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Phosphorus Transformations in an Oxisol under contrasting land-use systems: The role of the soil microbial biomass

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Abstract

It is generally assumed that phosphorus (P) availability for plant growth on highly weathered and P-deficient tropical soils may depend more on biologically mediated organic P (P_o) turnover processes than on the release of adsorbed inorganic P (P_i). However, experimental evidence showing the linkages between P_o, microbial activity, P cycling and soil P availability is scarce. To test whether land-use systems with higher soil P_o are characterized by greater soil biological activity and increased P mineralization, we analyzed the partitioning of P among various organic and inorganic P fractions in soils of contrasting agricultural land-use systems and related it to biological soil properties. Isotopic labeling was used to obtain information on the turnover of P held in the microbial biomass. Soil samples were taken from grass–legume pasture (GL), continuous rice (CR) and native savanna (SAV) which served as reference. In agreement with estimated P budgets (+277, +70 and 0 kg P ha^{-1} for CR, GL and SAV, respectively), available P estimated using Bray-2 and resin extraction declined in the order CR > GL > SAV. Increases in Bray-2 and resin P_i were greater in CR than GL relative to total soil P increase. Organic P fractions were significantly less affected by P inputs than inorganic fractions, but were a more important sink in GL than CR soils. Extractable microbial P (P_{chl}) was slightly higher in GL (6.6 mg P kg^{-1}) than SAV soils (5.4 mg P kg^{-1}), and significantly lowest in CR (2.6 mg P kg^{-1}). Two days after labeling the soil with carrier free ^{33}P, 25, 10 and 2% of the added ^{33}P were found in P_{chl} in GL, SAV and CR soils, respectively, suggesting a high and rapid microbial P turnover that was highest in GL soils. Indicators of P mineralization were higher in GL than CR soils, suggesting a greater transformation potential to render P_o available. Legume-based pastures (GL) can be considered as an important land-use option as they stimulate P cycling. However, it remains to be investigated whether crops planted in pasture–crop rotations could benefit from the enhanced P_o cycling in grass–legume soils. Furthermore, there is need to develop and test a direct method to quantify P_o mineralization in these systems.

Introduction

Phosphorus (P) deficiency is a major constraint to agricultural productivity and it affects an area estimated at over 2 billion hectares worldwide. The highly weathered acidic Oxisols and Ultisols account for more than 70% of P-deficient soils in the tropics, of which nearly 50% are found in tropical America (Fairhurst et al., 1999). If food security is to be attained in the tropics during the coming decades, the constraint of P deficiency must be addressed.

As a non-renewable resource with relatively low concentrations in the biosphere, use of P from fertilizers must be rationalized. This is especially true in the tropics where iron and aluminum oxides in Oxisols and Ultisols cause strong sorption of soluble fertilizer P. To improve the efficiency of P applications, it is imperative to maximize the recycling of P from crop residues, and organic and mineral fertilizers. In highly weathered tropical soils, the availability of P may depend more on the turnover of easily decomposable soil

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organic matter than on the desorption of inorganic P (P_i) (Tiessen et al., 1992; Gijsman et al., 1996; Tiessen and Shang, 1998). Processes such as immobilization of P_i by microbes and its gradual release via microbial turnover can protect P from physico-chemical adsorption reactions if this release is synchronized with the demand of growing plants and/or a subsequent generation of microorganisms (Magid et al., 1996). Furthermore, a significant part of the P held in soil microorganisms is a readily available P source for plants (Macklon et al., 1997).

Microorganisms play a key role in soil P_o transformations (Stewart and Tiessen, 1987; McLaughlin et al., 1988; Magid et al., 1996; Frossard et al., 2000) through excretion of phosphatase enzymes (Dighton, 1983; Sinsabaugh et al., 1993), mineralization of P from organic sources (Lopéz-Hernandez et al., 1998; Gressel et al., 1996), and synthesis and release of P_o (Seeling and Zasoski, 1993; Magid et al., 1996). In addition, microorganisms can solubilize sparingly soluble P_i forms (Kucey et al., 1989; Illmer et al., 1995). Soil microorganisms were found to be a major factor in controlling organic and inorganic P solution concentrations in temperate grassland topsoils (Seeling and Zasoski, 1993).

In recent years, substantial progress has been made in the selection and breeding of crop and forage plants genetically adapted to low-P soils (Pellet and El-Sharkawy, 1993; Rao et al., 1999b; Lynch and Beebe, 1995; Fageria and Baligar, 1997). The improved cultivars have been shown to efficiently utilize low inputs of fertilizer P (Rao et al., 1995; Friesen et al., 1997; Oberson et al., 1999). In addition to root attributes such as size, distribution and P uptake efficiency, which affect the ability of plants to absorb P (Rao et al., 1997, 1999a), rates of crop/forage residue decomposition differ among plant species (Thomas and Asakawa, 1993; Friesen et al., 1997; Gijsman et al., 1997a). The decomposition of plant residues is biologically driven, with soil microorganisms making by far the most important contribution in mineralization (Cadisch and Giller, 1997). A linkage between vegetative cover, soil biological activity and soil P availability was suggested by studies of Guggenberger et al. (1996) and Oberson et al. (1999) where changes in the P status of native savanna soils replaced by introduced grass–only (*Brachiaria decumbens*) and grass–legume (*Brachiaria decumbens* and *Pueraria phaseoloides*) pastures were investigated. Grass–legume pastures had higher reserves of labile P_o compounds (phosphonates and diester P) assessed by liquid state ^{31}P nuclear magnetic resonance spectroscopy (Guggenberger et al., 1996). The soils under grass–legume pasture maintained higher organic and available P levels with less temporal variation than grass–alone or native pastures (Oberson et al., 1999). With comparable fertilizer inputs and greater product exports than in grass–alone pastures, improved P availability in grass–legume pastures could not be due to differences in P budgets. It was suggested that greater turnover of roots and above-ground litter in legume-based pastures could provide for steadier P_o inputs and, therefore, higher P cycling and availability. The soil P_o fraction most affected was NaOH extractable P (Oberson et al., 1999). Beck and Sánchez (1994) concluded NaOH-P_o to be the primary source of plant available P in non-fertilized systems. Tiessen et al. (1984) found a close relationship between resin extractable P_i and P_o in Ultisols. These studies suggested that P_o contributes to available P_i.

If this is correct, land-use systems that increase soil P_o should enhance soil biological activity and therefore increase P mineralization. We tested this hypothesis by studying soil P transformations in an Oxisol under contrasting agricultural systems after 5 years of change in land-use. The contrasting land-use systems were grass–legume pasture (GL), continuous rice (CR) and native savanna (SAV) that served as reference treatment. First, chemical sequential P extraction was used to evaluate whether a higher partitioning of fertilizer P into organic fractions occurred in GL than in CR. To investigate whether P_o was immobilized or turned over, biological soil parameters related to P transformations such as size of the soil microbial biomass, amount of microbially bound P and C mineralization and acid phosphatase activity were assessed. Given the central role of microorganisms in the soil P cycle, microbial P turnover was studied using ^{33}P isotopic labeling.

Materials and methods

Field experiment

Soil samples were taken in a 5-year-old field experiment established in 1993 to investigate the sustainability of crop rotation and ley farming systems for the acid soil savannas (Friesen et al., 1997). The experiment was carried out at the CORPOICA-CIAT (Instituto Colombiano Agropecuario; Centro Internacional de Agricultura Tropical), Carimagua research

station, Meta, Colombia (4°30'N, 71°19'W), 150 m above sea level on the eastern plains of Colombia. The area is representative of the well-drained savannas. Rainfall averages 2240-mm annually, falling mainly from late March to mid-December. Mean annual temperature is 27°C. Soils are well drained silty clay Oxisols (tropeptic haplustox, isohyperthermic). Soil chemical characteristics of the 0–10 cm soil layer are summarized in Table 1.

The following treatments were included in the study:

(1) Native savanna (SAV) as reference: native grassland, burned once per year in February, not grazed.
(2) Grass–legume pasture (GL): pasture under-sown with rice in 1993, since then grass–legume pasture including *Brachiaria humidicola* CIAT 679, *Arachis pintoi* CIAT 17434, *Stylosanthes capitata* CIAT 10280 and *Centrosema acutifolium* cv Vichada CIAT 5277; partly resown for renovation in June 1996 with legumes (the same *Arachis pintoi* and *Centrosema acutifolium*; additionally *Stylosanthes guianensis* CIAT 11833); on average grazed with 2.7 steers ha^{-1} during 15-day followed by a 15 days ley re-growth phase.
(3) Continuous rice (CR) since 1993: *Oryza sativa* cv Oryzica Sabana 6, cv Oryzica Sabana 10 since 1996; fallow during second semester rain and dry season.

The experiment had a split-plot design with four replicates with treatment sub-plots of 0.36 ha size (200 × 18 m). Before establishing the treatments GL and CR on savanna, the soil was conventionally tilled after burning the native vegetation. The CR and GL plots were limed before starting the experiment in 1993 using 500 kg dolomitic lime ha^{-1}. Rate of fertilization (kg ha^{-1}) of rice was 80 N (urea, three split applications), 60 P (triple superphosphate), 100 K (KCl), 35 S (20 as elemental, 15 as $MgSO_4 \cdot H_2O$) and 10 Zn ($ZnSO_4 \cdot 6\ H_2O$). With the exception of fertilizer P placement in rice, all fertilizers were broadcast. While the introduced pasture received an additional 20 kg P ha^{-1} only when renovated in 1996, rice was fertilized annually (60 kg P ha^{-1}), resulting in the P inputs shown in Table 2. Native savanna was not fertilized. The systems also differ in soil cultivation (frequent, rare or no cultivation in CR, GL and SAV treatments, respectively) and in application of herbicides (frequent, rare or no application of herbicides for CR, GL and SAV treatments, respectively).

Phosphorus budgets were estimated by subtracting the P removed from the system by grain and/or with animal live weight gains from the P applied as mineral fertilizers. Phosphorus exports in grain were calculated by multiplying weighed rice grain yields with measured P contents in the grain. Phosphorus exported in the animals was assumed to be 8 g per kg of live weight gain (NRC, 1984). Live weight gains in GL were on average 68 kg ha^{-1} $year^{-1}$.

Soil sampling

Soils were sampled in the rainy season, in September 1998. For the sampling, 0.09 ha sub-plots (75 × 12 m) were delimited in each treatment and replicate to avoid border effects and disturbance by micro-plots laid out for special investigations. These sub-plots were divided into three 25 × 12 m sampling sub-sub-plots to assure that random cores were taken over the complete area. Within each sampling sub-sub-plot area, 20 cores (0–10 cm depth) were sampled using a 5 cm diameter auger. The cores were put into a bucket, homogenized, and roots removed. Subsequently, all 60 cores were mixed in another bucket and a sub-sample of about 2 kg soil (wet wt.) was filled into plastic bags. Collected soil samples were immediately (in the field) put into cool boxes and taken back to the station where they were kept at 4°C until they were sieved (4-mm) and the remaining visible plant residues were removed. Part of the soil was stored at 4°C and the remainder was air-dried.

Sampling in the 0–10 cm layer represented a precautionary method to avoid contamination with soil from deeper layers that was not affected by soil cultivation (usually to a maximum of 15 cm depth).

Soil phosphorus analyses

Soil P analyses were carried out on each field replicate using air-dried, 2-mm-sieved samples. Sequential P fractionation was carried out using the following extractants on 0.5 g soil samples and quantifying inorganic P (P_i), total P (P_t) and (by difference) organic P (P_o) in the extracts (Hedley et al., 1982, modified by Tiessen and Moir, 1993): H_2O with anion exchange resin in HCO_3-form, 0.5 M $NaHCO_3$, 0.1 M NaOH, and hot (80°C for 10 min) concentrated HCl. Total P in the soil residue after extraction was determined by digestion with hot concentrated perchloric acid. Changes in fractions in fertilized CR and GL soils

Table 1. Soil pH, total C, total N and exchangeable cations in the 0–10-cm soil layer of the contrasting land-use systems

Treatment	pH(H_2O)	Total C mg g^{-1}	Total N mg g^{-1}	Total P mg g^{-1}	Bray-2 P mg g^{-1}	Exchangeable cations $cmol_+$ kg^{-1}			
						Al	Ca	Mg	K
Savanna†	4.9b	26.1a	1.68	216a	1.4a	2.64b	0.21a	0.15a	0.09a
Grass–legume†	4.8b	28.4b	1.80	272b	3.4a	2.19a	0.57b	0.34b	0.14b
Continuous rice†	4.7a	24.7a	1.67	354c	15.6b	2.33ab	0.57b	0.28b	0.21c
F-test‡	**	***	ns	***	***	*	***	***	***

†Means of four field replicate samples per treatment. Means within a column followed by the same letter are not significantly different ($P = 0.05$) by Tukey's multiple range test.
‡*F*-test: $^{***}P < 0.001$, $^{**}P = 0.001$–0.01, $^{*}P = 0.01$–0.05; *ns*, not significant.

Table 2. Estimated P budget over 5 years (1993–1997) for contrasting land-use systems, and resulting changes in total P contents in the 0–10 cm soil layer

Treatment	Estimated P budget (kg P ha^{-1})			Increase in soil total P content		
	Input	Export	Balance	Bulk density† (Mg m^{-3})	Calculated from P balance (mg P kg^{-1})	Measured‡ (mg P kg^{-1})
Savanna	0	0	0	1.24	0	
Grass–legume	80	10	+ 70	1.24	56	52 (11)
Continuous rice	300	23	+ 277	1.15	241	152 (8)

†Source: CIAT (1999).
‡See Table 3; mean and SEM of four field replicates; pairs of SAV and GL, and SAV and CR, respectively, were formed at random.

were compared to non-fertilized SAV using:

$$\text{Increase (\%)} = 100 \frac{\text{(size of fraction in fertilised treatment} - \text{size of fraction in SAV)}}{(\text{Sum P}_t \text{ fertilized treatment} - \text{Sum P}_t\text{SAV})} \quad (1)$$

where:

$$\text{Sum P}_t = \text{Resin P}_i + \text{NaHCO}_3\text{P}_t + \text{NaOH P}_t + \text{HCl P}_t + \text{Resid P}_t \quad (2)$$

Bray-2 P and total P were determined at the Analytical Service Laboratory of CIAT. Bray-2 P was extracted using dilute acid fluoride (0.03 M NH_4F, 0.1 M HCl) at a 1:7 soil:solution ratio using 2.85 g soil and 40 s shaking time. Total soil P was determined by digestion with a mixture of 2 parts hot concentrated HNO_3 and 1 part concentrated $HClO_4$, using 5 ml per 0.5 g soil.

Particulate organic matter

Particulate organic matter (> 53 μm) was isolated by dispersing soil of each field replicate in 5% sodium hexametaphosphate and passing the suspension through a sequence of 2-mm, 250 μm and 53 μm sieves (Cambardella and Elliott, 1992; Gijsman, 1996). The fraction > 2-mm was discarded while the 250 and 53 μm fractions were dried at 50°C in an oven, finely ground and analyzed for total C and N using a flash combustion element analyzer (Carlo Erba Instruments, NA 1500, Rodano-Milano, Italy).

Biological and biochemical soil analyses

Microbial biomass C, N and P

Field moist soil (4-mm sieved) was incubated at 26°C for 15 days at a water content of 50% of field capacity (280 g H_2O kg^{-1} soil dry wt.) before the size of microbial C, N and P was determined. Microbial C and N were determined in duplicate and microbial P in triplicate on each field replicate.

Levels of microbial C and N were analyzed using the method of Vance et al. (1987) in which paired sub-samples of incubated soil were either immediately extracted with 0.5 *M* K_2SO_4 or fumigated with ethanol-free chloroform for 24 h in the dark at room temperature before extraction. Extracts were lyophilized and transported to FiBL Frick Switzerland where they were re-wetted before organic C and total N determination using a Dimatoc 100 apparatus (Dimatec, Essen, Germany). Glycine, EDTA and ammonium sulfate standards subjected to the same procedure showed that lyophilization had no effect on C and N content in the extracts. Microbial C and N released

during chloroform fumigation (C_{Chl}, N_{Chl}) were calculated from the difference in extractable C and N, respectively, before and after the fumigation. No conversion factors were used to convert C_{Chl} and N_{Chl} values to microbial biomass.

The portion of microbially bound P released during 1.25 h fumigation with chloroform (P_{Chl}) was determined by the procedure of Morel et al. (1996) as modified by Oberson et al. (1997). Microbial P released during fumigation was extracted by Bray-1 solution and a correction for P adsorption during fumigation was calculated based on recovery of P adsorbed from a series of P additions incubated for an equivalent time period (Fig. 1a).

Soil respiration, C mineralization and phosphatase activity

Carbon mineralized during soil respiration was determined for each field replicate by trapping and quantifying CO_2 evolved by 40 g sub-samples of humid soil (4-mm sieved) placed in sealed containers using the method of Zibilske (1994). The samples were incubated for 63 days during which evolved CO_2 was determined twice per week during the first 3 weeks of incubation, and weekly thereafter. Carbon mineralization was calculated as the CO_2-C produced during incubation. The metabolic quotient qCO_2 (Anderson and Domsch, 1990; Gijsman et al., 1997b) was calculated as the rate of CO_2-C production (μg) per mg C_{Chl} per h.

Acid phosphatase activity at pH 6.5 of soil samples was measured for each field replicate using 1 g air-dried soil (< 2-mm) according to the method of Tabatabai (1982).

Isotopic composition of extracted microbial P

To investigate the microbial P turnover in the absence of fresh organic matter additions, an isotopic labeling experiment was carried out under conditions of constant soil respiration by combining the approach of McLaughlin et al. (1988) with the P_{chl} determination method using Bray-1 extractant as described previously (Oberson et al., 1997).

Field moist samples (4-mm sieved) were pre-incubated at 26°C for 3 weeks by which time the respiration rate had become low and nearly constant (Fig. 2). Then, isotopically exchangeable P was labeled by thoroughly mixing the soil with carrier free ^{33}P labeled orthophosphate solution. The labeling amounted to 11.0, 8.6 and 12.4 kBq g^{-1} soil for SAV, GL and CR, respectively. Water content was only slightly raised by labeling. Soil water content was controlled by weighing and readjusted if required every other day during the ongoing incubation.

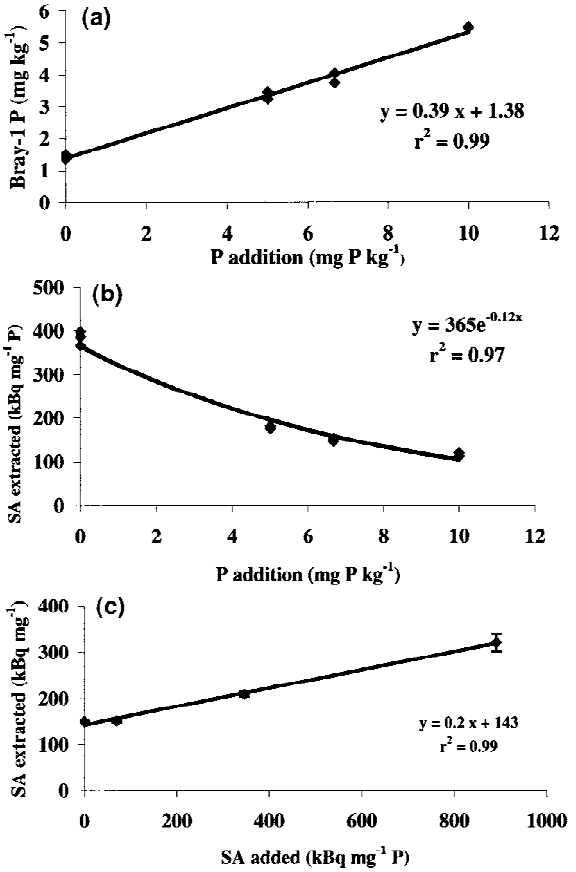

Figure 1. (a)Amounts of P extracted using Bray-1 as a function of the amount of unlabeled P added to the GL soil. Three (0 and 5 mg P kg^{-1} soil) or two analytical replicates per P addition. (b)Specific activity (SA) of Bray-1 extracted P as a function of the amount of unlabeled P added to the GL soil 2 days after labeling. Three (0 and 5 μg P/g soil) or two analytical replicates per P addition. (c)Specific activity (SA) of Bray-1 extracted P as a function of the SA of a constant amount of P (6.67 mg P kg^{-1}) added with increasing ^{33}P activities to the GL soil 2 days after labeling. Each point presents a mean of three analytical replicates.

Two and 8 days after labeling, chloroform released P was determined as described previously. In addition to colorimetric P_i determination, radioactivity in all Bray-1 extracts was determined by scintillation counting using 1 ml aliquots. Preliminary tests showed that neither the Bray-1 extractant nor possible residues of chloroform affected the counting.

The concentration of ^{33}P applied was very low (< 3.5×10^{-6} μg P g^{-1} soil) compared to ^{31}P. Therefore,

the specific activity (SA) of P_i in the extracts can be calculated as follows:

$$SA\ (Bq\mu g^{-1}P) = {}^{33}P/{}^{31}P \quad (3)$$

For the non-fumigated, non-P-amended samples, the SA (SA-$Bray_0$) can be obtained directly after decay correction to the date of labeling. While P_i sorption occurring during fumigation and extraction can be corrected by the P addition curve approach presented previously (Fig. 1a), correction for sorption and/or exchange of the ^{33}P isotope is much more complex (McLaughlin et al., 1988). As the disappearance or appearance of ^{33}P in solution depends not only on the isotope concentration, but also on the specific activity of P in solution, correction for isotope recovery cannot be calculated from the disappearance of an added spike of isotope. Figure 1b illustrates that the addition of increasing amounts of non-labeled P_i dilutes the SA of Bray-1 extractable P. Figure 1c demonstrates that the addition of the constant amount of P_i with increasing specific activities increases the specific activity of Bray-1 extracted P_i. The recovery of ^{33}P added with the spike solutions is not complete since some of it is immediately exchanged with ^{31}P located on the soil's solid phase. Therefore, the recovery of ^{33}P derived from lysed microbial cells will not be complete and has to be corrected (McLaughlin et al., 1988). This correction is obtained by the addition of spike solutions containing a P_i amount close to P_{chl} and a range of specific activities (Fig. 1c) (McLaughlin et al., 1988). However, because exchange processes are on-going (Fardeau, 1993), new correction curves had to be established at every sampling date. This renders the experiments very laborious. For this reason, composite samples containing equal portions of the four field replicates were used. The P_{chl} content of the composite samples did not differ from the mean value assessed on the four field replicates.

Statistical analysis

Statistical analysis was carried out using log transformed data in order to meet the assumptions of analysis of variance (i.e., additive effects, symmetric errors with equal variance). The effect of land-use system was tested by analysis of variance (ANOVA). If the *F*-test was significant ($P < 0.05$), the means were compared using Tukey's multiple range test.

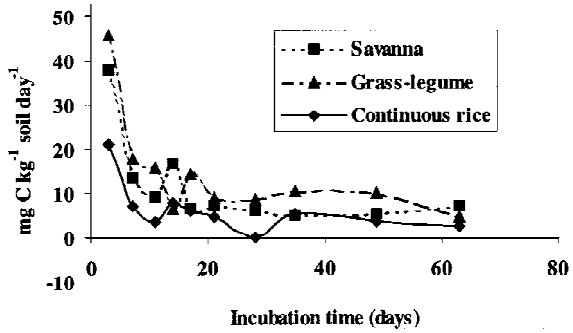

Figure 2. Changes in daily C mineralization rate during the incubation.

Results and discussion

Fate of applied P

Inputs by fertilizers exceeded P exports in GL and CR, resulting in positive P balances (Table 2). Total P determined by perchloric acid digestion (Table 1) was not significantly different from the sum of fractions extracted by the sequential procedure (Table 3). The total P content measured in the 0–10 cm soil layer was 52 and 152 mg P kg^{-1} higher in the GL and CR, respectively, than in the SAV topsoil (Tables 2 and 3). The increases in total P content calculated from the P balance (Table 2), assuming the bulk densities shown in Table 2, were 56 and 241 mg P kg^{-1} for GL and CR, respectively. These increases demonstrate full recovery of applied P in the 0–10 cm soil layer of GL while some P is unaccounted for in the CR soil. Due to soil cultivation in the CR treatment (usually to a maximum of 15 cm depth), part of the applied P may have been mixed into deeper soil layers or may have moved into the subsoil, as observed by Beck and Sánchez (1996) on a cropped Ultisol.

In agreement with the positive P balance, available P_i increased in the CR and GL soils in relation to the SAV reference (Tables 1 and 3). For tropical pasture species, amounts of Bray-2 P from 2 to 5 mg kg^{-1} (i.e., the range within the GL soils fall) are considered medium while they would be considered low for crops. For most tropical crops, Bray-2 P of 11–15 mg kg^{-1} are considered adequate and >15 high (I.M. Rao, unpublished), indicating that in the investigated CR soils, P is not a yield limiting factor.

Resin, $NaHCO_3$ (0.5 M) and NaOH (0.1 M) extractable P_i were higher in CR than in GL, both in absolute and relative terms (Table 3). NaOH P_i is confirmed to be the main sink for applied P, as observed

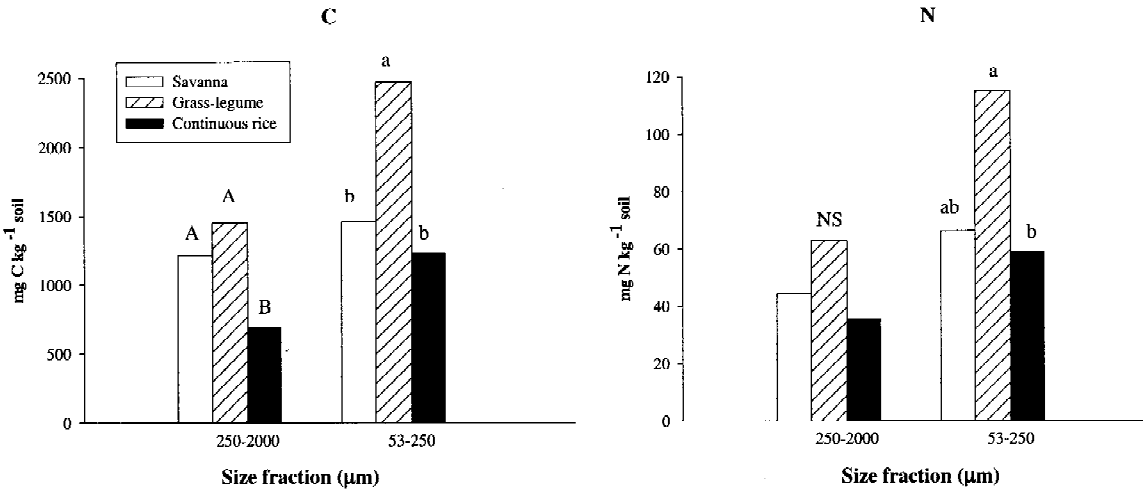

Figure 3. Particulate organic matter C and N in two size fractions in soils of contrasting land-use systems. Upper case letters denote significant differences among systems for the 250 μm–2-mm, lower case letters for the 53–250 μm size fraction.

in earlier studies on Oxisols at Carimagua (Friesen et al., 1997; Oberson et al., 1999) and on Ultisols in the Amazon basin (Beck and Sánchez, 1994). Using path analysis, Beck and Sánchez (1994) concluded that P desorbed from this fraction could maintain P availability in fertilized systems.

Organic P fractions were less affected by P inputs than P_i fractions. In addition, they were less affected in CR than in GL where especially NaOH P_o acted as a sink. Analysis of changes in fractions using Eq (1) shows that more than 30% of the increase over SAV went into organic fractions in GL while the corresponding portion was less than 11% in CR soils (Table 3). Differences in the partitioning of P in the fertilized treatments indicate that P transformation processes in CR soils differ from those in GL soils. Though changes in absolute size of organic fractions are not statistically significant (Table 3), the organic fraction most affected was NaOH P_o. This confirms a higher partitioning of P applied as fertilizer into organic fractions in GL soils (Guggenberger et al., 1996; Oberson et al., 1999). If NaOH P_o could contribute to plant available P_i, GL should be characterized by higher soil biological activity mediating P mineralization, than CR soils.

Biological activity in relation to P transformations

Size and composition of the microbial biomass

The amount of extracted microbial C, N and P was affected by the agricultural land-use system in the order GL $\sim$ SAV $>$ CR (Table 4). This confirms that, in Colombian savanna soils, the replacement of the native vegetation by grass–legume pasture rather increases the soil microbial biomass, while rice monocropping leads to a decrease (Gijsman et al., 1997b).

Although the fumigation extraction method is widely used to measure microbial biomass, there is still uncertainty about the conversion factors (k_{EC}, k_{EN}, k_P) to use in calculating the total soil microbial C, N and P contents. While techniques for k_{EC} and k_{EN} determinations are available (Sparling and Zhu, 1993; Jörgensen, 1996), recent studies agree on not using k_P factors (Morel et al., 1996; Oberson et al., 1997; Myers et al., 1999) because to date, no precise technique for its determination is available that could account for all the variation among different organisms and different soils. Sparling and Zhu (1993) found k_{EC} to vary from 0.10 to 0.90 on acid sandy soils of Western Australia. Feigl et al. (1995) obtained k_{EC} factors of 0.20–0.30 on a range of Amazon basin rain forest soils comprising Oxisols, Ultisols and an Alfisol with 18–80% clay content. If we convert C_{Chl} (Table 4) using a k_{EC} of 0.25, the estimated soil microbial biomass ranged from 289 (CR) to 689 (GL) mg microbial C kg^{-1} soil. This is less than reported for Oxisols under undisturbed tropical rain forest (Feigl et al., 1995), but is in the range reported for temperate pastures (Sparling, 1992) and arable soils (Fliessbach and Mäder, 1997). The same applies for the percentage of total soil C held in the microbial biomass (1.2–2.4%, assuming k_{EC} of 0.25) (Sparling, 1992), which suggests that the soil microbial population has adapted

Table 3. Distribution of P in various fractions in fertilized land-use systems (continuous rice, grass–legume pasture) 5 years after establishment on native savanna as assessed from sequential extraction. Relative changes (% increase) describe which percentage of total P increase in fertilised systems over native savanna was found in a given fraction (formula see footnote †)

Treatment	Resin	$NaHCO_3$		NaOH		HCl		Resid	Sum		
	P_i	P_i	P_o	P_i	P_o	P_i	P_o	P_t	P_t‡	P_i§	P_o¶
Savanna											
Mean mg kg^{-1}	2.6a	3.9a	11.3a	27.4a	45.3	35.6a	23.9	60.6	212a	69a	81.9
Grass–legume											
Mean mg kg^{-1}	4.8b	6.7b	14.6b	45.5b	51.0	46.5b	30.3	62.2	263b	103b	97.8
% Increase†	4.3	5.4	6.5	35.5	11.3	21.4	12.6	3.2	101	66.6	31.1
Continuous rice											
Mean mg kg^{-1}	14.3c	20.2c	17.1b	111.0c	42.7	54.3b	36.2	65.6	363c	200c	98.0
% Increase†	7.7	10.7	3.8	55.0	−1.7	12.3	8.1	3.3	100	85.8	10.6
F-Test	***	***	**	***	ns	*	ns	ns	***	***	ns

Means of four field replicates samples per treatment. Means within a column followed by the same letter are not significantly different ($P = 0.05$) by Tukey's multiple range test. *F*-test: $^{***}P < 0.001$, $^{**}P = 0.001$–0.01, $^{*}P = 0.01$–0.05; *ns*, not significant.

† Increase (%) = (size of fraction in fertilized treatment – size of fraction in SAV) / (Sum P_t fertilized treatment – Sum P_t SAV) 100.

‡Sum P_t = Resin P_i + $NaHCO_3$ P_t + NaOH P_t + HCl P_t + Resid P_t = Sum P_i + Sum P_o.

§Sum P_i = Resin P_i + $NaHCO_3 P_i$ + NaOH P_i + HCl P_i.

¶Sum P_o = $NaHCO_3 P_o$ + NaOH P_o + HCl P_o.

to the severe chemical constraints of highly weathered tropical soils.

While N_{Chl} values are also in a similar range to temperate cropped soils (Fliessbach and Mäder, 1997), $P_{Chl}$is significantly lower. As a consequence, C_{Chl}/N_{chl} ratios were similar while C_{Chl}/P_{Chl} was significantly higher. Though differences are not significant, the lower C_{Chl}/N_{Chl} ratios in GL and CR than SAV indicate higher N availability for microorganisms in CR and GL soils. While N fertilizers were applied in CR, N availability in GL was increased by biological N fixation (Cadisch et al., 1989; Thomas, 1992). The P availability according to Bray-2 P contents varied among the soils (Table 1) and was adequate to high in CR soils. Despite that, the C_{Chl}/P_{chl} ratios were not affected by the land-use system. Microbial C/P ratios from 12:1 under high to 45:1 under low available P conditions were found by Chauhan et al. (1981).

Because it had the highest total soil P content and lowest P_{Chl}, the P_{Chl}/total P ratio was lowest in the CR soil (Table 4). The CR soil was also characterized by the lowest C_{Chl}/total C and N_{Chl}/total N ratios, indicating a lower significance of microorganisms as nutrient pools in the CR than in the SAV and GL soils. The P_{chl} was lowest in CR despite the fact that this treatment had the highest content in Bray-2 and resin extractable P_i (Tables 1 and 3), confirming results obtained in another experimental site on the Eastern Plains of Colombia (Gijsman et al., 1997b). Higher P_{Chl} amounts under grass–legume pasture than savanna or grass–alone pasture were observed by Oberson et al. (1999). Consequently, in low P Oxisols, the quantity of extractable microbial P is determined by factors other than available P_i. Pastures containing legumes seem to exert a positive effect on microbial P through the input of plant material of higher quality (Thomas and Asakawa, 1993; Friesen et al., 1997). In addition, pasture soils are subjected to much less cultivation and herbicide applications than CR soils.

Size and composition of the microbial biomass in the various land-use systems agree with the higher importance of P_o in GL and SAV than CR soils suggested by the P fractionation data. However, the changes in the size of the microbial nutrient pools or P_o fractions cannot distinguish whether higher P_o means P immobilization, or if the increases go along with higher mineralization. Results of Rao et al. (1994), Thomas and Lascano (1995) and Oberson et al. (1999) suggest that the higher litter quality of grass–legume pastures may result in increased mineralization and nutrient turnover. While approaches to measure soil P mineralization are available for non-P limited soils with medium to low P sorption capacity (Grierson

Table 4. Size, composition and significance of the soil microbial biomass nutrient pool in an Oxisol under different land-use systems: amounts of extracted microbial C, N and P; microbial nutrient ratios and extracted microbial nutrients as percentage of the total nutrient content in the soil.†

Treatment	C_{Chl} mg kg^{-1}	N_{Chl} mg kg^{-1}	P_{Chl} mg kg^{-1}	C_{Chl}/N_{Chl}	C_{Chl}/P_{Chl} (%)	C_{Chl}/total C (%)	N_{Chl}/total N (%)	P_{Chl}/total P
Savanna	145.0b	26.4b	5.4b	5.4	27.4	0.6 ab	1.6 b	2.5 b
Grass–legume	172.3b	35.3b	6.6b	4.9	25.9	0.6 b	2.0 b	2.4 b
Continous rice	72.2a	17.5a	2.6a	4.1	27.6	0.3 a	1.1 a	0.7 a
F-Test	**	**	***	ns	ns	*	**	***

Means followed by the same letter are not significantly different ($p = 0.05$) by Tukey's multiple range test. *F*-test: *** $P < 0.001$, ** $P = 0.001$–0.01, * $P = 0.01$–0.05; *ns*, not significant.
† determined after a 15-day incubation period.

et al., 1999; Lopéz-Hernandez et al., 1998; Oehl et al., 2001), the P mineralization potential of low P acid Oxisols can be deduced only from indicators. We used C mineralization (Gressel et al., 1996) obtained from respiration measurements and acid phosphatase activity (Sinsabaugh et al., 1993; Renz et al., 1999).

Indicators of phosphorus mineralization

Cumulative C mineralization was higher in both grassland systems than in the CR soil (Table 5). While mineralization during the first 2 weeks of incubation was only slightly higher in SAV than GL (Fig. 2, Table 5), GL maintained higher mineralization thereafter.

Results of C mineralization were related to substrate availability indicated by particulate organic matter (Fig. 3). There was more C and N in both size fractions in GL than in CR soils (significant at $P = 0.05$ for all except N in the 250 μm – 2-mm fraction), and contents in SAV took an intermediate position. Particulate organic matter (POM) accounted for the majority of soil organic matter initially lost as a result of cultivation of North American grassland soils (Cambardella and Eliott, 1992). The POM-N content in moist savanna soils of West-Africa was shown to be influenced by organic matter additions and to contribute significantly to the maize N supply (Vanlauwe et al., 1999). That POM-N was highest in GL, therefore, suggests a higher input of mineralizable organic matter, while the decrease in POM-N when SAV was replaced by CR suggests a loss of organic nutrient reserves. In a recent study, Barrios et al. (1999) also observed a significant increase for light soil organic matter fractions and potential N mineralization when SAV was replaced by GL, while decreasing values were observed when SAV was replaced by CR. Phiri et al. (2001), using size density fractionation on a Colombian volcanic-ash soil, found that the amount of soil organic matter fractions correlated well with the amount of $NaHCO_3$ extractable P_i and P_o.

In contrast to microbial biomass and soil respiration, the metabolic quotient qCO_2 did not differ among treatments, neither for the unsteady respiration phase from day 1 to day 14, nor during the phase of basal respiration (Table 5). Thus, no difference in organic substrate use efficiency is suggested (Gijsman et al., 1997b; Fliessbach and Mäder, 1997).

Acid phosphatase activity, and in turn the potential to mineralize available phosphomonoesters (Renz et al., 1999), declined in the order SAV > GL > CR (Table 5). The higher activity in SAV than GL disagrees with results obtained on a nearby pasture experiment (Oberson et al., 1999), where phosphatase activity was significantly increased in introduced GL pasture soils. This might be due to differences in the botanical composition of the pastures (Rao et al., 1997). A significant decrease in acid phosphatase activity when native vegetation was replaced by sugar cane monocropping was found in similar soils from Brazil (examined by Feller et al. (1994), using the same method).

Soil acid phosphatases are exoenzymes of microbial and plant origin (Tabatabai, 1982). They can keep their functionality through stabilization by sorption and association on soil compounds (Leprince and Quiquampoix, 1996). Plant roots increase phosphatase secretion under conditions of P deficiency (Rao et al., 1999a; Almeida et al., 1999). In addition, acid phosphatase activity in soils is related to organic matter (Feller et al., 1994; Renz et al., 1999). Higher phosphatase activity in SAV than GL may be due to very low P_i availability in SAV soil. The lower values of acid phosphatase activity in CR soil could be due to high P_i availability. The intermediate values of GL could result from a combined effect of greater over-

Table 5. Carbon mineralization and specific respiratory quotient of two different incubation periods, and phosphatase activity in low P acid soils of different land-use systems.†

Treatment	Cumulative C mineralization (mg C kg^{-1} soil)		qCO_2 (μg CO_2-C mg^{-1} Cchl h^{-1})		Phosphatase activity (μg nitrophenol g^{-1} h^{-1})
	1–14 days	14–63 days	1–14 days	14–63 days	
Savanna	274b	299.3b	5.8	1.8	270.4c
Grass–legume	251b	416.8b	4.6	2.1	223.3b
Continuous rice	114a	161.5a	4.5	1.8	145.5a
F-Test	**	***	ns	ns	***

Means followed by the same letter are not significantly different ($P = 0.05$) by Tukey's multiple range test. *F*-test: *** $P < 0.001$, ** $P = 0.001$–0.01, * $P = 0.01$–0.05; *ns*, not significant.
† determined after a 15 days incubation period.

Table 6. Reactivity of microorganisms towards added ^{33}P compared with Bray-1 extractable P: percentage of ^{33}P recovered in extractable microbial P (P_{Chl}) and percentage of ^{33}P in the Bray-1 extractant of the non-fumigated, non-P-amended sample ($Bray_0$-P) at two sampling dates.

Treatment	Days since labeling†	$Bray_0$-P (mg kg^{-1})	P_{chl} (mg kg^{-1})	Percentage of ^{33}P found in	
				$Bray_0$-P	P_{Chl}
Savanna	2	0.4 (0.01)	5.3 (0.31)	4.9 (0.21)	9.6 (0.90)
	8	0.2 (0.01)	4.4 (0.14)	2.0 (0.08)	9.5 (0.15)
Grass–legume	2	1.4 (0.09)	4.4 (0.56)	5.8 (0.49)	24.7 (2.36)
	8	0.9 (0.04)	3.3 (0.23)	3.4 (0.04)	20.5 (1.86)
Continuous rice	2	7.4 (0.24)	2.2 (0.63)	6.9 (0.24)	1.6 (0.70)
	8	6.6 (0.24)	1.1 (0.05)	5.6 (0.26)	1.8 (0.24)

Mean and SEM (in brackets) of three analytical replicates.
† Before labeling, soils were incubated for 3 weeks.

all biological activity, low P_i availability and higher organic substrate availability.

In conclusion, results on C mineralization, substrate availability estimated by P_o fractions and POM, and phosphatase activity suggest that P mineralization is more important in delivering available P in GL soils than in SAV and CR soils.

Microbial P turnover

Two days after labeling the soil, 25, 10 and 2% of the added ^{33}P were recovered in extractable microbial P in GL, SAV and CR soils, respectively, with only slight changes occurring after this time (Table 6). Differences among the percentage of incorporated isotope were greater than among P_{Chl}. Extractable P_{Chl} was similar in GL and SAV soils during the incubation experiment, but lower in CR. Comparison of the P_{Chl} value assessed at the end of the pre-incubation time before labeling the soil (Table 4) with P_{Chl} values measured during the incubation experiment (Table 6) shows that the size of P_{Chl} did not increase, but rather decreased during incubation. Thus, incorporation of ^{33}P was not caused by an increase in P_{Chl} size, but shows that a part of P_{Chl} was renewed. More than 20% of the tracer found in P_{Chl} in the GL soil suggests that microorganisms had turned over significant amounts of P held in their biomass during the 2 days since labeling. This indicates a greater turnover of extractable microbial P in GL than in SAV and CR soils.

The high rates of ^{33}P incorporation into P_{Chl} in this experiment suggest that microbes can assimilate large amounts of soluble P_i within a very short time. The exact mechanisms of P assimilation by and P release from specific soil microorganism species have received scant attention. The P regulation of a few microbes, especially *Escherichia coli* (Wanner, 1996) and *Saccharomyces cerevisiae* (Oshima and Halvorson, 1994), is well known from laboratory studies. Inorganic orthophosphate is the preferred P source (Wanner, 1996). To cope with low P concentrations, *E. coli* as well as other microorganisms have developed an emergency system induced by gene expression (Torriani-Gorini, 1994). This system includes additional specific P_i transport mechanisms (Wanner,

1996), increased phosphatase production (Torriani-Gorini, 1994; Joh et al., 1996; Yazaki et al., 1997), and uptake of glycerol and hexose phosphates and phosphonates (Wanner, 1996). An increased rate of P recycling by organisms living in P deficient soils was suggested by Magid et al. (1996).

Substantial microbial P turnover can be deduced from other incubation studies as well. McLaughlin et al. (1988) found 25% of ^{33}P added with labeled medic residues incorporated into the microbial biomass within 7 days. Walbridge and Vitousek (1987) recovered between 7 and 44% of added isotope in P_{Chl} after wetting–drying cycles, but as the specific activity of acid fluoride extractable P_{Chl} was not corrected for sorption or desorption of the isotope, these values most probably underestimate actual isotope incorporation into microbial biomass. Friesen and Blair (1988) found 11% of added ^{33}P in the P_o pool of a moderately acidic low P podzol 1 day after addition and incubation.

Application of the sequential P fractionation procedure described previously on ^{33}P labeled GL soils showed that, 2 days after labeling, up to 10% of the added isotope was recovered in NaOH P_o (S. Bühler, unpublished). This concurs with the fast turnover of extractable microbial P deduced from ^{33}P incorporation. These findings suggest a great significance and velocity of P_o turnover in low P acid Oxisols, and agree with recent results showing that soil organic matter turnover was much faster in highly weathered tropical than temperate soils (Tiessen and Shang, 1998).

While other studies (*loc. cit.*) carried out with C additions and wetting–drying cycles caused flush effects that were accompanied by significant changes in the size of microbial P, our work focused on microbial P turnover under conditions of basal respiration and nearly constant P_{Chl} size. Nevertheless, it cannot be completely excluded that soil microbial activity was stimulated by the thorough mixing that occurred when labeling the soil, even though the size of P_{Chl} was not increased (Tables 4 and 6). Other factors that could result in overestimated turnover are the presence of labeled particulate or organic P since the calculation of ^{33}P found in P_{Chl} is based on specific activity determinations obtained by dividing total radioactivity by P_i in aliquots of the Bray-1 extracts. Radioactivity as well as P_i concentrations were the same whether 0.05 or 0.45 μm filters were used. This can be explained by centrifugation prior to filtration. Hence, interference of particulate P on the isotopic composition can be discounted. The P_o-to-P_i ratios were usually around 0.2 in Bray-1 extracts of soils from a nearby field experiment, and were only slightly reduced by fumigation (Oberson et al., 1997). Therefore, the effect of P_o on the isotopic composition of Bray-1 extracts during the 8 days incubation experiment has been assumed to be negligible.

Significance of microbial P and organic P cycling in contrasting land-use systems

In our study, the highest ^{33}P incorporation (Table 6) suggesting fastest microbial turnover, was found in GL, which also had the highest biological activity (Tables 4 and 5) and low to medium available P content (Tables 1 and 2). The lowest turnover rate was found in the CR soil, which also had the lowest biological activity and highest P status. The intermediate microbial turnover rate in SAV was accompanied by low available P and intermediate biological activity. Thus, in the investigated soils, biological activity rather than soil P_i availability seems to determine microbial P uptake and release, and thus microbial P turnover. The higher the microbial activity, the greater the microbial P turnover.

Beck and Sánchez (1994) concluded that, in fertilized Ultisols, P_i is the main source of plant available P. However, our results suggest that this applies only in the case of high fertilizer doses. In the case of the low P doses applied to GL, increases in biologically mediated processes play an important role in the maintenance of P availability and the efficiency of P cycling. These low P doses are maintained in the P cycle by the standing pasture plant biomass, which provide for steadier P_o inputs by root and above-ground litter than do crops. Through the decomposition process, plant litter P enters into the soil microbial biomass. The microbial biomass represents a rapidly cycling P pool whose turnover is affected by the agricultural land-use system. Thus, in the case of low P doses, the failure of P to enter P_o pools, including plant biomass, living and dead soil organic matter, can be thought to indicate a degrading system due to low level of P cycling (CIAT, 1999).

The present study cannot give a final conclusion on the impact of different microbial P turnover rates on soil P dynamics. The immobilization of P_i by microbes and its gradual release through microbial turnover protects P from physico-chemical adsorption reactions with soil particles if this release is synchronized with the demand of growing plants and/or of

a next generation of microorganisms (Magid et al., 1996). If not, substantial competition between soil microbes and plants, on the one hand, and soil inorganic particles (adsorption surfaces) on the other, could occur for the scarce P resource, as found by Umrit and Friesen (1994). While P_i availability was increased more in CR than in GL, both absolute and relative to total P increases in soils, the P_o availability was increased more in GL. Introduced GL pastures, in which soil microbial P turnover is highest, sustain a high productivity of high quality forage (Lascano, 1991; Thomas and Lascano, 1995; Lascano and Euclides, 1996). This suggests that increased microbial P contents do not inhibit improved forage germplasm (Rao et al., 1995; 1999b) from acquiring the P needed.

Conclusions

Agricultural land-use systems replacing native savanna on Oxisols affect the partitioning of P among inorganic and organic P fractions. Indicators of organic P mineralization suggest that organic P is more important for delivering available P in improved grass–legume pastures than in continuously cropped and cultivated soils while, in cultivated soils, much higher P fertilizer doses significantly increase available inorganic P contents with lesser impact of organic P pool sizes. The amount and turnover of P held in the soil microbial biomass is increased when native savanna is replaced by improved pasture while it was lowered when soils are cultivated and cropped continuously. Therefore, the study suggests an alternative strategy to cropping low P Oxisols through applications of high P fertilizer doses. The combination of low P fertilizer doses and grass–legume pastures composed of germplasm that is adapted to the chemical constraints of Oxisols promotes P cycling and efficient use of P inputs. Given the high productivity of grass–legume pastures, the soil microbial biomass cannot be seen as a pool competing with the plant but rather as an important part in the P cycle that supports a high P use efficiency of the non-renewable P resource. However, it remains to be investigated whether crops planted in pasture-crop rotations could benefit from the enhanced organic P dynamics in grass–legume pasture soils, and a direct method to quantify organic P mineralization should be developed and tested in these systems.

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