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

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.

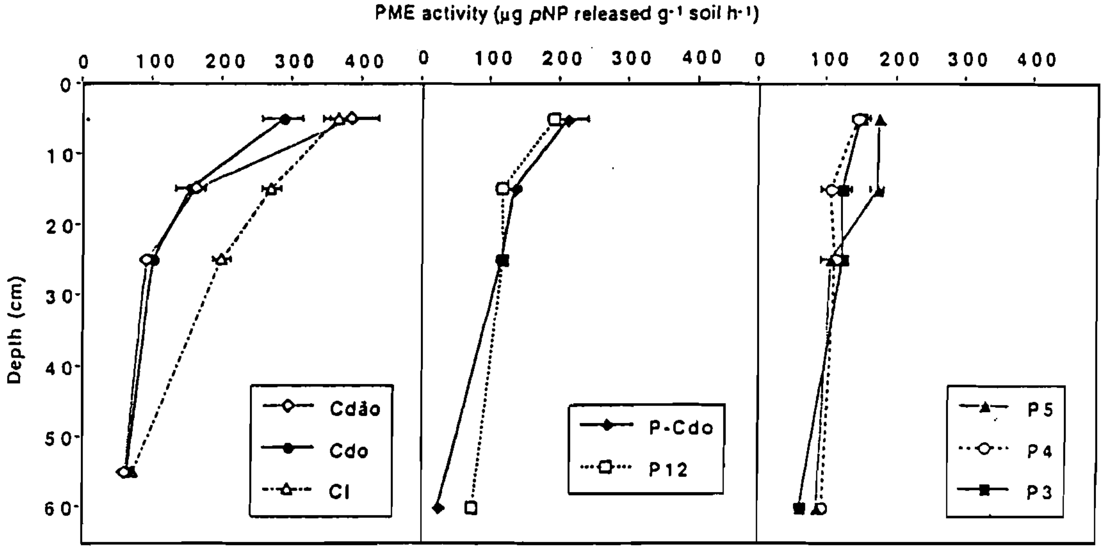

Fig. 2. Phosphomonoesterase activity at pH 6.5 as a function of soil depth (horizontal bars represent standard deviations of the means). Cdão (Cerradão), Cdo (Cerrado) and Cl (Campo limpo) are different physiognomies of Cerrado savanna, from closed to open type; P-Cdo is a pastured Cerrado used as the 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 pNP released g^{-1} soil h^{-1}). Soils under Cerrado, Cerradão, pastured Cerrado and 12-yr-old pasture then had intermediate values (300 µg pNP 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 pNP 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.

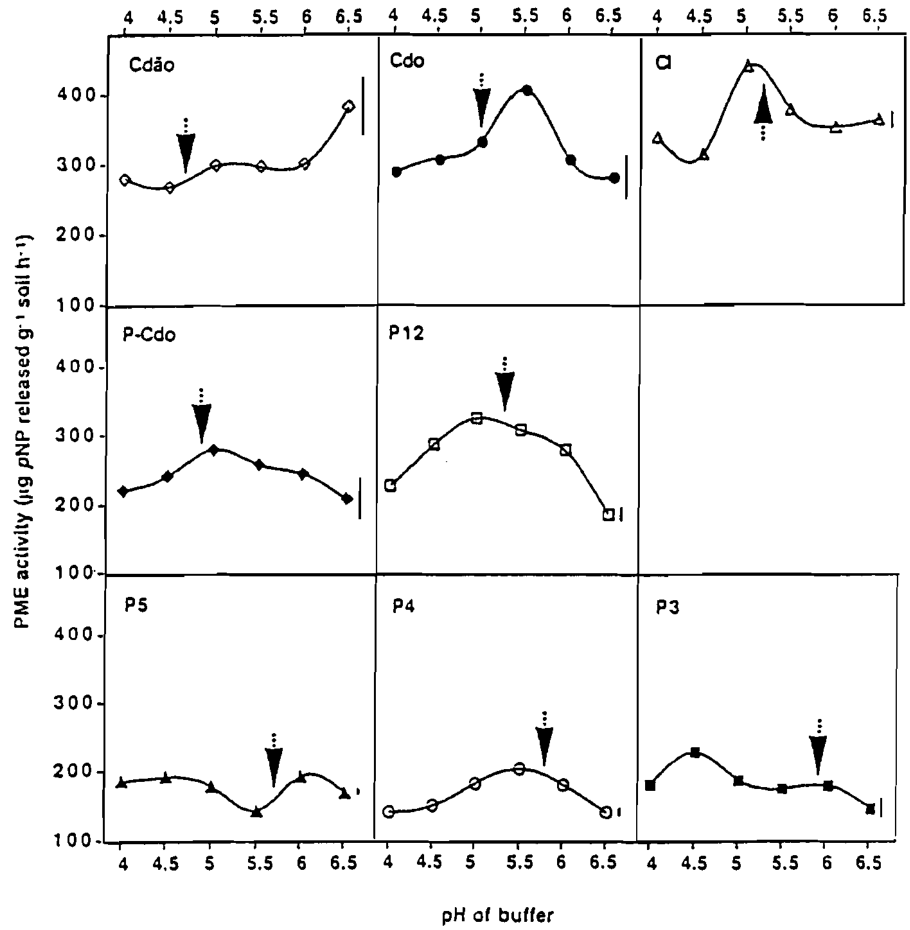

Fig. 3. Effect of buffer pH on PME activity of topsoils (vertical arrows represent the soil pH; vertical bars represent the maximum standard deviation of the means). Cdão (Cerradão), Cdo (Cerrado) and Cl (Campo limpo) are different physiognomies of Cerrado savanna, from closed to open type; P-Cdo is 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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