

# Phosphorus cycling in a small watershed in the Brazilian Cerrado: impacts of frequent burning

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**Abstract** Plant productivity in many tropical savannas is phosphorus limited. The biogeochemical cycling of P in these ecosystems, however, has not been well quantified. In the present study, we characterized P stocks and fluxes in a well-preserved small watershed in the Brazilian Cerrado. As the Cerrado is also a fire-dominated ecosystem, we measured the P stocks and fluxes in a cerrado *stricto sensu* plot with complete exclusion of fire for 26 years (unburned plot) and then tested some predictions about the impacts of fire impacts on P cycling in an experimental plot that was burned three times since 1992 (burned plot). The unburned area is an ecosystem with large soil stocks of total P (1,151 kg ha<sup>-1</sup> up to 50 cm depth), but the

largest fraction is in an occluded form. Readily extractable P was found up to 3 m soil depth suggesting that deep soil is more important to the P cycle than has been recognized. The P stock in belowground biomass (0–800 cm) was 9.9 kg ha<sup>-1</sup>. Decomposition of fine litter released 0.97 kg P ha<sup>-1</sup> year<sup>-1</sup>. Fluxes of P through bulk atmospheric deposition, throughfall and litter leachate were very low (0.008, 0.006 and 0.028 kg ha<sup>-1</sup> year<sup>-1</sup>, respectively) as was stream export (0.001 kg ha<sup>-1</sup> year<sup>-1</sup>). Immobilization of P by microbes during the rainy season seems to be an important mechanism of P conservation in this ecosystem. Fire significantly increased P flux in litter leachate to 0.11 kg ha<sup>-1</sup> year<sup>-1</sup>, and added 1.2 kg ha<sup>-1</sup> of P in ash deposition after fire. We found an increase of P concentration in soil solution at 100 cm depth (from 0.03 µg l<sup>-1</sup> in unburned plot to 0.3 µg l<sup>-1</sup> in the burned plot). In surface soils (0–10 cm) of the burned plot, fire decreased the concentrations of extractable organic-P fractions, but did not significantly increase inorganic-P fractions. The reduction of extractable soil organic P in the burned plot in topsoil and the increase of P in the soil solution at greater depths indicated a reduction of P availability and may increase P fixation in deep soils. Repeated fire events over the long term may result in significant net loss of available forms of phosphorus from this ecosystem.

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## Introduction

Soil phosphorus availability is thought to be a limiting factor to plant growth in many undisturbed mature ecosystems on highly weathered soils, including moist tropical forests, dry tropical forests, and savannas (Vitousek 1984; Kauffman et al. 1994; Haridasan 2001). In savanna ecosystems, fire plays a critical role in the ecology and biogeochemistry.

The savannas of central Brazil (Cerrado) are mainly on highly weathered Oxisols (Latossolos according to the Brazilian classification). Consequently, Cerrado soils usually are impoverished in plant-available forms of P (Lathwell and Grove 1986), with most of the P bound to Fe or Al oxides that are not available for plant uptake (i.e. occluded P). As such, the organic fractions of phosphorus, either associated to organic matter in mineral soils or in litter on the soil surface, are believed to have great importance for P cycling and plant growth (Lilienfein et al. 2000). Furthermore, the role of fire in mineralizing organic forms of P (i.e., pyromineralization) may be important for sustained P cycling.

The Cerrado has a highly seasonal climate with burning events occurring mainly in the end of the dry season (May through September) (Coutinho 1990). Burnings clearly release the nutrients retained in plant tissues or in litter on the soil surface. The rapid liberation of these organic nutrients can promote a temporary enrichment of the soils (e.g. in P) but can also cause the loss of nutrients through the export of ashes, erosion, or leaching through the soil profile and to streams (Kauffman et al. 1994). In addition to combustion of aboveground organic matter, increases in soil temperature during the fire front can combust soil organic matter, thus reducing phosphorus in organic fractions (Saá et al. 1994). As a whole, there is generally a transient increase in inorganic phosphorus availability after burning (Saá et al. 1994; Giardina et al. 2000) but the source of this P either as pyromineralized litter or soil organic P remains unclear (Galang et al. 2010) as does the fate of the P either re-immobilized into living biomass, retained in soils, or leached in soil solution.

The objective of this study was to quantify fluxes and stocks of P in woodland Cerrado areas in a small watershed that is well-preserved. Fluxes and stocks were initially characterized in a 10 ha plot protected from fire. We measured P inputs through bulk

atmospheric deposition and outputs through the stream. Additionally, fluxes of throughfall, litter leachate, decomposition, and soil solutions were measured as were stocks of soil phosphorus fractions, belowground biomass and fine litter. We hypothesized that input through atmospheric deposition and output through stream water would be low in comparison to internal biogeochemical cycling of P in this ecosystem. Potential impacts of fire on some key aspects of P cycling were then studied by comparison with a 10 ha plot nearby that was burned three times since 1992. We hypothesized that fire would not only affect P stocks in aboveground biomass and litter but would also change soil organic P fractions and P solution fluxes through the soil profile.

## Materials and methods

### Study area

The study was conducted in the IBGE Ecological Reserve (RECOR) located 35 km south of Brasília, Federal District, Brazil (15°56'41"S and 47°53'07"W Gr). The RECOR has a total area of 1,350 ha and an average altitude of 1,100 meters. Annual average rainfall is 1,470 mm (average of 23 years) and is highly seasonal. The reserve includes the typical plant formation gradient of the central Brazilian plains: from grasslands to dense savanna woodlands besides gallery forest and wetlands (*brejos* and *veredas*).

The RECOR is one of Brazil's Long Term Ecological Research sites, in which "The Fire Project", a large scale experiment on ecological effects of prescribed burning, is taking place. The project was initiated in 1989 in plots that had been protected against fire for 18 years. Since 1990 experimental plots of either 5 ha (for Cerrado grasslands) or 10 ha (for savanna and woodland savanna ecosystems) in size, have been burned experimentally according to different fire frequencies and season (early, middle or late dry season) (Gonzales et al. 1997).

The two experimental plots of 10 ha each (500 × 200 m) used for this study were located in a savanna woodland vegetation locally known as Cerrado (Eiten 1972). One plot had been protected from fire for 26 years (unburned plot), and one plot had been protected until 1992 and then was burned in

August (middle of the dry season) of 1992, 1996, and 1999. The distance between the burned and unburned plots is approximately 1,500 m. The soil in both plots was described as clayey Acrustox. We assumed that independent, randomly selected samples inside each plot were the units of replication as the dimensions of the studied plots are larger than the plots usually used for fire experiments. Although it is not the objective of the study to represent regional scale fire effects, the manipulated site is typical of other sites in the region based on a number of different variables (species composition, structure, soil type) and probably variations between sites are smaller relative to the treatment effects. We consider the fire experiment described here as an unreplicated experiment with strong and critical predictions as suggested by Oksanen (2001) for experiments with ecosystems or large scale communities involving very costly and/or intensive manipulations.

#### Belowground biomass, litter, ash and soil sampling

The determination of P stocks in belowground biomass was done only in the unburned plot. The coarse roots (2–5 mm, 5.1–10 mm, 10.1–20 and >20 mm) were collected between 0 and 800 cm depth in two pits (with dimension 200 × 200 × 800 cm) from: 0–10, 10–50, 50–100, 100–200, 200–300, 300–400, 400–500, 500–600, 600–700, and 700–800 cm. The fine roots (<2 mm) were from five different pits (with dimensions of 50 × 100 × 100 cm) in 0–10, 10–50, and 50–100 cm. In total 56 samples were analyzed for the different depths for coarse roots and 15 samples for fine roots.

The litter biomass stock was determined from nine 50 × 50 cm samples in both the unburned and burned plots. The samples were collected in August 1999 in the unburned and June 1999 in the burned, before the fire event. After the fire event, ash was collected in the burned plot in another nine 50 × 50 cm samples. The litter and ash samples were dried at 70°C and weighed.

Soil samples for chemical characterization were collected from burned and unburned plots with a 10-cm diameter auger along three 150 m transects starting 25 m from the plot edge, with samples collected every 50 m. Each sample along the transect was a composite from two random collection points.

The spacing between transects was about 100 m. The soil samples were collected for different purposes. Samples for soil P determinations until 500 cm depth were collected in May of 1998 at 0–10 ( $n = 6$ ); 10–20 ( $n = 6$ ); 20–50 ( $n = 6$ ); 50–100 ( $n = 3$ ); 100–200 ( $n = 3$ ); 200–300 ( $n = 3$ ); 300–400 ( $n = 3$ ); 400–500 ( $n = 3$ ). Samples for determination of microbial P in fresh soil were collected as three replications at 0–10, 10–20 and 20–50 cm depth in August of 1999 (dry season,  $n = 9$ ), December of 1999 (wet season,  $n = 9$ ), March of 2000 (wet season,  $n = 9$ ), and August of 2000 (dry season,  $n = 9$ ;  $n = 36$  total for all dates). Soil water content was measured by oven-drying soil samples at 105°C.

In order to evaluate the effects of fire over time in the burned plot, 21 surface soil samples (0–10 cm) were collected in the burned plot: before fire in August 1998 ( $n = 3$ ) and May 1999 ( $n = 3$ ), 1 day before fire in August 1999 ( $n = 3$ ), 1 day after fire in August 1999 ( $n = 6$ ) and in the subsequent wet season (December 1999 ( $n = 6$ )). Considering that the passage of fire through the plot is not uniform and may result in an increased heterogeneity, a larger number of samples were collected after the fire. Samples were collected in the unburned and burned plots in May 1999 ( $n = 3$ ), August of 1999 ( $n = 3$ ) and December of 1999 ( $n = 3$ ) for comparison between fire treatments.

Soil samples collected after fire were carefully collected so as to avoid contamination by ash. When necessary, the layer of ashes was carefully removed before collecting the soil samples.

#### Nutrient determinations in biomass and ash samples

The belowground biomass, fine litter and ash samples were digested in duplicate with perchloric acid and hydrogen peroxide at 300°C. The extract was analyzed on an ICP-AES (Trademark IRIS, in the Plant Tissue Analysis Laboratory of the EMBRAPA Cerrados, Planaltina, Brazil) using Yttrium as an internal standard.

#### Analyses of soil P fractions

A modified P sequential extraction (Tiessen and Moir 1993) that derives from the Hedley et al. (1982) P sequential extraction was carried out in duplicate with the following order of extractors: 0.5 M NaHCO<sub>3</sub>

(pH 8.5), 0.1 M NaOH, hot concentrated HCl, and residue digestion with H<sub>2</sub>SO<sub>4</sub> (residual fraction). The resin extraction was not performed because resin P is included in the NaHCO<sub>3</sub> fraction and literature largely considers them as a similar pool (see Cross and Schlesinger 1995; Johnson et al. 2003). Phosphorus extracted by exchange resin is readily available, making resin P more important for studies of crop fertility or effectiveness of P fertilization (Santos et al. 2008; Pavinato et al. 2009). The 1 M HCl step was also omitted, as weathered soils such as those at this site generally possess little P in primary minerals extracted with 1 M HCl (Cajuste et al. 1994). The P concentrations in all fractions were determined with molybdate blue chemistry (Murphy and Riley 1962). Specifically for in-depth characterization of P we did not determine the residual P fraction. For these samples an estimation of the residual fraction was obtained by the difference between total P and the sum of the other fractions. Certified Aldrich Phytic acid and ERA 667 Nutrients, PotableWatR with Orthophosphate P were used as quality control standards for the organic and inorganic P solution determinations, respectively. The NIST Montana Soil, Standard Reference Material 2711 was used as the soil total P quality control standard. We performed an independent total P determination with separate soil samples by total digestion with H<sub>2</sub>SO<sub>4</sub>.

Chloroform fumigation was used on fresh soil samples for the determination of microbial biomass P following Demetz and Insam (1999). The samples were collected and maintained in field moist condition under refrigeration at 4°C for a maximum of 3 weeks prior to analysis. The analysis to determine microbial P follows the steps of the sequential extraction, with duplicate samples, but one of the samples received 1 ml of chloroform to kill the microorganisms (Demetz and Insam 1999). The sum of the differences for each P fraction among the fumigated samples (sterilized) and non-fumigated samples is attributed to microbial biomass (Potter et al. 1991).

#### Solution sampling, analyses and flux estimates

The following solutions were collected every 2 weeks from January 1999 to December 1999: bulk precipitation ( $n = 66$ ), throughfall ( $n = 44$  and 41 in burned and unburned plots, respectively), and litter leachate ( $n = 43$  and 40, respectively). Bulk

precipitation was collected at 1.5 m from the soil surface using 16 cm funnels placed at two locations within IBGE Ecological Reserve. All bulk precipitation funnels fed through looped tubing to restrict evaporation. Volume-weighted mean annual P concentrations and inputs were estimated for each collector. The average of the two bulk precipitation collectors was used to describe P inputs through atmospheric deposition. Three small pits were randomly located in each of the plots (burned and unburned). At each pit two 16 cm funnels with bottles for throughfall were installed, one 240 cm<sup>2</sup> PVC collector was placed under the O horizon for litter leachate collection, and Prenart superquartz tension lysimeters were installed at 25 cm and 100 cm depths. Soil solutions were collected biweekly from December 1998 to May 2000, but a sample was not always present due to insufficient precipitation to generate enough infiltration. For the 25 cm lysimeters, 35 samples were collected from each plot, while for the 100 cm lysimeters, 27 and 20 samples were collected from the unburned and burned plot, respectively.

During collection, sample volume was measured and a subsample transferred to a previously acid-washed polypropylene bottle. The subsamples were filtered through 0.4 µm polycarbonate filters and stored cold (4°C). All samples were analyzed for total P with a ICP-AES ERLAN 6200, Perkin Elmer, using Rhodium as an internal standard. To estimate P flux in solution, volume-weighted mean annual concentration was multiplied by the hydrologic flux of water. For bulk precipitation, throughfall, and litter leachate, the water flux was estimated from the volume collected. We did not measure water flux in soil and consequently did not determine soil solution flux.

#### Streamwater sampling and flux estimates

Stream water grab samples were collected from the Roncador Stream, a second order stream that drains a 1,200 ha watershed in the IBGE Ecological Reserve. Over the course of study, collections were made on approximately 50% of all days from May 1998 to May 1999 ( $n = 168$ ) while samples were collected weekly from May 1999 to May 2000 ( $n = 56$ ). The stream gage height was measured on all days of collection ( $n = 224$ ) and the stream discharge was estimated from a gage height versus discharge relationship

established for the stream in 1999 following the methods of Rantz (1982). Total dissolved P was measured as above. P flux in the stream was estimated by multiplying annual discharge and volume-weighted mean annual concentration.

### Statistical analyses

Data were tested for normality, and when necessary, we used a non-parametric Mann–Whitney *U* test and a Kruskal–Wallis test to compare means, with a significance level at 5%. Tukey post-hoc test was used after the Kruskal–Wallis test (Zar 1996) for comparison of microbial P at different depths and P concentration in bicarbonate fraction before and after fire. We performed a Principal Component Analysis to compare burned and unburned plots (samples collected in 1999—before and after burning—and 2000) using soil P fractions after data were  $\log_e$  transformed.

## Results

### Phosphorus fluxes and stocks in the unburned plot

Soil of the study area had a low pH at the surface with increasing values with depth. Soil carbon content was 3.18% in the surface layer, decreasing with depth to a value of 0.39% in the 400–500 cm soil layer (Table 1). Nitrogen followed the same pattern, but decreased sharply in relation to carbon and was below

analytical detection limits beyond 100 cm depth. The available Mehlich I P was low for the entire profile and varied between 0.1 and 0.3  $\mu\text{g g}^{-1}$ . Available cations (extracted with Mehlich I) were low in the surface layer and also decreased with depth.

The total phosphorus at the soil surface was 356  $\mu\text{g g}^{-1}$  decreasing to 170  $\mu\text{g g}^{-1}$  at the 400–500 cm layer (Fig. 1). The predominant soil P fraction at all depths was the residual form (Fig. 1). The available organic and the inorganic ( $\text{NaHCO}_3$ ) fractions followed a similar pattern as total P, decreasing with depth (Fig. 2).  $\text{NaHCO}_3$ -Pi decreased from 2.9 to 1.1  $\mu\text{g g}^{-1}$ , while  $\text{NaHCO}_3$ -Po decreased from 9.9 to 0.1  $\mu\text{g g}^{-1}$ . NaOH extractable P also showed decreasing patterns with depth but had higher concentration than the  $\text{NaHCO}_3$ . NaOH-Pi ranged from 35.4 to 4.3  $\mu\text{g g}^{-1}$  while NaOH-Po ranged from 64.6 to 3.1  $\mu\text{g g}^{-1}$  (Fig. 2). Concentrated HCl extractable Pi also showed similar patterns but the decrease with depth was not so sharp (81.9–46.2  $\mu\text{g g}^{-1}$ ), while HCl-Po concentrations were low throughout the profile (5.0–0.2  $\mu\text{g g}^{-1}$ ). The sum of all the fractions indicated that 22% of total P at the 0–10 cm soil layer was organic, contrasting with only 2% at the 400–500 cm layer (Fig. 2).

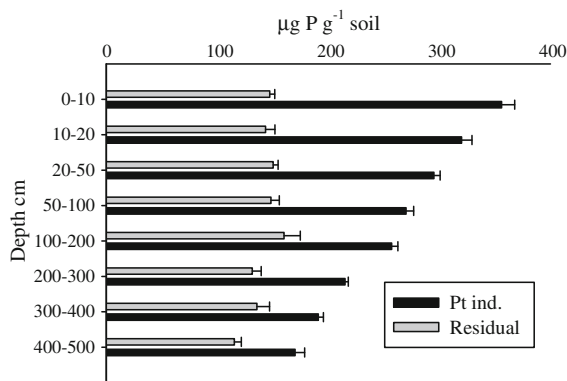
Concentrations of microbial P in the unburned plot varied with soil depth only during the wet season (Kruskal–Wallis test,  $\chi^2 = 7.6$ ,  $P < 0.05$ ) (Fig. 3) with the wet season concentration being larger at the 0–10 cm layer when compared to the 20–50 cm layer (Tukey test,  $P < 0.05$ ). The microbial P fraction also varied between dry and wet seasons. There was a

**Table 1** Soil chemical characterization for the unburned Cerrado plot, IBGE Ecological Reserve, Brasília, Brazil

Depth (cm)	pH <sup>a</sup> water	pH <sup>a</sup> CaCl <sub>2</sub>	C (%)	N (%)	C/N	P Mehlich I ( $\mu\text{g g}^{-1}$ )	K <sup>+</sup> (cmolc kg <sup>-1</sup> )	Ca <sup>2+</sup> (cmolc kg <sup>-1</sup> )	Mg <sup>2+</sup> (cmolc kg <sup>-1</sup> )
0–10	4.33 (0.09)	3.72 (0.09)	3.18 (0.06)	0.17 (0.01)	19	0.2 (0.1)	0.090 (0.013)	0.072 (0.004)	0.075 (0.007)
10–20	4.61 (0.02)	3.86 (0.07)	2.45 (0.18)	0.11 (0.01)	22	0.2 (0.1)	0.059 (0.009)	0.062 (0.006)	0.055 (0.005)
20–50	5.01 (0.05)	4.07 (0.04)	1.69 (0.07)	0.07 (0.01)	24	0.1 (0.1)	0.029 (0.01)	0.051 (0.007)	0.029 (0.009)
50–100	5.23 (0.09)	4.37 (0.05)	1.11 (0.06)	0.04 (0.00)	27	0.2 (0.2)	0.013 (0.001)	0.052 (0.003)	0.016 (0.002)
100–200	5.44 (0.07)	4.69 (0.13)	0.84 (0.06)	–	–	0.1 (0.0)	0.009 (0.004)	0.055 (0.005)	0.013 (0.004)
200–300	5.60 (0.05)	5.01 (0.12)	0.69 (0.01)	–	–	0.1 (0.0)	0.007 (0.001)	0.054 (0.001)	0.010 (0.002)
300–400	5.74 (0.08)	5.23 (0.02)	0.52 (0.03)	–	–	0.1 (0.0)	0.006 (0.001)	0.044 (0.006)	0.008 (0.001)
400–500	5.91 (0.11)	5.36 (0.02)	0.39 (0.03)	–	–	0.3 (0.4)	0.009 (0.004)	0.053 (0.010)	0.011 (0.003)

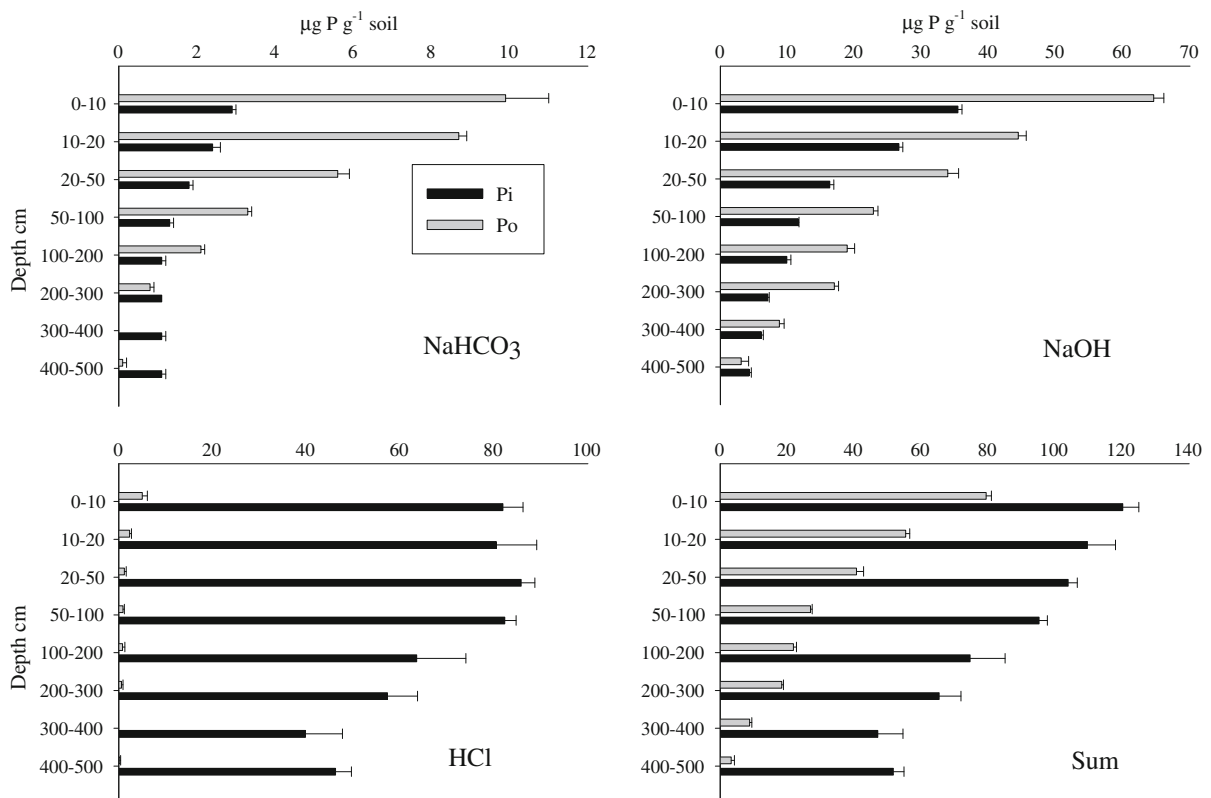
Samples collected in May 1998. The standard error is in parentheses

<sup>a</sup> Soil pH in 1:2 solution of deionized water or 0.01 M CaCl<sub>2</sub>



**Fig. 1** Concentration of total phosphorus (independent digestion; “Pt Ind.”) and residual phosphorus fraction at different soil depths in an Acrustox in the unburned area of Cerrado in IBGE Ecological Reserve, Brasília, Brazil. The samples were collected in May 1998. The standard error is represented by the bar. *Pt ind.* total independent phosphorus

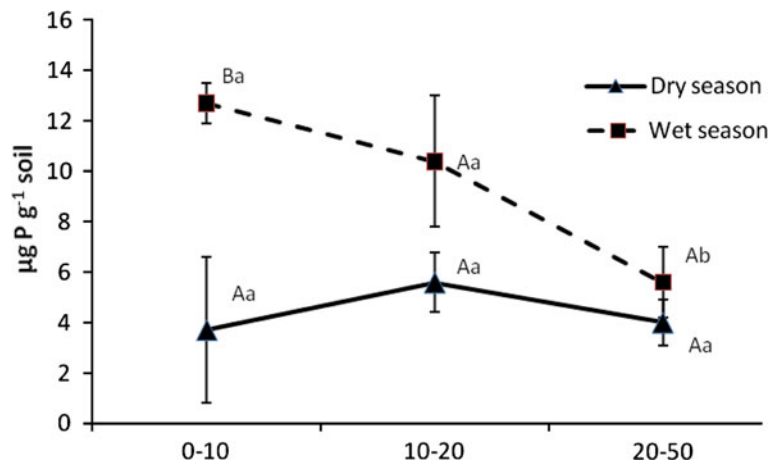
significant increase at the 0–10 cm layer, from 3.7  $\mu\text{g g}^{-1}$  in the dry season to 12.7  $\mu\text{g g}^{-1}$  in the wet season (Mann–Whitney *U* test,  $U = -2.6$ ,  $P < 0.05$ ).



**Fig. 2** Concentration of phosphorus fractions at different soil depths in an Acrustox in the unburned area of Cerrado in IBGE Ecological Reserve, Brasília, Brazil. The samples were collected in May 1998. The standard error is represented by the bar



**Fig. 3** Concentrations of microbial P in an Acrustox with fire exclusion in the IBGE Ecological Reserve, Brasília, Brazil for different depths (in cm) and seasons. Horizontal bars indicate  $\pm 1$  standard error. Uppercase letters indicate differences between seasons in the same depth, lowercase letters indicate difference between depths in the same season



**Table 2** Stocks of phosphorus in the unburned area of Cerrado in the IBGE Ecological Reserve, Brasília, Brazil

Compartment	P stock (kg ha <sup>-1</sup> )
Plant biomass	
Aboveground <sup>a</sup>	12.6
Belowground <sup>b</sup>	9.9
Fine litter	4.6
Sum	27.1
Soil microbial biomass (0–50 cm) <sup>3</sup>	
Rainy season	27.9
Dry season	14.3
Soil (0–50 cm) <sup>c</sup>	
Total P	1,151.0
Organic P	259.0
Available P (Mehlich I)	0.6

<sup>a</sup> Aboveground nutrient stock includes nutrient stock of the herbaceous layer (Batmanian and Haridasan 1985) and of the woody layer (Silva 1990) modified from Nardoto et al. (2006)

<sup>b</sup> Up to 800 cm depth and includes fine and coarse roots

<sup>c</sup> Soil bulk density in 0–10 cm = 0.66 g cm<sup>-3</sup>, 10–20 cm = 0.68 g cm<sup>-3</sup>, 20–50 cm = 0.68 g cm<sup>-3</sup>

total discharge was  $3.5 \times 10^6$  m<sup>3</sup> year<sup>-1</sup> of water. Considering the discharge weighted total dissolved P as 0.370 µg l<sup>-1</sup>, the P export through stream water was 0.001 kg ha<sup>-1</sup> year<sup>-1</sup> (Fig. 4b).

#### Effect of fire on P cycling

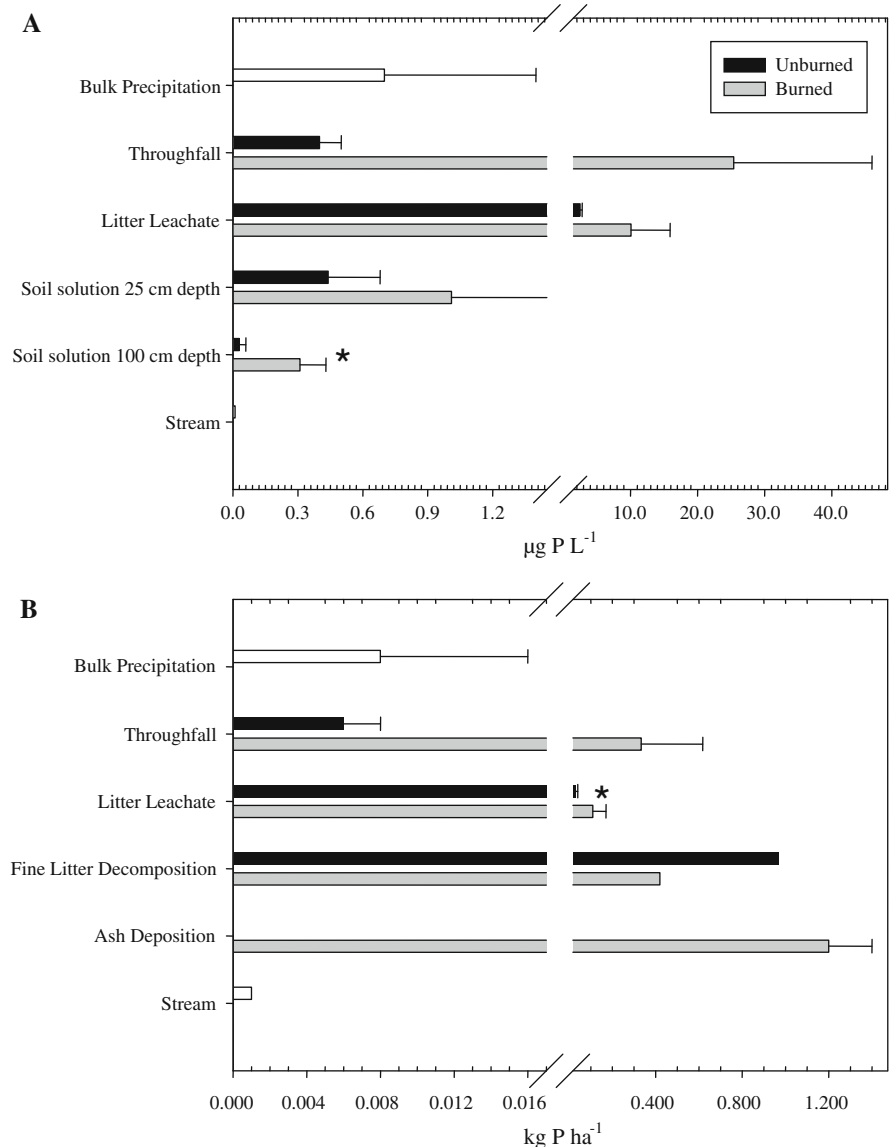
The comparison of the P fractions within the burned plot before and after the third quadrennial fire in 1999 showed significant differences in the NaHCO<sub>3</sub>-Pi fraction (Kruskal–Wallis test  $\chi^2 = 9.64$ ,  $P < 0.05$ )

and NaHCO<sub>3</sub>-Po (Kruskal–Wallis test  $\chi^2 = 11.58$ ,  $P < 0.05$ ) (Fig. 5). The other fractions did not differ significantly. There was a tendency for decreases in the organic and inorganic NaHCO<sub>3</sub> fractions, 4 months after the fire and during the rainy season (676 mm of accumulated rain after fire event).

In the Principal Component Analysis, which included soil samples collected in both burned and unburned plots, axis 1 and axis 2 explained 43 and 31% of the variance, respectively. The variables that presented the highest correlation coefficients with axis 1 were NaHCO<sub>3</sub>-Po (0.779), total organic P (0.362) and NaOH-Po (0.341) (Table 3). The axis 2 presented the highest correlation coefficients with HCl-Po (0.794) and HCl-Pi (0.345) (Table 3). The variance was mostly explained by NaHCO<sub>3</sub> and NaOH organic P fractions. The PCA analysis discriminated between the two fire treatments (Fig. 6). Burned and unburned soil samples were separated along axis 1 with unburned soil samples presenting higher concentrations of P in NaOH and NaHCO<sub>3</sub> organic fractions.

Soil P fractions (0–10 cm depth) from unburned and burned plots were compared for May 1998, August 1999 and December 1999. The residual fraction, the sum of the fractions and an independent measure of total P (an estimate of total P in independent samples) did not differ between plots (Fig. 7). Total P (determined independently) varied between 352 and 367 µg g<sup>-1</sup>. The mean concentration of residual P was 148 µg g<sup>-1</sup>. The HCl-Pi was almost invariable, between 71 and 73 µg g<sup>-1</sup> and did not differ between plots (Fig. 7).

**Fig. 4** Phosphorus concentrations (a) and fluxes (b) in the unburned and burned area of Cerrado in IBGE Ecological Reserva, Brasília, Brazil. The samples were collected in 1999 for bulk precipitation, throughfall and litter leachate and in 1999–2001 for soil solutions. Horizontal bars represent  $\pm 1$  standard error. Mann–Whitney  $U$  test was used for comparison of P concentrations in throughfall and litter leachate while Student's  $t$  test was used to test soil solution. Asterisk indicates difference between treatments. Phosphorus flux from decomposition was calculated by multiplying the total P stock in fine litter by the decomposition rate of  $0.21 \text{ year}^{-1}$  found by Resende (2001)



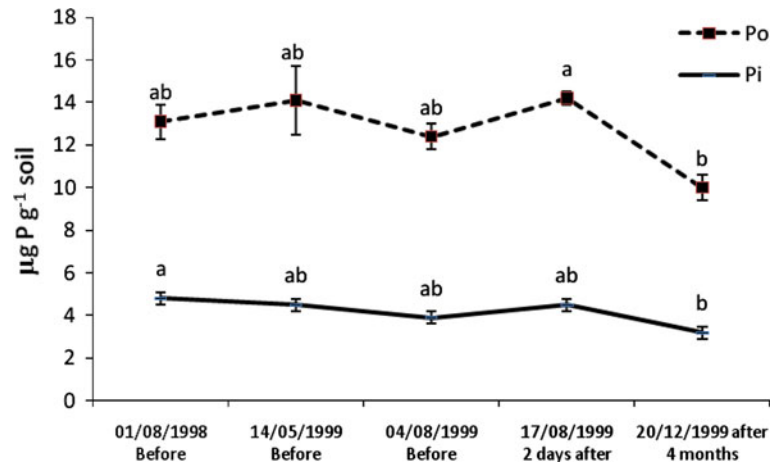
In contrast to residual P and total P, we found a significant difference in the sum of the organic fractions (the sum of  $\text{NaHCO}_3\text{-Po}$ ,  $\text{NaOH-Po}$  and  $\text{HCl-Po}$ ), from  $91.7 \mu\text{g g}^{-1}$  to  $79.3 \mu\text{g g}^{-1}$  (Mann–Whitney  $U$  test,  $U = -2.6$ ,  $P < 0.05$ ; Fig. 7) for the unburned and the burned plots respectively. Burning caused a significant decrease of P concentration in the  $\text{NaHCO}_3\text{-Po}$  fraction (from  $11.8$  to  $8.5 \mu\text{g g}^{-1}$  in unburned and burned plots, respectively, Fig. 7) (Mann–Whitney  $U$  test,  $U = -2.4$ ,  $P < 0.05$ ). Similarly the decrease of P in the  $\text{NaOH-Po}$  fraction from  $74.4$  to  $64.2 \mu\text{g g}^{-1}$  was significant (Mann–Whitney  $U$  test,  $U = -2.6$ ,  $P < 0.05$ , Fig. 7).

Total inorganic P (the sum of  $\text{NaHCO}_3\text{-Pi}$ ,  $\text{NaOH-Pi}$ ,  $\text{HCl-Pi}$ ) did not differ between plots ( $112 \mu\text{g g}^{-1}$  for the unburned and  $116 \mu\text{g g}^{-1}$  for the burned plot, Fig. 7). The increases of P in  $\text{NaHCO}_3\text{-Pi}$  (from  $3.6 \mu\text{g g}^{-1}$  in the unburned to  $4.0 \mu\text{g g}^{-1}$  in the burned plot) and in  $\text{NaOH-Pi}$  fraction (from  $37.3 \mu\text{g g}^{-1}$  in the unburned to  $38.8 \mu\text{g g}^{-1}$  in the burned plot) were not significant.

The fluxes of P in the burned plot in throughfall ( $0.33 \text{ kg P ha}^{-1} \text{ year}^{-1}$ ) and in litter leachate ( $0.11 \text{ kg P ha}^{-1} \text{ year}^{-1}$ ) increased relative to the unburned plot, but this was significant only for litter leachate (Mann–Whitney  $U$  test,  $U = -2.0$ ,



**Fig. 5** Concentration of phosphorus bicarbonate fraction in a Cerrado area (Acrustox soil) burned in IBGE Ecological Reserve, Brasília, Brazil. Soil samples (0–10 cm depth) collected in 1999. The prescribed fire occurred on August 15, 1999. Vertical bars indicate  $\pm 1$  standard error; letters indicate differences between collection dates



**Table 3** Correlation coefficients between P fractions and the first two axes of PCA

P fraction	Axis 1	Axis 2
NaHCO <sub>3</sub> -Pi	0.231	0.146
NaHCO <sub>3</sub> -Po	<b>0.779</b>	-0.090
NaOH-Pi	0.113	0.195
NaOH-Po	<b>0.341</b>	0.251
HCl-Pi	0.010	<b>0.345</b>
HCl-Po	-0.232	<b>0.794</b>
Total organic	<b>0.362</b>	0.261
Total inorganic	0.051	0.047
Residual	0.053	-0.154
Total P (sum of fractions)	0.128	0.095
Total P (independent)	0.063	0.135

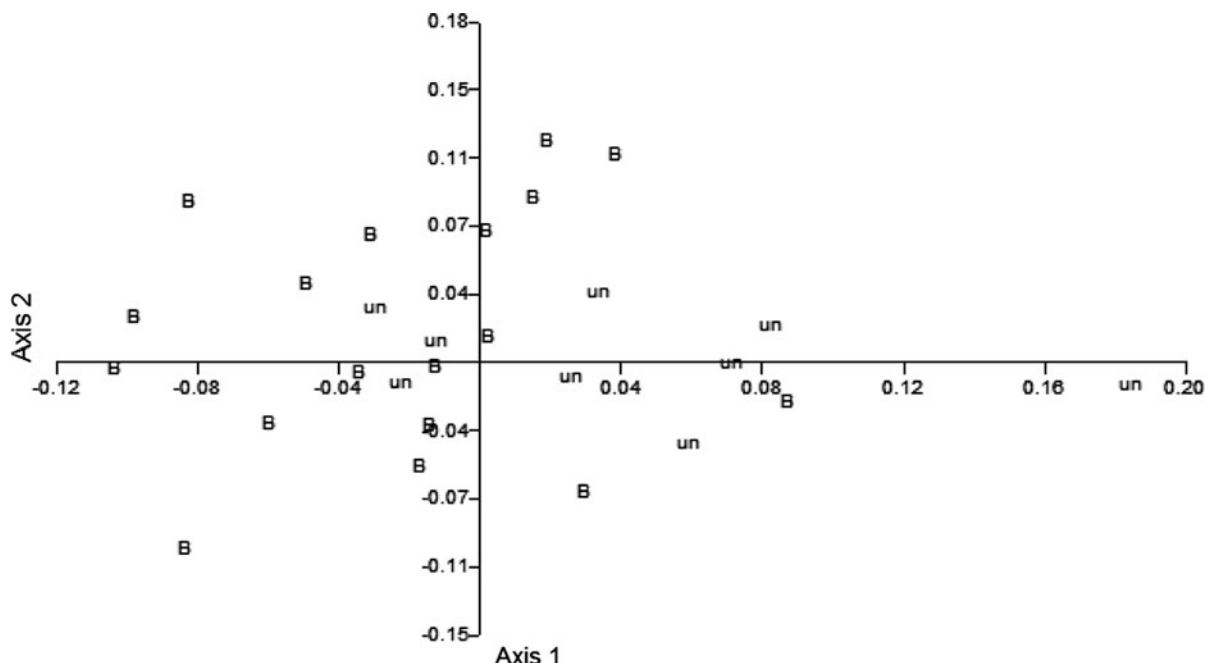
$P < 0.05$ ) (Fig. 4b). P in soil solution was higher in the burned plot compared to unburned plot (Fig. 4a). At 25 cm depth the concentration of P was  $1.01 \mu\text{g l}^{-1}$ , but it did not differ significantly from the unburned plot. However, at 100 cm depth the P concentration in the burned plot ( $0.31 \mu\text{g l}^{-1}$ ) was significantly higher than in the unburned plot ( $0.03 \mu\text{g l}^{-1}$ ; Student's  $t$  Test  $t = -2.33$ ,  $\text{df} = 21$ ,  $P < 0.05$ ). Fine litter in burned plot was estimated in  $5.6 \text{ Mg ha}^{-1}$  resulting in a stock of  $2.0 \text{ kg P ha}^{-1}$ . The flux through fine litter decomposition was  $0.42 \text{ kg P ha}^{-1} \text{ year}^{-1}$  (Fig. 4b) (decomposition rate of  $0.21 \text{ year}^{-1}$  according to Resende 2001). After fire combustion of biomass, there was a mean deposition of  $1,990 \pm 381$  (SE)  $\text{kg ha}^{-1}$  of ash. Phosphorus concentration in ash was  $0.6 \text{ mg g}^{-1}$ , resulting in a deposition of  $1.2 \text{ kg P ha}^{-1}$  in the burned plot (Fig. 4b).

## Discussion

### Soil P

The main source of P in soils is apatite (Lindsay et al. 1989). Walker and Syers (1976) described the evolution of P availability during the weathering of rocks containing apatite. As a consequence of weathering dynamics, the dominant P forms in highly weathered tropical soils are the occluded forms associated with oxide minerals (Cross and Schlesinger 1995). Many highly weathered tropical soils, including the soils of the Cerrado region of this study, have reached the “terminal steady state” (Walker and Syers 1976) where soil organic P is the determinant of availability (Johnson et al. 2003).

At the soil surface layer, the distribution of P fractions in the studied Acrustox is similar to those found for other Oxisols also under native Cerrado vegetation (Araújo et al. 1996; Lilienfein et al. 1996; Lilienfein et al. 2000; Neufeldt et al. 2000). In general, Oxisols with larger clay contents have larger total P contents. In all soils, total P contents and concentrations tend to decline with depth. In the present study, the total P concentration was 51% lower at 400–500 cm depth compared to the superficial layer (0–10 cm). Similarly, the available fractions, NaHCO<sub>3</sub> and NaOH, also decreased with depth. Accumulation of available P (and of total P) in the superficial horizons is an indication of bioaccumulation (accumulation of essential nutrients like P caused by plants) (Jobbágy and Jackson 2001) exceeding the processes of P loss that occurred during pedogenesis (Lekeman et al. 1996).



**Fig. 6** Score plot for the principle component analysis of phosphorus fractions in soil samples collected in the unburned and burned plot. *un* Unburned, *B* burned. Axis 1 explained 43% of variance, axis 2 explained 31% of variance

The data further indicate that the decrease with depth of the organic portion is concomitant with an increase in the relative percentage of total P that is inorganic. Furthermore, the  $\text{NaHCO}_3\text{-Po}$  fraction declines more rapidly with depth than the remaining organic fractions, and it disappears at 300–400 cm depth. This fraction is more sensitive to biological activities because it is related to both plant growth and microorganism activity, and can broadly be considered as the most available organic fraction.

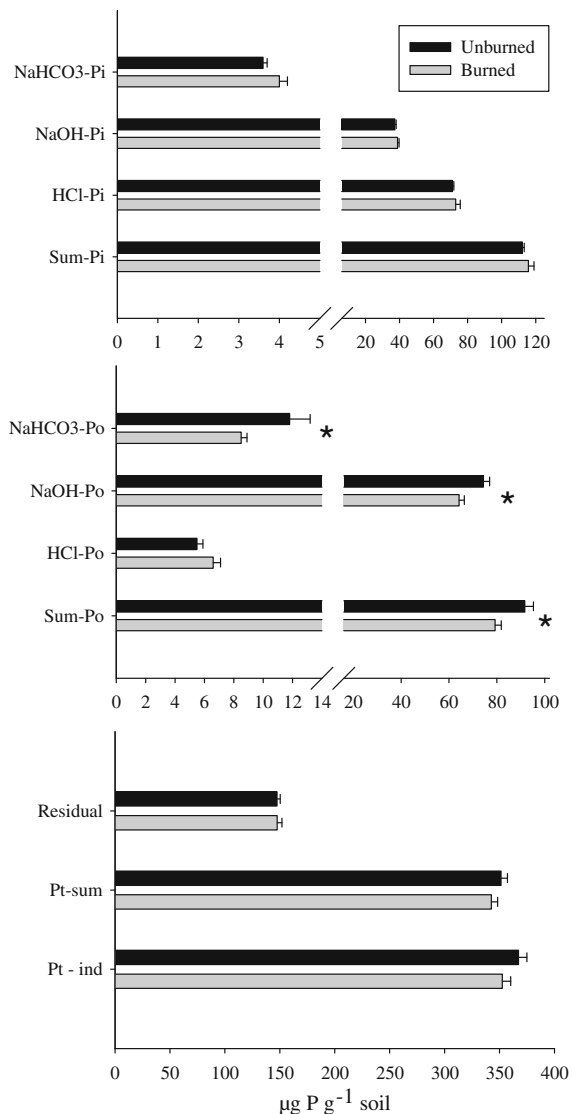
Occluded P concentrations (i.e. concentrated HCl and residual) decreased more subtly with depth than organic and available inorganic fractions that declined sharply. Those occluded fractions have low solubility and consequently low mobility, and therefore are not readily available for plant absorption and are not leached from the ecosystem. The relative proportion of occluded fractions increases with depth relative to the available P fractions (as described by Smeck 1973).

Despite declining available and total P concentration with depth, our results suggest that deep soil is more important to the P cycle than has been recognized. Most studies evaluate the P cycle only in the superficial soil horizons (0–20 cm), but the

persistence of labile organic P fractions (i.e.  $\text{NaHCO}_3\text{-Po}$ ) to at least to 300 cm depth in the present study demonstrates that P cycling is also important in deep soil. The  $\text{NaHCO}_3\text{-Pi}$  fraction is also present along the whole profile. Even at 500 cm depth, we still have a measurable concentration of readily extractable P ( $1 \mu\text{g g}^{-1}$ ). Because roots are often found to several meters depth in deeply weathered Oxisols, including under Amazonian forest (Nepstad et al. 1994) and under Cerrado (Oliveira 1999), deep root uptake and turnover may be important for determining the pool of P that is potentially available to the vegetation.

#### Effects of fire on soil P

Results from the burned and unburned plot comparison indicate that repeated burning decreased the organic-P fraction and P availability in these soils at 0–10 cm. For this long-term burned vs. unburned plot comparison (Figs. 6 and 7) the decrease of organic P was probably caused by the subsequent decrease of organic inputs by litter (litter stock in the plots decreased from  $13.3\text{--}5.6 \text{ Mg ha}^{-1}$ ), rather than a direct effect of soil P transformations during the fire



**Fig. 7** Comparison of the soil phosphorus fractions between the two treatments, unburned ( $n = 9$ ) and burned ( $n = 18$ ) Cerrado plots in IBGE Ecological Reserve, Brasília, Brazil. Soil samples collected in 1999 in the 0–10 cm depth. *Pt ind* independent phosphorus determination. Horizontal bars indicates  $\pm 1$  standard error, asterisk indicate difference between treatments

event. The decrease of the  $\text{NaHCO}_3\text{-Po}$  and  $\text{NaOH-Po}$  fractions due to the repeated fires indicates a loss of P bioavailability in the burned plot for both microbial activity and plant absorption.

A similar pattern of organic P loss as an immediate response to fire has been described for a caatinga dryland soil of Northeast Brazil (Lessa 1998) and for a dry tropical forest soil in Mexico (Dockersmith

et al. 1999). In the present study, we also observed our lowest  $\text{HCO}_3\text{-Po}$  value after the fire, although the response was not significant and was delayed by 4 months. In addition, contrary to observations in the caatinga and dry forests, we did not observe a significant increase in the inorganic available fractions following fire in the superficial soil layer. In fact, after 4 months  $\text{HCO}_3\text{-Pi}$  was at its lowest observed value. The presence of  $1.2 \text{ kg ha}^{-1}$  of inorganic P in ash deposition after fire did not cause an increase in soil Pi in the surface soil layer.

Changes in soil organic and inorganic P fractions are related to fire characteristics (Galang et al. 2010). Cerrado fires depend on the existence of fine fuel (leaves and branches up to 6 mm in diameter) (Miranda et al. 1996). In general, the fire front is relatively fast with a lower heat pulse (Miranda et al. 1996) leading to small changes in soil temperature during the fire front (Miranda et al. 1993; Castro-Neves and Miranda 1996). These rapid and relatively cool Cerrado fires clearly contribute ash to the soil surface, but apparently do not significantly alter soil organic matter. In limited areas (e.g. near burning branches) more intensive fire may cause larger modifications in the soil P fractions (Saá et al. 1994). The deposition of ash from charred trees and shrubs may also contribute to localized P input, thus increasing the heterogeneity of burned areas. Finally, in the present case, the two previous burns had already depleted mineral soil organic P, which may limit the potential for further declines during this third quadrennial burn.

#### P fluxes

In Cerrado ecosystems P fluxes are low and the largest P reservoir is the soil, although mainly as unavailable P forms. The fluxes of dissolved P through atmospheric deposition, throughfall and litter leachate are very low. The litterfall-P flux is also low ( $0.4 \text{ kg P ha}^{-1} \text{ year}^{-1}$  in the fine litter) partly as a result of P resorption (Nardoto et al. 2006). Phosphorus resorption rates vary according to species but can be as high as 70% for some species and indicate a stronger limitation by P than by N (Nardoto et al. 2006). Litterfall also has a high C/P ratio (up to 1,300) that leads to very low decomposition rates. Given these low P fluxes, the decrease of extractable soil organic P caused by repeated burning may reduce

the available P stocks over the long term. This can contribute to a possible decline of plant productivity and soil microbial activity in Cerrado ecosystems. Likewise, if much of the ash produced during burning was lost to water and wind erosion rather than entering the soil, then repeated fire with frequencies <4 years could lead to reduction of available-P pools.

The observed increase in P concentration of soil solutions at 100 cm depth may also indicate a pathway for P loss. Leaching of P beyond the major rooting zone is possible, although unlikely given the low concentrations in soil solution. Uptake of P by deep roots is possible but reaction of solution P at depth with soil iron and aluminum oxides is more likely (Leal and Velloso 1973; Lopes and Cox 1979; Bahia-Filho et al. 1983; Fontes and Weed 1996). The distribution of soil nutrients with depth is determined by plant cycling and leaching processes. Plants exert a dominant control on the vertical distribution of the most limiting elements, such as available P, causing its concentration in the topsoil (Jobbágy and Jackson 2001). Frequent fires in Cerrado ecosystems could be a factor increasing P leaching and changing the distribution of P fractions in the soil profile.

Despite the absence of direct measurements of P absorption by plant roots, measurements indicate a seasonal variation in water demand according to soil depth. During the dry season, the deep soil compartment can contribute as much as 83% of the total water used in a tree-dominated Cerrado community (Oliveira et al. 2005) but uptake from deep soil layers ceases soon after the upper layers are wetted (Quesada et al. 2004). Water in soil superficial layers is more readily available to the vegetation than that in the deeper layers (Meinzer et al. 1999).

A potentially underappreciated dynamic of P cycling in the Cerrado ecosystems is microbial retention of P. In seasonal climates, microbial P fluctuates seasonally, caused in this case by soil moisture variation between dry and wet seasons (Grierson and Adams 2000). In the unburned plot, an increase of microbial P concentration occurred at the surface soil (0–10 cm depth) in the rainy season. This is an indication that, where plant and microorganisms compete for available P, microbial assimilation during the growing season may keep soil available P levels low, avoiding leaching and chemical fixation by the iron and aluminum oxides. This is a

conservative feature of the P cycle in an ecosystem with large P stocks in the soil, but a small available P fraction.

Nardoto and Bustamante (2003) in a study conducted at the IBGE Ecological Reserve, found that microbial biomass can be very dynamic and strongly associated with rain events. Short dry spells can increase the mortality of microorganisms in the soil and the subsequent mineralization of nutrients that then become available to plants upon wet-up. At the beginning of the rainy season there is nitrogen immobilization that is followed by an increase of net nitrogen mineralization at the end of the rainy season (Nardoto and Bustamante 2003). The ratio between microbial C and microbial P can be an important indicator of P availability. Considering the concentration of microbial C (Nardoto and Bustamante 2003) and the average concentration of microbial P we estimated that the C/P microbial ratio is approximately 56. This is a very low value compared to the C/P<sub>o</sub> ratio of 400 we calculated for the soil. Therefore, microbial P may be much more easily mineralized, being an important potential source of available P for plant growth. Rates of P mineralization are unknown for Cerrado ecosystems, but this attribute is crucial for the understanding of the role of the microbial biomass on P cycling and prevention of ecosystem losses.

The effect of fire on microbial biomass and activity in a woodland savanna in Ethiopia was dependent on fire intensity (Jensen et al. 2001). Moderate fire caused an increase in nutrient availability, and then a short-term increase in microbial carbon. Theoretically, microbes would be a sink of nutrients in the period between fire and plant regrowth (Jensen et al. 2001). In Cerrado soils microbial biomass may present similar transient short-term effects.

Forests growing on nutrient-poor substrates present an array of nutrient conserving mechanisms to maintain plant biomass and productivity. In such ecosystems, a closed nutrient cycle might develop with a positive internal accumulation, but low inputs by atmospheric deposition and low hydrological outputs. The low P inputs by atmospheric deposition coupled to a very low P output to stream, as described in this study, indicates such a closed nutrient cycle. Taken together, the low P availability in the soil, low decomposition rates, and low P fluxes in solutions

indicates that Cerrado terrestrial and aquatic systems are very conservative with P.

## Conclusions

In this tropical savanna on deep, highly weathered Oxisols we found indications of P availability up to 3 m soil depth, which may be caused by biological activity of the P cycle that has not been anticipated for Cerrado soils before. In addition, mechanisms of P conservation in this P limited ecosystem include P immobilization by microbes during the rainy season. Repeated fire on a 4 years return frequency caused an increase in P leaching to deeper soil horizons and a reduction of P stocks in litter and organic P in soil. Consequently repeated fires might decrease P availability in the long-term. The output of P to the stream is low, indicating a closed nutrient cycling in the Roncador watershed with strong limitation by P.

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