability (provided by $NaHCO_3$ and NaOH extractions) showed little difference between natural vegetation and pastures, but the proportions of Po extracted suggest the importance of organically bound P as a source of plant-available P. All NMR spectra showed signals of organic P (monoesters and diesters) and inorganic P forms (orthophosphate and pyrophosphate), with little influence of land use. Organic P appears to be mainly in the form of stable phosphomonoesters. The most marked effect of land use was a decrease in PME activity under recent pastures, indicating an increase of PME stability in topsoils after the maize-grass ley plantation. The importance of the pH of the PME activity measurement is also discussed.

Key words: Organic P, labile P, ^{31}P-NMR, phosphosmonoesterase, Cerrado, Oxisols.

Chapuis-Lardy, L., Brossard, M. et Quiquampoix, H. 2001. **Détermination du statut du phosphore organique d'oxisols de la région des Cerrados (Brésil) par RMN du ^{31}P et mesures d'activité phosphomonoestérase**. Can. J. Soil Sci. **81**: 591–601. La production des savanes brésiliennes connues sous le nom de Cerrados est principalement limitée par une faible disponibilité du phosphore présent dans les sols. Malgré l'importance croissante de cette région pour l'agriculture brésilienne, le statut du phosphore reste peu étudié. Cette étude a été réalisée sur des échantillons d'oxisols prélevés sous végétation naturelle de savanes et sous pâturages. Les formes du phosphore et leur stabilité ont été étudiées par résonance magnétique nucléaire (RMN du ^{31}P) et mesures de l'activité phosphomonoestérasique (PME). Le contenu en phosphore des horizons superficiels s'échelonne respectivement de 301 à 456 mg kg^{-1} sol pour le phosphore total (Pt) et de 84 à 194 mg kg^{-1} pour le phosphore organique (Po) ; les valeurs les plus élevées étant observées sous végétation naturelle. La quantification des formes de phosphore plus ou moins assimilables présentes dans les sols (extraites par $NaHCO_3$ et NaOH) montre peu de différences entre la végétation naturelle et les pâturages ; Les quantités obtenues suggèrent cependant l'importance des formes organiques comme source de phosphore assimilable par les plantes. Tous les spectres obtenus par RMN révèlent la présence de formes organiques (monoesters et diesters) et inorganiques (orthophosphates et pyrophosphates) ; Le mode d'utilisation des terres ne semble pas avoir de véritable influence sur la distribution de ces différentes formes de P. Dans ces sols, le phosphore organique est essentiellement sous la forme stable de phosphomonoesters. L'activité phosphomonoestérase (PME) est plus faible dans les sols des pâturages récents que sous végétation naturelle ; cette diminution est indicatrice d'une stabilité accrue des phosphomonoesters consécutivement à la mise en pâturage. L'importance du choix du pH pour la mesure enzymatique est également discuté.

Mots clés: Phosphore organique, Phosphore assimilable, ^{31}P-RMN, Phosphosmonoestérase, Cerrado, Oxisols

The Cerrado region is an area about 200×10^6 ha, which has been submitted to intense agricultural expansion since the 1970s. Low P supply is known to be a major agronomic constraint in the highly weathered soils of Cerrados (Goedert 1983), caused by the high phosphate sorption capacity of the clay fraction and the behaviour of phosphate ions in such soils. The mineralization of inorganic P from organically bound P is fundamental to plant nutrition in tropical soils (Harrison 1987). Sodium bicarbonate (Olsen et al. 1954) extracts a combination of inorganic P (Pi) in the soil solution, some Pi held on exchange complexes and labile (easily mineralized) organic P (Po) substrates (Bowman and Cole 1978a). This method is commonly used to establish labile organic and inorganic P levels in soils. Given the importance of labile Po in natural systems for the P supply of plants, we have quantified the Po content in the $NaHCO_3$ extracts. Extraction with sodium hydroxide (NaOH) provided the moderately labile P (retained by Fe- and Al-oxides) in addition to the forms obtained by bicarbonate extraction. Since the work of Newman and Tate (1980), ^{31}P-NMR

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analysis has been used to examine the structural composition of alkali-soluble P (Hawkes et al. 1984; Condron et al. 1990; Guggenberger et al. 1996a). These authors and others have shown that Po in alkali extracts of topsoils consists mainly of phosphomonoesters. This form of Po appeared to be relatively resistant to mineralization.

Phosphatases perform an important function in soil by transforming organic P into inorganic phosphate (HPO_4^{2-}, $H_2PO_4^-$), which is available to plants and microorganisms. Acid and alkaline PME, distinguished by their pH optima, are present in soils (Tabatabai 1982) and transform phosphomonoesters into inorganic P. Acid phosphatases would generally be expected to be more common in acidic soils (Eivazi and Tabatabai 1977), and thus in Cerrado soils because the soil pH is less than 6.0. Few reports are currently available on PME activity in Oxisols under the savanna types known as Cerrados. Phosphatase research in Cerrado soils has been based on a single pH condition of 6.5, as recommended by Tabatabai and Bremmer (1969) to assay enzyme activity at its optimum value. We tested PME activity at different pH values and especially at 6.5 to provide a comparison with data from the literature. Although ^{31}P-NMR spectroscopy is a powerful tool to assess the structural composition of soil Po, few data are available on forms of organic P in Cerrado soils. We have, therefore, characterized the organic P fraction that is potentially available to plants using ^{31}P-NMR spectroscopy.

Because the turnover of organic P in soil depends on microbial activity, the presence of labile Po is susceptible to change in land use (Condron et al. 1990; Guggenberger et al. 1996a). Pastures have become an increasingly widespread form of land use following deforestation or cultivation of tropical savanna soils. With the development of exotic grass plantations during the past 25 yr, *Brachiaria* ssp. probably accounts for 85% of the total area planted in Cerrado region (Macedo 1995). We therefore examined organic P status in soil under native savannas and plantations of *Brachiaria brizantha.*

Knowledge about the status of P, especially of the organic fraction, is essential for the proper understanding and practical management of the P cycle in the oxisols of the Cerrado region. The main goal of this work was to obtain information on the organic P by combining ^{31}P-NMR with enzymatic measurements for field situations.

MATERIALS AND METHODS

Natural Vegetation

Four sites were selected under native vegetation in the ecological reserve of Roncador near Brasilia (15°57′S, 47°52′W; alt. 1120 m). The mean annual temperature is 26°C, and the average annual rainfall is about 1600 mm with more than 50% from December to March. Within the general term "cerrado", savanna presents a gradient of physiognomies, from the grassland type (the "campo limpo", denoted Cl) to a closed tree canopy (the "cerradão", denoted Cdão) (Eiten 1972). Between these, there are intermediate physiognomies, including a typical savanna ("cerrado" sensu stricto, denoted Cdo).

Pastures

Pastures were selected in experimental systems of Embrapa-Cerrados also located in the Great Plateau of Central Brazil (15°38′S, 47°45′W). These sites presented climate and soil type similar to those of natural systems.

A part of the area was kept as natural vegetation of Cerrado (sensus stricto) to become a control (P-Cdo) and sometimes used as natural pasture. In 1982, four plots were limed, fertilized and planted with *Brachiaria brizantha* (P12). In order to slow pasture degradation, three of these four plots were limed and fertilized again and a single maize-grass ley was grown, respectively in 1990, 1991, 1992. When the soil was sampled in May 1995, subsequent pastures were aged 12 (P12), 5 (P5), 4 (P4) and 3 (P3) years.

Soil Sampling

Soil samples were collected with a shovel from 10 points randomly located within each plot, avoiding a 2-m zone around the edge of the plot, and then bulked by depth: 0–10, 10–20, 20–30, 30–50 cm for all sites and 50–60 or 50–70 cm, respectively, for natural sites and pastures. Samples were air-dried and screened through a 2-mm stainless steel sieve. Both native and pasture sites are oxisols (Dark Red Latosol according to the Brazilian soil classification).

Soil Analysis

General Analyses

The clay contents of the soil samples were determined after dispersion and sedimentation using the pipette method. The pH was measured in H_2O at a soil:solution ratio 1:2.5 using a glass electrode. The cationic exchange capacity and base saturation were determined after extraction by barium chloride (Rhoades 1982) and analysis using atomic absorption spectrometer. Exchangeable aluminium was extracted with 1 M KCl. Amorphous and crystallized oxide forms of Fe and Al were removed using oxalate solution [Tamm method as described by Blakemore et al. (1987)] and dithionite-bicarbonate-citrate method (Mehra and Jackson 1960), respectively. The most important properties of the soils are given in Table 1. Complete chemical characterization of samples P3, P4 and P5 (3-, 4- and 5-yr-old pastures) was not carried out for practical reasons, but preliminary trials showed that their texture, cation exchange capacity and mineralogy did not differ from the 12-yr-old pasture.

Whole Soil Pt, Po, C

Total P was determined by the colorimetric method (John 1970) after combustion at 550°C and digestion in concentrated HNO_3 of a 2-g sample (Laurent and Brossard 1991). Total organic P (Po) was determined after combustion at 550°C and extraction with 2 N H_2SO_4 (Anderson 1960). Three replicates were analyzed. Total Pi concentration was calculated as the difference between Pt and Po. Total C content was obtained by dry combustion using an ANA 1500 Carlo Erba analyzer. The relative analytical error of C concentrations was ± 2%.

$NaHCO_3$ Pt, Pi

A 1.5-g sample of soil was shaken with 60 mL of 0.5 M sodium bicarbonate adjusted at pH 8.5 for 17 h at 25°C. The

suspension was centrifuged for 20 min at 12 500 rpm, acidified with concentrated H_2SO_4 and filtered through an ashless filter. Inorganic P was determined in the filtrate by a colorimetric method (John 1970). For determination of Pt, 5 mL of the solution was digested with acidified potassium persulphate at 120°C in an autoclave for 60 min as described by the Environmental Protection Agency (1971). The organic P content was calculated as the difference between Pt and Pi. Assays were conducted in triplicate on topsoil (0–10 cm) samples, except for Cerrado (Cdo).

NaOH Pt, Po

Three 25-g subsamples (air-dried, < 2 mm) of 0–10 cm layers (except for Cdo) were shaken in centrifuge tubes with 100 mL 0.5 M NaOH for 17 h, centrifuged (12 500 rpm, 20 min), and the supernatant filtered through an ashless filter paper (slow filtration, Prolabo). Methods used to determine P contents were the same as in the $NaHCO_3$ extraction.

^{31}P-NMR Spectroscopy

Alkali-extraction (NaOH) has been used for ^{31}P-NMR investigations by numerous workers (e.g., Newman and Tate 1980; Hawkes et al. 1984; Condron et al. 1990; Guggenberger et al. 1996a) as this procedure extracts labile forms of P, which are of interest in studies of cycling and availability to plants. The NaOH extracts of the topsoil samples were concentrated on a rotary evaporator from 60 mL to 6 mL. A Varian Unity Inova 500 spectrometer operating at 202.42 MHz for ^{31}P was used to give non-^{1}H-decoupled spectra after collection of 1000 scans. Additional recording conditions were: temperature, 25°C; spectral width, 25.3 kHz; pulse angle, 90°; recycle time, 25 s; acquisition time, 0.63 s. With this set of acquisition parameters, a peak corresponding to a P compound of 1 μg P mL^{-1} can be detected and, since the recycle time is more than five times the spin-lattice relaxation time, T_1, the peaks on the NMR spectrum are proportional to their P content. Samples contained of 3.15 mL of concentrated NaOH extract to which 0.35 mL D_2O was added for the ^{2}H field/frequency lock, and they were run in 10-mm-diameter tubes. The quantities of the various forms of P were calculated from peak area ratios, and peaks were assigned according to literature data (Newman and Tate 1980; Condron et al. 1990). No signal was detected outside the 10 to −10 ppm range.

Assay of Phosphomonoesterase Activity

The PME (EC 3.1.3.2.) activity in soils was assayed using sodium ***p*-nitrophenol phosphate (*p*NPP)** as a substrate (Tabatabai 1982). A 1-g sample of soil was incubated at 37°C for 1 h in 4 mL **modified universal buffer (MUB)** (Skujìns et al. 1962), 0.2 mL toluene and 1 mL 0.025 M *p*NPP. Then 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were added to the mixture. Addition of $CaCl_2$ prevents dispersion of clay during the subsequent treatment with NaOH. The addition of NaOH is necessary to extract the **p-nitrophenol (*p*NP)** from the soil because *p*NP is partially adsorbed by soil under neutral or slightly acidic condition (Pettit et al. 1977). The soil suspension was swirled and filtered through a Whatman No. 2v folded filter paper. The optical density was measured with a spectrophotometer (Spectronic Genesys 5, Milton Roy Co.) at 410 nm. The number of analytical replicates per sample has been established by preliminary trials. Assays were in triplicate and PME activity was expressed as the amount of colorimetrically determined *p*NP released during 1 h incubation per gram of dry soil (μg *p*NP released g^{-1} soil h^{-1}). Controls were performed by adding 1 mL *p*-NPP to suspension containing soil, MUB and toluene after the alkalinisation with $CaCl_2$ and NaOH.

Distribution of PME Activity

Assays were performed with the MUB solution buffered at pH 6.5 described by Tabatabai and Bremmer (1969) as the pH of optimum acid PME activity. Soil samples at different depths, from 0 to 60 or 70 cm, respectively, for natural sites and pastures were assayed.

Optimum pH for PME Activity.

To obtain the pH range in which optimum PME activity occurred in these soils, a series of MUB solutions with pH values between 4.0 and 6.5 were used. Assays were carried out with 0–10 cm samples.

Statistics

For chemical analysis and PME activity measurements, error variances were estimated from the measured data (triplicates). For the ^{31}P-NMR we assumed population variances of 5% (Preston 1987). *t*-tests were performed and values were considered as different when probability was lower than 5%.

RESULTS AND DISCUSSION

Soil Analysis

Soils were very deep, well drained, acid and had a low cation exchange capacity (Table 1). The composition of the clay-sized fraction (kaolinite, gibbsite and iron oxides) determined the behaviour of these soils in terms of ion exchange, water-holding characteristics and phosphate adsorption.

The C and Pt contents of natural and pasture topsoils were in the ranges 24.0–48.2 g C kg^{-1} soil and 301.0–456.1 mg Pt kg^{-1} soil, respectively (Table 2). Carbon and Pt concentrations in soils under natural systems do not differ from values given in other studies for similar vegetation [for C, review in Brossard et al. (1997); for Pt, see Guerra et al. (1996)]. Therefore, the Pt contents in the topsoils of the grassland (Campo limpo, Cl, 456 mg kg^{-1}) and the typical savanna (Cerrado, Cdo, 436 mg kg^{-1}) were significantly ($P < 0.05$) larger than others (321–376 mg kg^{-1}), and lower in the 12 yr-old pasture (P12, 301 mg kg^{-1}). The Po contents of 0–10 cm samples varied between treatments with significantly ($P < 0.05$) larger values under natural vegetation (in the order: Cl > Cdão > Cdo). Differences between natural vegetation and pastures in whole soil total and organic contents are significant only in the topsoils. Organic P showed no significant difference between natural vegetation and pastures in the 10–20 cm soil layer (64–103 mg kg^{-1}) and in the 30–50 cm samples (53.6–79.4 mg kg^{-1}). Organic P rep-

Table 1. Some properties of soils

Site[z]	Horizon	Depth (cm)	Clays (g kg^{-1})	pH (H_2O)	Effective CEC (mmol kg^{-1})	Al^{3+} (KCl) (mmol kg^{-1})	Base saturation (%)	Fe_d[y] (g kg^{-1})	Fe_o[y] (g kg^{-1})	Al_d[y] (g kg^{-1})	Al_o[y] (g kg^{-1})
Cdão	A	0–15	689	4.7	43.3	111	34.0	8.98	0.25	3.12	0.62
	BA1	30–55	704	4.9	36.4	ND[x]	31.7	9.16	0.17	2.96	0.55
Cdo	A	0–15	672	5.0	39.0	66	41.4	11.40	0.26	3.44	0.66
	BA1	30–55	728	5.3	31.1	ND	60.3	10.35	0.16	3.01	0.54
Cl	A	0–10	474	5.2	29.0	47	66.3	8.78	0.35	3.84	0.95
	BA1	20–35	436	5.3	24.3	ND	45.3	10.47	0.29	4.10	0.72
P-Cdo	A	0–20	716	4.9	29.9	26	22.1	9.74	0.13	2.45	0.51
	AB	20–60	724	5.1	36.4	ND	12.9	9.41	0.16	2.53	0.58
P12	A	0–10	488	5.3	33.7	7	99.3	8.55	0.20	2.55	0.54
	AB	10–55	635	5.0	17.8	ND	73.3	8.52	0.16	2.35	0.52

[z]Cdão (Cerradão), Cdo (Cerrado), Cl (Campo limpo): from closed to open savanna; P-Cdo, pastured Cerrado, the control for pastures; P12, 12-yr-old pasture of *Brachiaria brizantha.*

[y]Fe_d, Fe_2O_3 in dithionite-bicarbonate-citrate extract; Fe_o, Fe_2O_3 in oxalate extract; Al_d, Al_2O_3 in citrate-bicarbonate-dithionite extract; Al_o, Al_2O_3 in oxalate extract.

[x]ND, no determination.

Table 2. Whole soil P and C contents and pH values

Site[z]	Depth (cm)	Pt (mg kg^{-1})	Po (mg kg^{-1})	Po/Pt[y] (%)	C (g kg^{-1})	pH (H_2O)
Cdão	0–10	359.4 (2.2)[x]	147.0 (5.9)	41	42.3	4.7
	10–20	329.0 (2.2)	103.5 (8.3)	31	25.8	4.7
	30–50	270.9 (1.7)	63.9 (8.7)	24	17.7	4.9
Cdo	0–10	436.8 (4.6)	114.8 (6.7)	26	33.5	5.0
	10–20	385.2 (4.6)	95.5 (2.9)	25	26.5	5.1
	30–50	281.5 (9.3)	72.3 (3.4)	26	17.1	5.3
Cl	0–10	456.1 (9.1)	194.7 (8.0)	46	48.2	5.2
	10–20	373.2 (9.1)	86.0 (1.9)	23	41.0	5.2
	30–50	283.4 (13.8)	63.6 (3.6)	22	21.1	5.3
P-Cdo	0–10	343.7 (25.7)	96.7 (4.9)	28	33.3	4.9
	10–20	324.7 (29.8)	85.7 (1.2)	26	26.7	4.9
	30–50	271.4 (29.1)	79.4 (5.7)	29	17.2	5.1
P12	0–10	301.0 (5.3)	91.1 (2.3)	30	28.9	5.3
	10–20	272.4 (2.1)	64.6 (3.0)	24	25.0	5.6
	30–50	234.0 (5.7)	53.6 (1.9)	23	15.1	5.0
P5	0–10	321.3 (2.0)	84.5 (14.2)	26	24.0	5.8
	10–20	309.4 (5.4)	90.8 (2.7)	29	23.8	5.7
	30–50	285.7 (5.6)	68.6 (2.9)	24	14.4	5.8
P4	0–10	376.5 (2.1)	87.8 (9.4)	23	24.5	5.8
	10–20	311.7 (46.3)	64.0 (5.2)	21	24.2	5.8
	30–50	246.9 (13.3)	64.6 (4.5)	26	16.2	5.6
P3	0–10	345.5 (29.9)	91.9 (5;1)	27	31.2	5.9
	10–20	320.2 (26.3)	87.5 (3.5)	27	29.3	5.7
	30–50	251.2 (3.7)	59.4 (0.9)	24	17.9	5.6

[z]Cdão (Cerradão), Cdo (Cerrado), Cl (Campo limpo): from closed to open savanna; P-Cdo, pastured Cerrado, the control for pastures; P12, 12-yr-old pasture of *Brachiaria brizantha*; P5, P4, P3 correspond to interruption of *Brachiaria brizantha* with a single maize culture 5, 4 and 3 yr, respectively, before sampling.

[y]Proportion of organic P.

[x]Values in parentheses are standard deviations.

resented at least 21% of total P, independently of depth. In a synthesis of data from the world literature, Harrison (1987) reports smaller organic P contents in savanna soils. As similar concentrations of C and organic P were observed under pastured Cerrado and pastures, the land-use effect at these levels seems to be limited. Adsorption onto clay minerals probably protected organic matter against fast microbial degradation (Greenland 1965; Lilienfein et al. 1998).

Table 3. Contents of 0–10 cm samples of Pt and Po in the $NaHCO_3$ and NaOH extracts

	$NaHCO_3$			NaOH		
Site[z]	Pt (mg kg^{-1})	Po (mg kg^{-1})	Po/Pt[y] (%)	Pt (mg kg^{-1})	Po (mg kg^{-1})	Po/Pt[y] (%)
Cdão	14.2*a* (4%)[x]	11.2*a* (8%)	79	89.6*b* (25%)	37.6*c* (26%)	42
Cl	20.7*b* (5%)	17.8*c* (9%)	86	145.6*c* (32%)	66.8*e* (34%)	46
P-Cdo	11.3*a* (3%)	9.2*a* (9%)	81	67.7*a* (19%)	23.1*a* (24%)	34
P12	8.9*a* (3%)	7.8*a* (9%)	88	65.6*a* (22%)	30.2*b* (33%)	46
P5	11.0*a* (3%)	9.0*a* (11%)	82	68.3*a* (22%)	29.8*b* (35%)	44
P4	22.2*b* (6%)	13.2*b* (15%)	59	132.3*c* (35%)	30.5*b* (35%)	23
P3	10.1*a* (3%)	7.8*a* (8%)	77	83.7*b* (25%)	43.4*d* (47%)	52

[z]Cdão (Cerradão), Cl (Campo limpo): closed and open savanna types; P-Cdo, pastured Cerrado, the control for pastures; P12, 12-yr-old pasture of *Brachiaria brizantha*; P5, P4, P3 correspond to interruption of *Brachiaria brizantha* with a single maize culture 5, 4 and 3 yr, respectively, before sampling.
[y]Proportion of organic P in the extract.
[x]Values in parentheses indicate proportions of whole soil content found in extracts.
a–e Within each column, means followed by the same letter are not significantly different ($P < 0.05$).

A much larger proportion of the whole soil Pt was extracted in the topsoil by NaOH (19–35%) than by $NaHCO_3$ (3–6%) (Table 3). These extracted proportions are greater than those reported by de Araújo et al. (1996) and Guerra et al. (1996) for air-dried samples of oxisols under Cerrado vegetation or pasture. No ultrafiltration was carried out after extraction; therefore, some colloidal P may be present in the extracts. The same trend is observed for topsoil Po, but the proportions extracted are even greater, particularly for the bicarbonate extract: 26–47% in hydroxide extract and 8–15% in the bicarbonate extract. Total P content in $NaHCO_3$-extracts is significantly ($P < 0.05$) higher in 4-yr-old pasture and Campo limpo (respectively, P4, 22.2 mg kg^{-1} and Cl, 20.7 mg kg^{-1}) than in the other topsoils (8.9–14.2 mg kg^{-1}, Table 3). Extracted organic P contents followed a similar order: Cl > P4 > others. Bicarbonate extractable organic P has been found to be highly labile and available to plants and microbes in laboratory incubations (Bowman and Cole 1978b) and comparative field studies (Tiessen et al. 1984; Gahoonia and Nielsen 1992). In our study, there was a small difference for this P fraction between natural systems and pastures, but no net trend of land-use effects as demonstrated by Oberson et al. (1993) for cultivation systems. In NaOH-extracts, total P amounted to 65.6–145.6 mg kg^{-1} soil while organic P represented 23.1–66.8 mg kg^{-1} soil (with a lower value for P-Cdo, the typical savanna used as a control for pasture systems). In $NaHCO_3$ extracts, Po accounted for 77–88% of the extracted Pt, except for the 4-yr-old pasture (P4) topsoil, which was somewhat lower (59%), whereas proportions of Po in NaOH-extracts were lower (23% for P4 and 34–52% for the other topsoils). In a summary of the literature, Cross and Schlesinger (1995) suggested that the bicarbonate Po as a percentage of the total labile P forms (resin Pi, bicarbonate Pi and Po) represents a minimum index of the fraction of P that may be easily mineralized through biological processes. These authors showed that bicarbonate Po might represent nearly 80% of the total labile P in oxisols. Hydroxide organic P is more stable and turns over more slowly in the field (Bowman and Cole 1978a), but it may also contribute directly to plant-available P (Gahoonia and Nielsen 1992). Organic P extractable by NaOH has been used as an indicator of the P status and fertility of soils. This pool is thought to represent overall changes in soil organic matter and organic P levels by functioning as an active reservoir and source and sink of P when the soil is stressed by cultivation and net P export (Stewart and Tiessen 1987; Magid and Nielsen 1992; Tiessen et al. 1992, 1994; Beck and Sanchez 1994; Paniagua et al. 1995). In our study, the proportions of organic P in both extracts suggested the importance of organically bound P as a source of P for plants.

^{31}P-NMR Analysis of Alkali Extracts

The ^{31}P NMR spectra revealed several distinct forms of P in the alkali extracts (Fig. 1). All samples showed similar resonances in the spectra. Percentage peak areas indicating the proportion of total spectral area assigned to the different forms are given in Table 4. Intense, sharp signals at δ = 6.0–6.2 ppm were due to orthophosphate (inorganic P, Pi). Signals relating to pyrophosphate, another form of Pi, were observed in the range δ = –4.3 to –4.2 ppm. Phosphomonoesters, a group comprising inositol phosphates, sugar phosphates and mononucleotides, resonated at δ = 4.8–5.2 ppm. Signals at 0.4 to –1.0 ppm are due to phosphodiesters, e.g., phospholipids and DNA (Newman and Tate 1980), which caracterized a labile soil Po fraction. One signal was observed in this region between –0.3 and –0.1 ppm and accounted for 4.3 to 11.4 % of extracted P (Table 4). The spectra did not show typical signal of polyphosphates (δ around –20 ppm), an inorganic P accumulation form.

The inorganic P contents (52–74%, Table 4) as determined by ^{31}P-NMR spectroscopy (sum of orthophosphate and pyrophosphate) are in good agreement with the results derived from conventional chemical analyses (inorganic P = 54–77%, Table 3). The higher orthophosphate concentration of 4-yr-old pasture presumably results in part from the mineralization of diester-P forms, which are present in a lower proportion than in other pastures. The reason for the distribution of P forms in the 4-yr-old pasture is not known.

The predominance of monoester forms in the organic fraction in all spectra concurs with that reported in several

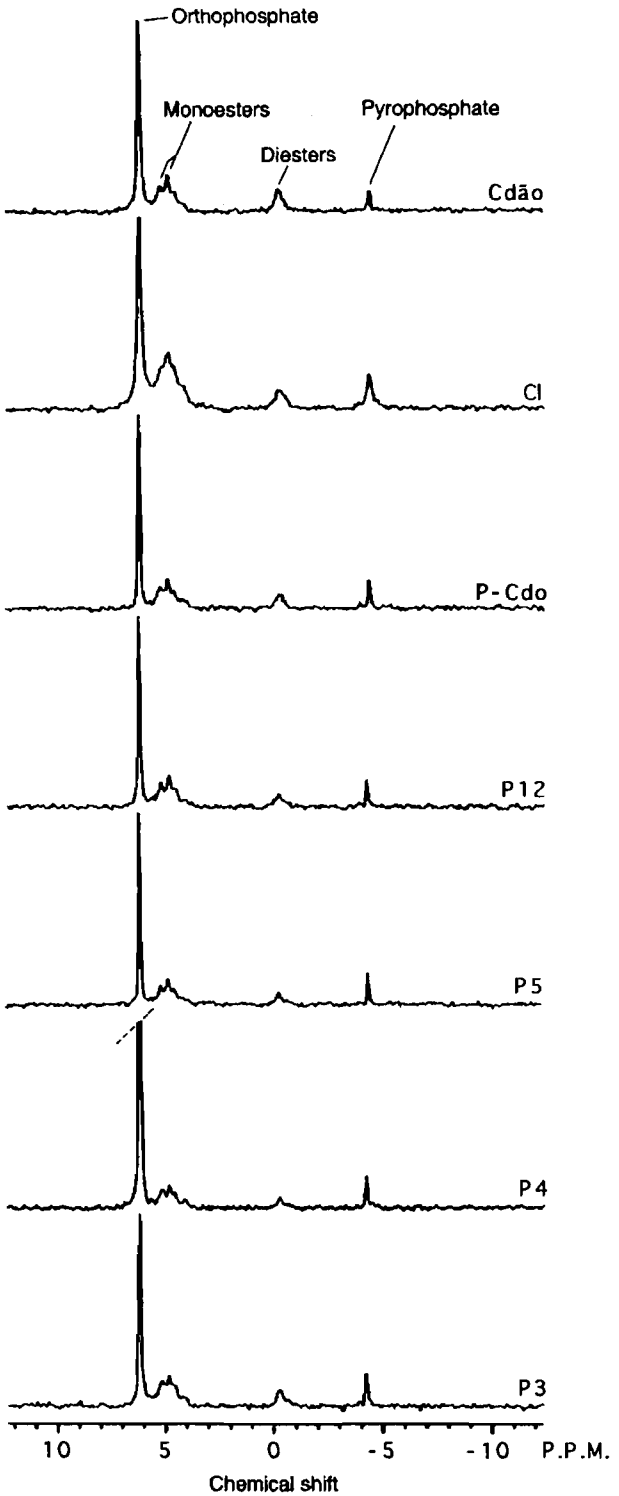

Fig. 1. ^{31}P-NMR spectra of NaOH extracts obtained from the 0–10 cm samples. Cdão (Cerradão), Cdo (Cerrado) and Cl (Campo limpo) are different physiognomies of Cerrado savanna, from closed to open type; P-Cdo is a pastured Cerrado used as the control for pastures; P12 represent the 12-yr-old pasture of *Brachiaria brizantha*; P5, P4, P3 correspond to interruption of *Brachiaria brizantha* with a single maize culture 5, 4 and 3 yr, respectively, before sampling.

previous NMR studies of organic P forms in soils of Europe, Mexico, New Zealand, Canada and Spain (e.g., Tate and Newman 1982; Hawkes et al. 1984; Condron et al. 1990; Guggenberger et al. 1996a; Turrión et al. 2001). Hinedi et al. (1988) showed that the monoesters are stable while the diesters are relatively rapidly converted to inorganic forms. The protection of organic matter in clayey soils, mainly by adsorption onto clay minerals, is often discussed. The high stability of monoesters against microbial and enzymatic attack is caused by their strong interactions with soil minerals because of their high charge density and precipitation as sparingly soluble Al- and Fe-salts (Anderson 1980). In our study, the dominance of monoester forms can also be related to the mineralogy of clayey soils, which is dominated by gibbsite (50–63%), goethite (13–20%), kaolinite (10–20%) and hematite (6–8%) as the rapid adsorption on soil minerals, and extensive interaction with sesquioxides protected monoesters from degradation (Tate 1984). Turrión et al. (2001) underlined the positive relationship of the concentration of phosphomonoesters in alkali-extracts to the Fe forms extracted by bicarbonate-dithionite-citrate method and with the percentage of silt plus clay, indicating a stabilization of monoester with fine fractions of soil and sesquioxides. A signal in the diester region between 1.0 and 0.4 ppm and tentatively assigned to sugar diesters has been observed by Guggenberger et al. (1996b) in oxisols under tropical pastures following native savanna. In our study, there was no distinct signal in this region. These organic esters can be hydrolyzed to pyrophosphate during extraction with 0.5 M NaOH (Leinweber et al. 1997). There is little information on the role of pyrophosphates, although Gressel et al. (1996) suggested that they represent a fairly available pool of biological origin. The teichoic acids (3.0–1.0 ppm) are another form of diesters and originate exclusively from the cell walls of Gram-positive bacteria (Schlegel 1993). The absence of signals accounted for teichoic acids and phosphonates (δ around 19 ppm), another microbially derived form, indicates a small proportion of bacterial P in the samples and can be explained by the use of air-dried samples. Topsoil under the savanna with closed tree canopy (Cerradão, Cdão) contained more phosphodiesters and less phosphomonoesters and pyrophosphate than the other topsoils. These differences are not explained, but could be due to differences in the composition of plant species growing in the soils as a large part of monoesters originates from decomposition of plant materials whereas the major organic form in microorganisms is phosphodiesters (Gressel et al. 1996). The monoester to diester ratio is a measure of organic P lability, diesters being more labile and plant accessible than P-monoester. The ratios ranged from 2.8 to 5.3 and showed no clear trend as a result of changing land use. These values were higher than those reported by Neufeld and Zech (1996) and Guggenberger et al. (1996b) for oxisols from Brazil and Colombia, but lower than those reported by Turrión et al. (2001) for Spanish soils.

PME Activity

Phosphatase enzymes are a good indicator of the organic P mineralization potential and biological activity of soils

Table 4. Distribution of various NaOH-soluble P species in topsoils as estimated from ^{31}P-NMR spectra

Site[z]	Orthophosphate[y]	Pyrophosphate[y]	Monoesters[y]	Diesters[y]	Po (monoesters + diesters)[y]	Monoesters: diesters ratio
Cdão	54.5	4.7	31.5	11.4	42.9	2.8
Cl	44.1	9.0	39.5	7.4	46.9	5.3
P-Cdo	44.6	7.5	38.1	9.7	47.9	4.0
P12	44.9	7.6	38.2	9.2	47.5	4.1
P5	43.5	9.0	37.4	10.1	46.5	3.7
P4	67.2	6.5	22.0	4.3	26.3	5.1
P3	46.7	8.7	34.8	9.7	44.5	3.6

[z]Cdão, Cerradão; Cl, Campo limpo; P-Cdo, pastured Cerrado, the control for pastures; P12, 12-yr-old pasture of *Brachiaria brizantha*; P5, P4, P3 correspond to interruption of *Brachiaria brizantha* with a single maize culture 5, 4 and 3 yr, respectively, before sampling.
[y]Percentages of whole NMR spectra area.

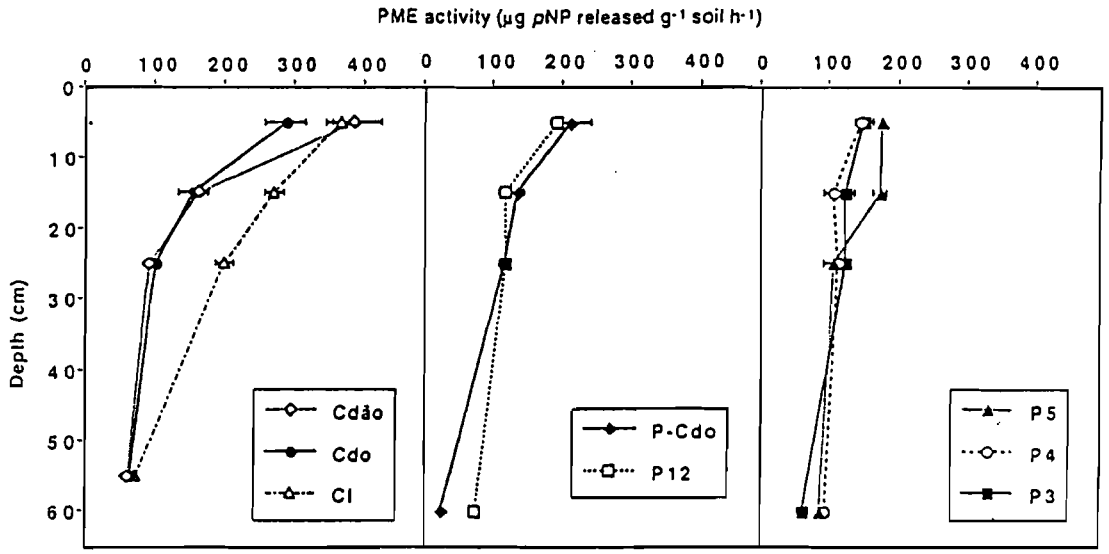

Fig. 2. Phosphomonoesterase activity at pH 6.5 as a function of soil depth (horizontal bars represent standard deviations of the means). Cdão (Cerradão), Cdo (Cerrado) and Cl (Campo limpo) are different physiognomies of Cerrado savanna, from closed to open type; P-Cdo is a pastured Cerrado used as the control for pastures; P12 represent the 12-yr-old pasture of *Brachiaria brizantha*; P5, P4, P3 correspond to interruption of *Brachiaria brizantha* with a single maize culture 5, 4 and 3 yr, respectively, before sampling.

(Speir and Ross 1978; Dick and Tabatabai 1993). However, their use is subject to potential experimental artefacts [review in Malcolm (1983) and Sinsabaugh et al. (1991)]; one is the soil pre-treatment. Ideally, field-moist soil should be used for enzymatic assays, as soon as possible after sampling, but this is sometimes not possible for practical reasons. The use of air-dried samples that are stable for long periods facilitates storage and transport and follows the same trends for a number of enzyme assays (Bandick and Dick 1999).

The organic P fraction extracted by NaOH consists mainly of phosphomonoesters. In this study, the PME activity was first assayed in the modified universal buffer at pH 6.5 because in literature this is often the standard condition. The PME activity decreased with depth (Fig. 2) following the same trend of decreasing total C, total and organic P contents (Table 2). Other authors also observed a decrease of PME activity with increasing soil depth and underlined that this decrease was associated with a decrease in C content (Deng and Tabatabai 1997; Baligar et al. 1988).

In comparison to the pastures, the soils under natural vegetation have a higher PME activity in the surface horizons. Enzymes of the topsoil produced between 140 and 420 µg *p*NP released g^{-1} soil h^{-1}. Three distinct groups are significantly different ($P < 0.05$); the highest activities were given by enzymes in soils under Campo limpo (Cl, the grassland type of savanna) and Cerradão (Cdão, the savanna type with closed tree canopy). Topsoils under 3-, 4- or 5-yr-old pastures presented the lowest activities. Intermediate values were developed in descending order under natural or pastured Cerrado (Cdo and P-Cdo) and 12-yr-old pasture. The values obtained in topsoils for natural vegetation were higher than those reported by Baligar et al. (1999) for similar Cerrados soils. The differences in PME activity between sites declined in the mineral horizons (Fig. 2) as differences in C and organic P contents (Table 2). At 60 cm depth, the activity was statistically the same in all sites (about 74 µg *p*NP released g^{-1} soil h^{-1}) except for the pastured Cerrado (P-Cdo, 23 µg *p*NP released g^{-1} soil h^{-1}). Bayan and Eivazi (1999) reported that iron oxides and

goethite enhanced acid phosphatase activity, probably due to the chemisorption of inorganic P produced by the organic P mineralization. On the other hand, the PME activities were reduced in the presence of clay-sized phyllosilicates, as the catalytic activity of the enzymes, which are adsorbed on these particles, can be significantly reduced (Leprince and Quiquampoix 1996; Rao et al. 2000). Nevertheless, kaolinite, which is the only phyllosilicate in the soils studied, has been shown to inhibit catalytic activity less than other clay-sized particles, such as illite and montmorillonite (Bayan and Eivazi 1999). The PME activity was positively correlated with soil total C and organic P contents (with respectively, $r = 0.88$ and $r = 0.87$; $P = 0.0001$). Similar dependences have been reported in the literature (Juma and Tabatabai 1978; Speir and Ross 1978; Feller et al. 1994; Baligar et al. 1999). A significant and negative correlation between inorganic P content and PME activity was observed ($r = 0.47$; $P = 0.006$). The negative effect of orthophosphate on PME activity is again demonstrated. The three groups identified appear to be related to the fertilization rate: no fertilization in the natural vegetation sites and pasture control; one application, which is no longer effective, in 1982 for the 12-yr-old pasture; and one application before establishment of pasture, which is still effective, for the more recent pastures. Feller et al. (1994) also observed the decrease in PME activity from natural savanna to cultivated soils of different regions. The higher acid PME activity in natural Cerrado as compared to recent pastures is in contrast to the findings of Oberson et al. (1995), who analyzed pastures and a native savanna in Colombia. But, in both studies, PME activity is associated with organic P content, with higher PME activity values corresponding to greater Po contents. Kulinska et al. (1982) measured a PME activity that was four times higher in a native Cerradão than in a deforested plot and demonstrated that enzyme activities were correlated with the quantity and quality of litter material. Many authors have reported that the PME activity in soils is associated with vegetation, root exudation and living biomass. For example, Tarafdar and Jungk (1987) found that phosphatase activity in the rhizosphere varies with plant species. Since phosphatases are adaptative enzymes, the intensity of their exudation by plant roots is, to some extent, influenced by the plant requirement for P (Silberbush et al. 1981). PME activity increased with the age of plants as the development of the root system with age might have resulted in an increased production of phosphatases of plant origin due to the increase in total root surface area; it may also be attributed to a gradual build-up of a microbial population in the root region (Tarafdar and Jungk 1987). Although there is little evidence in our study to confirm their observations, the differences in PME activity between sites could also be associated with the microbial populations living in the soil and the type and age of the vegetation. Lime can also explain the decrease of PME activity from natural systems to recent pastures as addition of Ca can reduced enzymatic activities (Halstead 1964).

Optimum pH for PME Activity in 0–10 cm Samples

The PME measurements were carried out at pH 6.5, which is often used as a standard condition. However, the soils are all acidic (Table 1), and the study of pH effects on PME activity shows that the pH behaviour is quite complex (Fig. 3).

For all pH values, three groups of PME activities can also be observed in the surface layer of soils: natural vegetation (Cl, Cdo and Cdão) > pastured Cerrado (P-Cdo) and old pasture (P12) > more recent pastures (P5, P4 and P3).

Because studies using different substrates (e.g., Skujìns et al. 1962; Halstead 1964; Tabatabai and Bremmer 1969) have indicated that most soils exhibit maximal PME activity near neutral pH (6.2–7.0), many authors employed these methodologies with no regard to pH of PME optimum activity. As indicated by Burns (1978), assays should always be performed at, or close to, the optimum pH for activity. In our study, this optimum varies within the samples. The topsoil under Cerradão (Cdão) presented an maximum PME activity at pH 6.5 (the highest pH measure) and lower values at pH below 6.5. For the soil under Cerrado (Cdo) and Campo limpo (Cl), the optimum activity occurred at pH 5.5 and 5, respectively.

For pastures, PME activity presented an optimum with values of the buffer pH lower than 6.5. For the topsoil under pastured Cerrado (P-Cdo) and 12-yr-old pasture (P12), the maximum activity was observed at pH 5. For the 5-yr-old pasture, there were two regions of higher activity, at pH lower than 4.5 or near 6. The pastures aged 4 and 3 developed an optimum activity, respectively, with a pH value of 5.5 and 4.5. Near the soil pH value, the significantly ($P < 0.05$) greatest enzymatic activity was measured for Campo limpo (Cl, 440 μg *p*NP released g^{-1} soil h^{-1}). Soils under Cerrado, Cerradão, pastured Cerrado and 12-yr-old pasture then had intermediate values (300 μg *p*NP released g^{-1} soil h^{-1}). The lowest activity near soil pH value occurred under the more recent pastures (P3, P4 and P5; 185 μg *p*NP released g^{-1} soil h^{-1}). For Cerradão (Cdão), the activity at pH 6.5 is well above that at soil pH. For Cerrado (Cdo) and pastured Cerrado (P-Cdo), and even more so for Campo limpo (Cl) and 12-yr-old pasture (P12), the values at pH 6.5 are much lower than at soil pH. For the recent pastures (P3, P4 and P5), the discrepancies are smaller, as the curves are rather flat. Data obtained near the soil pH show less difference than those obtained at pH 6.5, among the undisturbed systems, the pastured Cerrado (P-Cdo) and the 12-yr-old pasture (P12), but a greater difference between P-Cdo and the more recent pastures (P3, P4 and P5). Malcolm (1983) strongly recommended either the optimum pH or the soil pH, depending on the study objectives. In our study on acid soils, the optimum pH is often near the soil pH. We also recommend that the measurement of PME activity in acid tropical soils should be carried out at the soil pH unless the effect of pH on PME activity is determined. Nevertheless the importance of this issue should not be exaggerated because the pH-dependence of the PME activity in a natural soil has a much broader range of pH and a flatter optimum, as shown in the present study, than for a purified PME from a particular plant root or microorganism (Leprince and Quiquampoix 1996). This results from the diversity of the biological sources and associated diversity of pH-dependence of catalytic activity of individual PME.

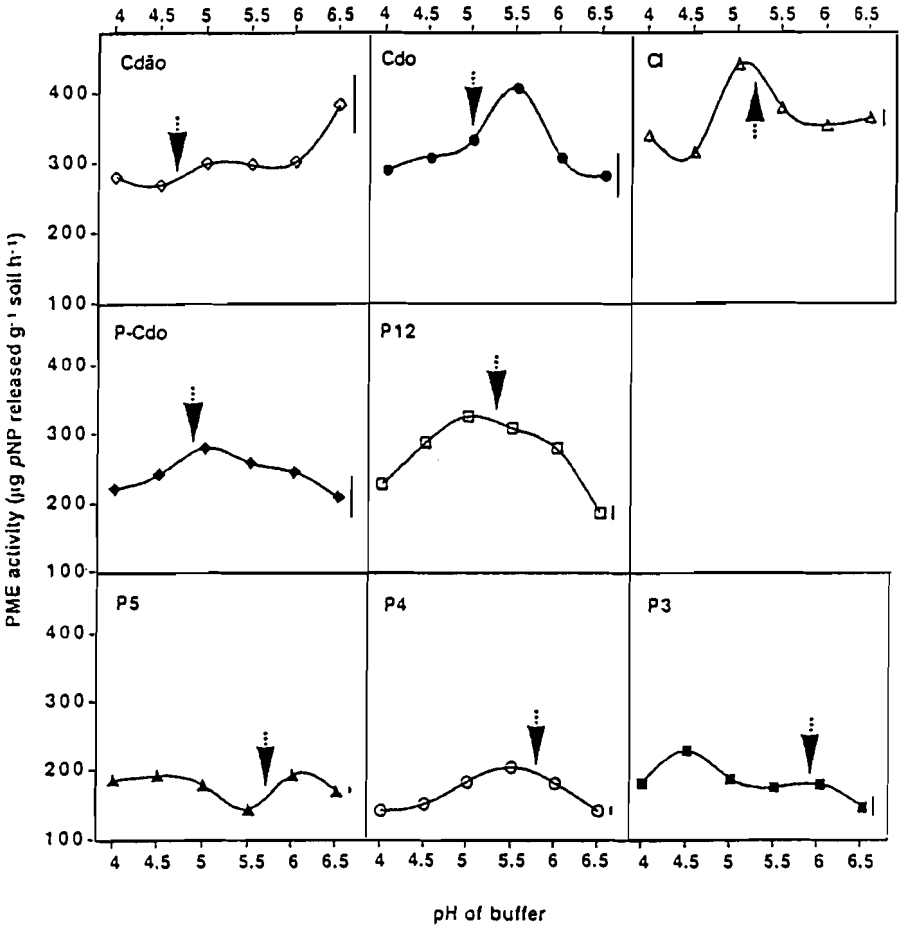

Fig. 3. Effect of buffer pH on PME activity of topsoils (vertical arrows represent the soil pH; vertical bars represent the maximum standard deviation of the means). Cdão (Cerradão), Cdo (Cerrado) and Cl (Campo limpo) are different physiognomies of Cerrado savanna, from closed to open type; P-Cdo is a pastured Cerrado used as the control for pastures; P12 represent the 12-yr-old pasture of *Brachiaria brizantha*; P5, P4, P3 correspond to interruption of *Brachiaria brizantha* with a single maize culture 5, 4 and 3 yr, respectively, before sampling.

CONCLUSIONS

This work is one of the first studies on the status of organic P in oxisols from the Cerrado region. The effect of land use on C and organic P concentrations does not appear to be strong. The bicarbonate extracted-P fraction may undergo rapid turnover, with little net difference between systems. The ^{31}P-NMR spectra of alkali extracts showed a similar distribution of labile organic P forms in topsoils from natural vegetation, pastured Cerrado and pastures and emphasized the dominance of phosphomonoesters, a stable form of Po. However, the effect of land use is clearly visible on the PME activities in surface horizons. The maize-grass ley plantation, therefore, resulted in increased phosphomonoester stability in topsoils as illustrated by the decreased PME activity. Liming or P fertilization is probably at least partly responsible for the decreased PME activity from pastured Cerrado to pastures, either by direct inhibition of the soil enzymes or by repression of PME synthesis by microorganisms and other providers.

These oxisols derived from Cerrados vegetation present greater organic P contents than those reported in literature for other savannas. The transformations of organic P contribute to the replenishment of the available inorganic P pool. In pastures, the nature of the extractable organic P, mainly phosphomonoesters, and its stability, demonstrated by low PME activity of soil, showed that the transformations of organic P are slowed down.

We consider that a better understanding of the stability of the Po pool in acid tropical soils can be gained from enzymatic investigations (PME and others) coupled with measurements of microbial biomass and activity.

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