Sequential Phosphorus Extraction of a ^{33}P-Labeled Oxisol under Contrasting Agricultural Systems

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ABSTRACT

Chemical sequential extraction is widely used to divide soil phosphorus (P) into different inorganic (P_i) and organic (P_o) fractions, but the assignment of these fractions to pools of differing plant availability, especially for low P tropical soils, is still matter of discussion. To improve this assignment, the effect of land-use systems and related P fertilizer inputs on size of P fractions and their isotopic exchangeability was investigated. A Colombian Oxisol, sampled from a long-term field experiment with contrasting management treatments, was labeled with carrier free ^{33}P and extracted after incubation times of 4 h, 1, and 2 wk. Phosphorus concentrations and ^{33}P recovery in fractions sequentially extracted with anion exchange resin (P_i), 0.5 *M* $NaHCO_3$ (Bic-P_i, Bic-P_o), 0.1 *M* NaOH (P_i, P_o), hot concentrated HCl (P_i, P_o), and residual P were measured for each incubation time. Resin-P_i, Bic-P_i, NaOH-P_i, and hot HCl-P_i were increased with P fertilization, with the highest increase for NaOH-P_i. The recovery of ^{33}P in the treatments with annual P fertilization clearly exceeding P outputs indicate that resin-P_i, Bic-P_i, and NaOH-P_i represented most of the exchangeable P. In these treatments, label P transformed with increasing incubation time from the resin to the Bic-P_i and NaOH-P_i fractions. The organic or recalcitrant inorganic fractions contained almost no exchangeable P. In contrast, in soils with low or no P fertilization, more than 14% of the ^{33}P was recovered in NaOH-P_o and HCl-P_o fractions 2 wk after labeling, showing that organic P dynamics are important when soil P_i reserves are limited.

PHOSPHORUS IS AN ESSENTIAL NUTRIENT for plants and often the first limiting element in acid tropical soils. Profound understanding of the P dynamics in the soil–plant system and especially of the short- and long-term fate of P fertilizer in relation to different management practices is essential for the sustainable management of tropical agroecosystems (Friesen et al., 1997). Chemical sequential extraction procedures have been and still are widely used to divide extractable soil P into different inorganic and organic fractions (Chang and Jackson, 1957; Bowman and Cole, 1978; Hedley et al., 1982; Cross and Schlesinger, 1995). The underlying assumption in these approaches is that readily available soil P is removed first with mild extractants, while less available or plant-unavailable P can only be extracted with stronger acids and alkali. In the fractionation procedure developed by Hedley et al. (1982) and modified by Tiessen and Moir (1993), the P fractions (in order of extraction) are interpreted as follows. Resin-P_i represents inorganic P (P_i) either from the soil solution or weakly adsorbed on (oxy)hydroxides or carbonates (Mattingly, 1975). Sodium bicarbonate 0.5 *M* at pH 8.5 also extracts weakly adsorbed P_i (Hedley et al., 1982) and easily hydrolyzable organic P (P_o)-compounds like ribonucleic acids and glycerophosphate (Bowman and Cole, 1978). Sodium hydroxide 0.5 *M* extracts P_i associated with amorphous and crystalline Al and Fe (oxy)hydroxides and clay minerals and P_o associated with organic compounds (fulvic and humic acids). Hydrochloric acid 1 *M* extracts P_i associated with apatite or octacalcium P (Frossard et al., 1995). Hot concentrated HCl extracts P_i and P_o from more stable pools. Organic P extracted by concentrated HCl may also come from particulate organic matter (Tiessen and Moir, 1993). Residual P that remains after extracting the soil with the already cited extractants, represents very recalcitrant P_i and P_o forms.

Several studies have related these different P fractions in tropical soils to plant growth (Crews, 1996; Guo and Yost, 1998) or showed the influence of land use and the fate of applied fertilizers (Iyamuremye et al., 1996; Linquist et al., 1997; Lilienfein et al., 1999; Oberson et al., 1999), and partly resulted in contrasting assignments of fractions to pools of different availability. By comparing the amounts of P extracted from the surface horizons of Brazilian Oxisols that had been under different land-use systems for 9 to 20 yr, either unfertilized or fertilized with mineral P fertilizer applications, Lilienfein et al. (1999) showed that most of the fertilizer was recovered in the Bic- and NaOH-P_i fractions, irrespective of the land-use system (resin-P_i was not measured). In a 4-yr field study conducted on a Hawaiian Ultisol, Linquist et al. (1997) recovered, 1 yr after fertilizer application, almost 40% of the applied triple super phosphate fertilizer in the hot HCl and H_2SO_4 fractions. Oberson et al. (1999) showed that in an Oxisol managed as a legume–grass pasture for 15 yr, resin-P_i, Bic- and NaOH-P_i, as well as NaOH-P_o levels were maintained at a higher level over the whole year in comparison with the same soil with the same total P content but managed as a grass only pasture. Iyamuremye et al. (1996) found an increase in resin-P_i, Bic-P_i and -P_o, as well as NaOH-P_i after addition of manure or alfalfa (*Medicago sativa* L.) residues to acid low-P soils from Rwanda. In the study of Guo and Yost (1998), resin-P_i, Bic-P_i, and NaOH-P_i were most depleted by plant uptake on highly weathered soils. NaOH-P_i was important in buffering available P

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Abbreviations: Bic-P, 0.5 *M* HCO_3-extractable P; C_{Chl}, microbially bound carbon released by chloroform; CIAT, Centro Internacional de Agricultura Tropical; CORPOICA, Corporacion Colombiana de Investigacion Agropecuario; C_p, P concentration in the soil solution; E_t, quantity of P isotopically exchangeable in one minute (mg kg soil^{-1}); N_{Chl}, microbially bound nitrogen released by chloroform; P_{Chl}, microbially bound phosphorus released by chloroform; P_i, inorganic P; P_o, organic P; P_{sum}, sum of P extracted in all fractions of the sequential fractionation; P_t, total P in a defined P fraction; P_{tot}, total soil phosphorus extracted with H_2O_2 and H_2SO_4; SA, specific activity (^{33}P/^{31}P).

supply while significant depletion of organic fractions could rarely be measured.

A possible method of gaining information about the availability of different P fractions is to label soil P, P fertilizers, or plant residues before applying the sequential fractionation scheme (MacKenzie, 1962; Weir and Soper, 1962; Dunbar and Baker, 1965). Two studies followed the movement of labeled P from plant residues to soil P fractions applying a modified Hedley (Daroub et al., 2000) or the Chang and Jackson (1957) fractionation procedures (Friesen and Blair, 1988). They found that at 6 or 11 d after plant residue addition, respectively, between 20 and 50% of the label was extractable as P_i with resin (Daroub et al., 2000) or with NH_4Cl and NH_4F (Friesen and Blair, 1988). For longer incubation periods up to 34 d, Daroub et al. (2000) showed a subsequent movement of the label from the resin-P_i fraction to the NaOH-P_i fraction. The results obtained in all these studies suggest that, in tropical soils, the amounts of P in the different pools measured by sequential P extraction procedures and the fluxes of P between pools are controlled both by physico chemical factors (sorption–desorption) and by biological reactions (immobilization–mineralization). However, the importance of these different reactions for different land-use systems, such as monocropping, pasture, or intercropping, remain largely unknown.

The objective of this study was to assess the effect of different land-use systems (native savanna, rice [*Oryza sativa* L.] monocropping, rice green manure rotation, grass legume pasture) on some physico chemical and biological reactions involved in P cycling in a Colombian Oxisol.

MATERIALS AND METHODS

Soils included in the study were sampled during the rainy season in September 1997 from a field experiment (Friesen et al., 1997) located at CORPOICA-CIAT (Corporacion Colombiana de Investigacion Agropecuario; Centro Internacional de Agricultura Tropical) research station, Carimagua, Meta, Colombia (4°30′N, 71°19′W). Mean annual temperature is 27°C and average rainfall is 2200 mm. The soils are well drained Oxisols (Kaolinitic, isohyperthermic Typic Haplustox) of clay loam texture (Table 1).

The surface soil layer (0–20 cm) was sampled in the long-term "Culticore" field experiment, which was established in 1993 with the objective to test the effect of different farming systems on plant productivity and soil fertility (Friesen et al., 1997). The experiment had a split-plot design with four replicates with treatment subplots of 0.36-ha size. Soil samples used for this study were taken at random in two replicates of each treatment and the replicates were mixed for laboratory analysis. For our study, the following treatments were included.

1. SAV (Native savanna): native grassland annually burned in February, not grazed; no fertilizer application.
2. GL (Grass–legume pasture): rice (cv. Oryzica Sabana 6) in 1993, with undersown pasture, since 1993 grass–legume pasture with *Brachiaria humidicola* (Rendle) Scheickerdt CIAT 679, *Centrosema acutifolium* Bentham cv. Vichada CIAT 5277, *Stylosanthes capitata* J. Vogel CIAT 10280, and *Arachis pintoi* CIAT 17434. The pasture was partly resown for renovation in June 1996 with legumes (the same *Arachis pintoi* Krapovickas & Gregory, and *Centrosema acutifolium* and additionally *Stylosanthes guianensis* (Aubl.) Sw., CIAT 11833). Grazing intensity was on average 2.7 steers ha^{-1} during 15 d followed by a 15 d pasture regrowth phase.
3. CR (Continuous rice): rice (cv. Oryzica Sabana 6, cv. Oryzica Sabana 10 since 1996) grown in monoculture; one crop per year followed by a weedy fallow incorporated with early land preparation at the beginning of the rainy season before sowing rice.
4. RGM (Rice green manure rotation): Rice followed by cowpea *Vigna unguiculata* (L.) Walp. var. ICA Menegua] in the same year. The legume was incorporated at the maximum standing biomass level in the late rainy season before sowing rice in the following rainy season.

Before establishing the treatments GL, CR, and RGM on savanna, the soil was conventionally tilled after burning the native vegetation. At the beginning of the experiment all treatments except SAV received 500 kg dolomitic lime ha^{-1}. Annual fertilization of rice consisted of 80 kg N ha^{-1} (urea, divided among three applications), 60 kg P ha^{-1} (triple superphosphate). In addition, 99 kg K as KCl, 15 kg Mg and 20 kg S (as $MgSO_4$), and 10 kg Zn ha^{-1} were applied at establishment and at recommended rates thereafter. With cowpea, an additional 20 kg N and 40 kg P ha yr^{-1} and 60 kg K, 10 kg Mg, 13 kg S, and 10 kg Zn ha^{-1} were applied at establishment and at recommended rates thereafter. The introduced pasture (GL) received additional fertilization only in 1996 [per ha: 20 kg P, 20 kg Ca (lime), 10 kg Mg (lime), 10 kg S (elemental), and 50 kg K (KCl)]. Phosphorus input-output balances were estimated by subtracting the P removed from the system by grain and/or with animal live weight gains from the P applied in mineral fertilizers. Phosphorus exports in grain were calculated by multiplying weighed rice grain yields with measured P content in the grain. Phosphate exported in the animals was assumed to be 8 g per kg of live weight gain. Live weight gains in GL were on average 68 kg ha^{-1} yr^{-1} (Oberson et al., 2001). The systems differed in soil cultivation (frequent [CR and

Table 1. Selected chemical and physical properties of the surface layer (0–20 cm) of the Colombian Oxisol under different land-use systems. Values are the average of four analytical replicates, except Fe- and Al-contents (three replicates).

Treatment†	Total C	Total N	pH in water	Al-Saturation	Fe_d‡	Fe_{ox}§	Al_d‡	Al_{ox}§	Clay	Bulk density
	g kg^{-1}			%	g kg^{-1}				%	Mg m^{-3}¶
SAV	27a#	1.64a	4.8b	86.8b	26.7a	3.6a	7.8a	2.0a	35.0a	1.27a
GL	29a	1.55a	4.9b	71.7a	26.4a	3.6a	7.7a	2.0a	39.3b	1.27a
CR	26a	1.45a	4.3a	75.4a	26.2a	3.7a	7.6a	2.0a	39.9b	1.21a
RGM	26a	1.49a	4.3a	76.3a	26.9a	3.5a	7.8a	2.0a	39.0b	1.24a

† SAV: Native savanna, GL: Grass-legume pasture, CR: Continuous rice cropping, RGM: Rice-green manure rotation.
‡ Extraction with dithoinite after Mehra and Jackson (1960).
§ Extraction with oxalate after McKeague and Day (1966).
¶ Source: CIAT (1999).
# Means within the same column followed by the same letter are not significantly different ($P = 0.05$) by Tukey's multiple range test.

RGM], rare [GL], or no cultivation [SAV]) and in application of herbicides (frequent [CR and RGM], rare [GL], or no application of herbicides [SAV]). Cultivated soils were tilled to a maximum of 15-cm depth.

Topsoil samples (0–20 cm) were air dried and sieved to pass a 2-mm sieve before chemical analysis in the analytical service laboratory of CIAT or shipped to Switzerland where they were stored in an air-dried condition until use for the fractionation experiment in 2000.

Soil Characterization

Bray-II P (0.03 *M* NH_4F, 0.1 *M* HCl) was extracted from 2-g subsamples of soil at a 1:7 soil solution ratio and 40 s shaking time (Bray and Kurtz, 1945). Total soil P (P_{tot}) was determined on subsamples of 0.25 mg soil with the addition of 5 mL concentrated H_2SO_4 and heating to 360°C on a digestion block with subsequent stepwise (0.5 mL) additions of H_2O_2 until the solution was clear (Thomas et al., 1967). Microbial nutrient pool sizes of P, C, and N (P_{Chl}, C_{Chl} and N_{Chl}) were estimated on the preincubated samples by extraction, of chloroform fumigated and unfumigated samples, with the Bray I extract (0.03 *M* NH_4F, 0.025 *M* HCl) (P_{Chl}) (Oberson et al., 1997) or K_2SO_4 (C_{Chl} and N_{Chl}) (Vance et al., 1987). No k-factors (Brookes et al., 1982; Hedley and Stewart, 1982; McLaughlin et al., 1986) were used to calculate the total microbial nutrient pools from measured P_{Chl}, C_{Chl} and N_{Chl} as there exist no proper estimates for these in acid tropical soils (Gijsman et al., 1997). P_{Chl} was corrected for sorption of released P according to Oberson et al. (1997). Dithionite-citrate-bicarbonate extractable and oxalate extractable Fe and Al (Fe_d, Fe_{ox}, Al_d, Al_{ox}) were determined according to Mehra and Jackson (1960) and McKeague and Day (1966). The mineralogy of the soils was determined on total soil samples, pretreated with H_2O_2 to remove organic C, by X-ray diffraction analysis (XRD). The samples were ground under acetone in a tungsten carbide vessel of a vibratory disk mill (Retsch RS1) for 10 min. Longer grinding times were not applied because of the detrimental effect that further grinding can have on the crystallinity of minerals, especially Fe (hydr)oxides (Weidler et al., 1998). For the Cu Kα radiation, the Bragg-Brentano geometry was chosen as an XRD routine setup. The measurements were carried out on a Scintag XDS 2000 (Scintag Inc., Ecublens, Switzerland) equipped with a solid state detector from 2 to 52° 2θ with steps of 0.05° 2θ and counting times of 16 s.

Sequential P Fractionation of Labeled Soils

Before starting the sequential P fractionation, the samples were preincubated in a climate chamber (24°C and 65% relative atmospheric humidity, no light) for 2 wk in portions of 100 g at 50% of their water holding capacity (300 g water kg^{-1} soil dry weight). Soil water content was controlled and adjusted every other day by weighing.

Subsamples of preincubated soils were labeled in portions of 15 g with 120 MBq ^{33}P (half-life 25.4 d) kg^{-1} which were added with 10 μL deionized water per g soil. The mass of P introduced with the ^{33}P label can be neglected ($<2.5 \times 10^{-3}$ μg P g^{-1} soil, Amersham product specification, July 2000). Therefore, the term 'P concentration' always refers to ^{31}P and specific activities (SA) are calculated as:

$$SA\ (Bq\ \mu g^{-1}\ P) = {}^{33}P/{}^{31}P \qquad [1]$$

Soil P was fractionated sequentially, after three different incubation times after labeling (4 h, 1 wk, and 2 wk), with three replicates per treatment following the modified method of Hedley et al. (1982), as described in Tiessen and Moir (1993), with HCO_3-saturated resin strips (BDH #55164, 9 × 62 mm), followed by 0.5 *M* $NaHCO_3$ (referred to as Bic-P), 0.1 *M* NaOH, (these first three steps each with an extracting time of 16 h) and concentrated hot HCl at 80°C for 10 min. The step using diluted cold HCl was omitted, as Ca-phosphates are only present at very low levels or are absent in highly weathered acidic soils (Agbenin and Tiessen, 1995), as shown for the soils used in this study by Friesen et al. (1997). Residual P was extracted as described previously for determination of P_{tot}.

The amount of soil extracted was doubled from 0.5 to 1 g with the original volumes of extractants (2 resin strips in 30 mL H_2O, 30 mL $NaHCO_3$, 30 mL NaOH, 15 mL concentrated HCl, 5 mL conc. H_2SO_4) used to get higher ^{33}P-concentrations in the extracts. This was preferred to the alternative of higher label application as the radiation might affect microbial growth (Halpern and Stöcklin, 1977). After each extraction, the samples were centrifuged at 25 000 × *g* for 10 min before filtering the solutions of the Bic- and the NaOH-extracts through 0.45-μm pore size Millipore filters (Millipore Corporation, Bedford, MA, cellulose acetate), and the hot HCl and residual P extracts through Whatman No. 40 filter paper.

Phosphorus concentrations in all extracts were measured after neutralization by the Murphy and Riley (1962) method. This method was used directly for the P recovered from the resin strips and for P_i determination in the HCl extracts. Organic matter was first precipitated by acidification in the Bic- and the NaOH-extracts prior to P_i determination (Tiessen and Moir, 1993). Total P (P_t) in the Bic-, the NaOH- and the HCl-extracts was measured after digestion of P_o with potassium persulfate (Bowman, 1989). Organic P was calculated as the difference between P_t and P_i in the Bic-, NaOH-, and hot HCl extracts.

To partition soluble $^{33}P_i$ and $^{33}P_o$ in the Bic-, the NaOH-, and the hot HCl-extracts into separate solutions before counting, 5 mL of the extracts were shaken with acidified ammonium molybdate dissolved in isobutanol (Jayachandran et al., 1992). By this method, P_i is extracted into the isobutanol while P_o remains in the aqueous phase. The complete recovery of P_i in the isobutanol phase was verified with the addition of a standard amount of ^{33}P in 0.5 *M* HCO_3, 0.1 *M* NaOH, and in 2.3 *M* HCl; recovery rates of added ^{33}P in the isobutanol phase were between 97 and 103%, which was not significantly different from 100%. Counts in the aqueous phase were 1.1% (HCO_3), 0.3% (NaOH), and 0.1% (HCl) of the original solutions showing that hardly any P_i went into this phase. Determination of total P in the aqueous phase is not possible because the presence of the molybdate interferes with the analysis (Jayachandran et al., 1992).

The radioactivity in each phase was determined with a liquid scintillation analyzer (Packard 2500 TR) using Packard Ultima Gold scintillation liquid in the ratio (extract to liquid) 1:5. The values were corrected for radioactive decay back to the day of soil labeling. All extracts were tested for possible quenching effects by adding defined ^{33}P spikes. Quenching in the acid resin eluate could be prevented by dilution of 250 μL eluate with 750 μL deionized water for counting. The quench effect in the hot concentrated HCl extract could be avoided by counting solutions separated with acidified isobutanol because the separated phases were not affected by quenching. All other extracts were not affected by quench effects.

The recovery of the label calculated as the sum of all fractions, including residual P, was never complete. Therefore, subsamples of the soil residue after final acid digestion were dried and weighed into scintillation vials. These subsamples were then counted after addition of 1 mL water and 5 mL of scintillation cocktail.

Isotopic Exchange Kinetics

The procedure of isotopic exchange kinetics was used to assess the exchangeability of P_i in the soils sampled in the different land-use systems. The method was conducted as described by Fardeau (1996). Suspensions of 10 g of soil and 99 mL deionized water were shaken for 16 h on an overhead shaker to reach a steady state equilibrium for P_i. Then, at t = 0, 1 mL of carrier free $H_3^{33}PO_4$ tracer solution containing 1.2 MBq was added to each continuously stirred soil water suspension. Three subsamples were taken from each suspension after 1, 10, and 100 min, immediately filtered through a 0.2- m pore size micropore filter, and the radioactivity in solution was measured by liquid scintillation as described previously. To determine the ^{31}P concentration in the soil solution (C_p, mg P L^{-1}) 10 mL of the solution were filtered through a 0.025-μm filter (Schleicher & Schuell GmbH, Dassel/Relliehausen, Germany, NC 03) at the end of the experiment. The smaller filter pore size was used to exclude any influence of suspended soil colloids on C_p determination (Sinaj et al., 1998). The P concentration in the filtrate was measured in a 1-cm cell by the Malachite green method (Ohno and Zibilske, 1991) with a Shimadzu UV-1601 spectrophotometer (Shimadzu Corp., Kyoto, Japan). Phosphorus concentrations in solutions from the SAV, GL and CR treatments were close to the detection limit and they were measured again in samples concentrated by evaporation (5:1). This procedure resulted in C_p values that were not significantly different from the non-concentrated solutions.

Assuming that at any given exchange time the specific activity (SA) of inorganic phosphate in the solution is equal to the SA of the total quantity of phosphate which has been isotopically exchanged, it is possible to calculate the amount of isotopically exchanged P (E_t, mg P kg^{-1} soil). The amount of P exchangeable within one minute (E_1), indicating the immediately available P, is expressed as (Fardeau, 1996):

$$E_1 = R \times 10 \times C_p/r_1 \quad [2]$$

where R is the introduced radioactivity and r_1 is the radioactivity remaining in solution after 1 minute of isotopic exchange. The factor 10 results from the soil solution ratio of 1:10.

Statistical Analysis

The effects of land-use systems and incubation time after labeling on P fraction size were tested by SYSTAT (Systat, 1997) by two-way ANOVA and Tukey's multiple range test over all treatments and times of fractionation, with exception of the resin-P_i fraction where the interaction between treatment and time was highly significant. Therefore, for resin-P_i the ANOVA and Tukey's multiple range test for the treatment factor were calculated separately for each time. Percentage recovery data were log-transformed to meet the requirements of analysis of variance. Time and treatment influences on the SAs of each fraction were tested by a two-way ANOVA and, as the interaction time × treatment was significant for all fractions, the treatment influence was tested for each repetition in time of sequential fractionation, separately.

RESULTS AND DISCUSSION

The mineralogy and Fe and Al (oxy)hydroxides contents of the surface soil from the four treatments were normal for this type of soil (Gaviria, 1993). On average of all treatments, the soil contained 68% quartz, 23% kaolinite, 4% anatase, 3% gibbsite, 2% rutile, and <1% vermiculite. There were no significant differences among the different land-use systems (SAV, GL, CR, RGM). This implies that any difference seen in P dynamics among land-use systems was mainly due to the land-use system and not to differences in soil mineralogy.

Total Soil P and P Balance Induced by the Different Treatments

The amounts of total P directly extracted from the soil samples (P_{tot}) were not significantly different from the total sum of P (P_{sum}) extracted in the different fractions of the sequential extraction for SAV and CR while the direct extraction led to significantly higher values (P = 0.05) for GL and RGM (Table 2). To evaluate whether differences in total P content were related to P fertilization, the increase in P_{tot} (calculated as the difference between P_{tot} extracted from fertilized GL, CR or RGM and P_{tot} extracted from non fertilized SAV) was compared with the estimated P balance of these treatments (significant correlation, $r^2 = 0.87$; $P < 0.001$). The increases in P_{tot} were of the same order of magnitude as the calculated P balance. Given the imprecision of the methods used to determine total P (O'Halloran, 1993) and of the estimations made to calculate the P balance, these results suggest that most of the P added as fertilizer and not taken up by plants remained in the surface layers of the soils. Except for the CR treatment these results agree well with Oberson et al. (2001). In their study, for CR only, about half of the calculated positive P balance was recovered in total P. Their sampling depth of 0 to 10 cm might explain this difference: soil tillage may have mixed P in the 0- to 10-cm soil

Table 2. Phosphorus status and calculated P balances of the studied Oxisol under different land-use systems. Total P was calculated as the sum of the sequential P fractionation (P_{sum}) or extracted directly with H_2O_2 and H_2SO_4 (P_{tot}).

Treatment†	Bray II P‡	P_{sum}‡	Δ P_{sum}§	P_{tot}‡	Δ P_{tot}§	P-Balance¶
	mg kg^{-1}					
SAV	0.9a	165aA	0	172aA	0	0
GL	2.0b	190bA	25	213bB	41	28
CR	17.2c	290cA	125	293cA	121	92
RGM	35.5d	335dA	170	376dB	205	153
F-test (soil)	***	***		***		

*** Indicates significance at P = 0.001.
† SAV: Native savanna, GL: Grass-legume pasture, CR: Continuous rice cropping, RGM: Rice-green manure rotation.
‡ P concentrations followed by the same lower case letter (within columns) or upper case letter (comparison of P_{sum} and P_{tot} within rows) are not significantly different (P = 0.05) according to Tukey's test.
§ Δ P calculated as the difference between P_{sum} or P_{tot} of fertilized treatments − SAV.
¶ Calculated by subtracting the P removed by grain and/or animals from the P applied with mineral fertilizer.

Table 3. Parameters of isotopic exchange kinetics influenced by the different treatments†.

Treatment‡	r_1/R§	C_p¶	E_1#
		mg L^{-1}	mg kg^{-1}
SAV	0.02a	0.0015a	0.7a
GL	0.03a	0.002b	0.6a
CR	0.04a	0.003c	0.8a
RGM	0.055b	0.015d	2.7b
F-test	***	***	***

*** Indicates significance at $P = 0.001$.
† Values are the average of three replications.
‡ SAV: Native savanna, GL: Grass-legume pasture, CR: Rice monoculture, RGM: Rice-green manure rotation.
§ Ratio of radioactivity remaining in soil solution to radioactivity added at time 0 after 1 minute of isotopic exchange.
¶ P concentration in the soil solution measured at soil:water ratio 1:10.
# Quantity of P exchangeable within 1 minute.

layer with soil in the 10- to 20-cm layer, resulting in incomplete recovery of P in the 0- to 10-cm sampling depth.

Isotopic Exchange Characteristics

The effect of the four land-use systems on P_i exchangeability in the surface layer of the Colombian Oxisol is illustrated in Table 3. The ratio r_1/R, which is inversely correlated to the P sorbing capacity of soils (Frossard et al., 1993), was below 0.05 for all treatments, suggesting that these soils have a high P sorbing capacity (Frossard et al., 1993). Furthermore, the r_1/R-values of the four treatments were positively correlated to P_{tot} ($r^2 = 0.76$; $P < 0.001$). This suggests that the different land-use systems have resulted, through P fertilization and cropping, in different sorption rates of P_i on soil minerals. Since in Oxisols P sorption is mainly governed by Al and Fe (oxy)hydroxides (Fontes and Weed, 1996), these treatments probably induced different degrees of P_i saturation on the (oxy)hydroxides such as gibbsite, which was identified in the soil from these treatments.

The P_i concentration in the soil solution (C_p) was close to the detection limit in the SAV, GL and CR treatments (Table 3). C_p was significantly increased in all fertilized treatments ($P < 0.001$). In SAV, GL and CR, C_p was much lower than the critical concentration needed to sustain optimal growth for a large range of crops (Kamprath and Watson, 1980; Fox, 1981). The variation in the amount of P_i isotopically exchangeable in one minute (E_1) followed the same trend as the variation in C_p.

P Concentrations in Different Fractions of the Sequential Extraction

The positive P balances of the fertilized GL, CR and RGM treatments resulted in significantly higher P concentrations ($P < 0.001$) compared to the savanna soil in all fractions except the organic fractions and residual P (Table 4). This agrees with the results of Friesen et al. (1997) and Oberson et al. (2001), who fractionated P forms according to the same method in the same field experiment, and studies conducted using other tropical soils (Beck and Sanchez, 1994; Linquist et al., 1997). Our results show that resin-P_i, Bic-P_i, and NaOH-P_i increased with P fertilizer input, with the NaOH-P_i fraction being the main sink for the applied P. The function of the NaOH-P_i fraction can be explained by the adsorption of P_i through ligand exchange with hydroxyl groups (Sposito, 1989) located on the surface of Fe and Al (oxy)hydroxides (Ainsworth et al., 1985; Parfitt, 1989; Torrent et al., 1992) and by the desorption of P_i from the surface of (oxy)hydroxides in the presence of 0.5 *M* NaOH (Houmane et al., 1986; Cross and Schlesinger, 1995).

During the continuous 2-wk incubation of the soil samples, resin-P_i and Bic-P_i fractions increased significantly ($P < 0.05$) between the first and second fraction-

Table 4. Distribution of P in various fractions according to the modified Hedley et al. (1982) fractionation procedure in the different treatments with and without P fertilization, at three times of incubation after soil labeling.

Treatment‡	Incubation Time	Resin	Bicarbonate		NaOH		Hot HCl		Residual	Total	Total
		P_i	P_i	P_o	P_i	P_o	P_i	P_o	P_t	P	P_o
		mg kg^{-1}									
SAV	4 h	0.9d†	1.4g	12.4a	22de	46a	37b	6.1ab	44ab	172ef	65a
GL	4 h	2.0c	2.8fg	11.8a	27de	56a	34b	8.6a	43b	185ef	76a
CR	4 h	4.8b	9.7def	15.0	102b	48a	56a	9.1a	49ab	298cd	72a
RGM	4 h	10.0a	21.4bc	6.7a	100bc	62a	65a	5.2abc	47ab	321abc	74a
SAV	1 wk	2.0c	4.3fg	5.7a	20e	42a	36b	4.1bc	42b	157f	52a
GL	1 wk	2.4c	6.4efg	10.0a	33d	47a	38b	3.3bc	43ab	184ef	61a
CR	1 wk	8.0b	14.3cde	14.3a	89c	47a	53a	2.5bc	50ab	279d	64a
RGM	1 wk	16.4a	29.8a	12.8a	119a	40a	63a	3.3bc	54ab	338ab	56a
SAV	2 wk	2.0d	4.1fg	6.3a	20e	42a	36b	4.1bc	48ab	164f	52a
GL	2 wk	4.2c	6.4efg	10.3a	33d	49a	38b	2.9bc	62a	207e	62a
CR	2 wk	7.5b	16.6cd	11.0a	90bc	56a	58a	1.2c	61ab	305bcd	68a
RGM	2 wk	15.8a	27.5ab	15.9a	118a	45a	63a	4.3bc	62a	354a	65a
ANOVA:											
Treatment		***	***	n.s.	***	n.s.	***	**	**	***	n.s.
Time		***	***	n.s.	n.s.	n.s.	n.s.	***	***	**	n.s.
Treatment × time		***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

† Values within a column followed by the same letter do not differ significantly ($P = 0.05$) according to Tukey's test. For resin-P_i a one-way ANOVA was calculated for the influence of treatment at each time and means were compared by Tukey's test.
‡ SAV: Native savanna, GL: Grass-legume pasture, CR: Continuous rice cropping, RGM: Rice-green manure rotation.
** Indicates significance at $P = 0.01$.
*** Indicates significance at $P = 0.001$.

ation date for all treatments (between 4 and 14 mg kg^{-1} for the sum of resin-P_i and Bic-P_i). There was no significant decrease in any fraction although total extractable P_o tended to decline (between 8 and 18 mg kg^{-1}) for all soils (Table 4). The absence of a significant movement of P out of P_o fractions may be due to the high variability of the results, especially for the organic fractions where coefficients of variation for Bic-P_o ranged from 13 to 70% and for NaOH-P_o from 7 to 45%. Since P_o is determined by the difference between P_t and P_i for a given extract, there are multiple sources of error. High variability of repeated measuring of Bic-P_o and NaOH-P_o were reported by Magid and Nielsen (1992). Problems in the determination of P_i are mentioned in Tiessen and Moir (1993), especially the possibility that P_i is precipitated along with the organic matter upon acidification and erroneously determined as P_o ($P_t - P_i$). On the other hand, P_o compounds could be hydrolyzed in the acidic solution during the measurement of P during the colorimetric measurement (Condron et al., 1990; Gerke and Jungk, 1991).

Increases in resin-P_i and Bic-P_i between 4 h and 1 wk of incubation suggest that mineralization of P_o led to the release of labile P_i from P_o fractions. As the first fractionation was started 4 h after labeling, the disturbance by mixing the soil with the label and the increased humidity may have stimulated microbial activity in spite of the preincubation. A temporary stimulation of the

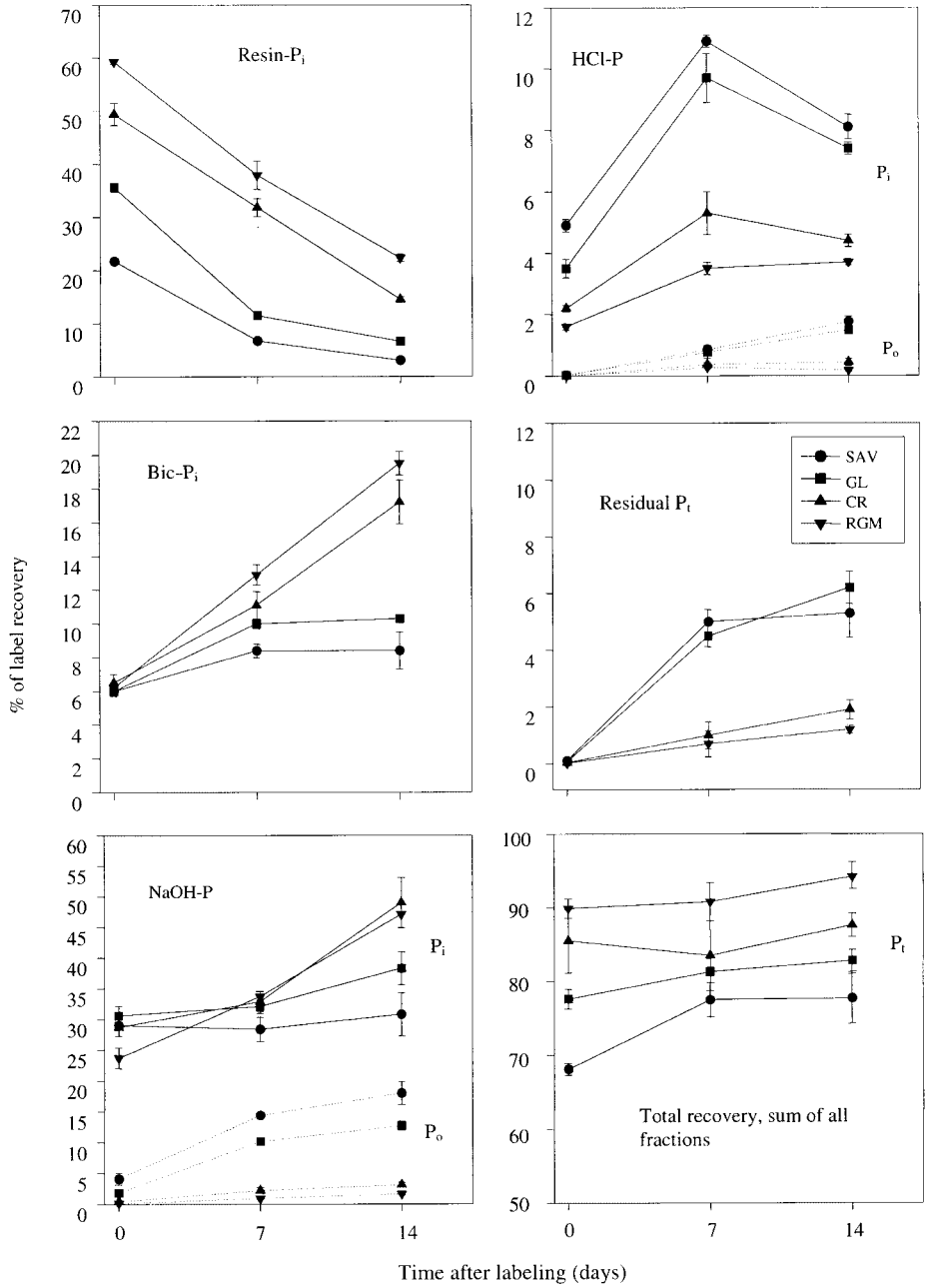

Fig. 1. Percentage of label recovery in the different fractions of the sequential P extraction and in the sum of all fractions at 4 h, 1, and 2 wk after labeling soil. Recovery in Bic-P_o in all soils at all sampling times <1% (means of three replicates ±SD).

microbial activity by mixing when labeling the soil was indicated in microbial turnover studies conducted on soils from the same field experiment (Oberson et al., 2001). This assumption seems likely, as there were little changes in fraction sizes between the second and the third fractionation indicating a stabilization of the system.

Distribution of ^{33}P Among P Fractions and Dynamics over Time

The fraction of ^{33}P recovered in the resin-P_i fraction 4 h after labeling varied between 22% in SAV and 60% in RGM (Figure 1). The ^{33}P recovery in this fraction was positively correlated to P_{tot} ($r^2 = 0.87$; $P < 0.001$, 4 h after labeling). The corresponding decrease with time of ^{33}P in the resin fraction in RGM and CR coincided with an increase in label recovery in Bic-P_i and NaOH-P_i, while in SAV and GL the decline in resin ^{33}P was accompanied by an increase in ^{33}P in NaOH-P_o (GL also NaOH-P_i), HCl-P_i and residual-P. For SAV and GL, the label recovered in resin-P_i decreased only slightly but significantly between the 1st and the 2nd wk, and the label recovery in Bic-P_i did not change significantly in this time. The amount of ^{33}P in NaOH-P_i was almost stable over the entire incubation time with a small but significant increase between the first and the second week for GL. This shows that in SAV and GL the label was rapidly exchanged between these fractions and that equilibrium with the (labeled) soil solution was reached. In contrast, ^{33}P in the Bic-P_i and the NaOH-P_i of CR and RGM was still increasing after 1 wk while resin-$^{33}P_i$ continued to decrease, showing that the exchange between these fractions was incomplete.

The data for $^{33}P_o$ were, because of the determination after the separation from P_i with the isobutanol method, not affected by the inherent problems in determination of the P_o fractions in the Hedley fractionation scheme as described previously. Only small amounts of the label were found in organic fractions after 4 h, but there were already significant differences in NaOH-$^{33}P_o$ ($P < 0.001$) in the order:

$$\text{SAV (4\%)} \approx \text{GL (2\%)} > \text{CR (0.4\%)} \approx \text{RGM (0.1\%)}.$$

This might be due to differences in microbial activity as observed by Oberson et al. (2001) in the same field experiment. Microbial biomass in the incubated soils, indicated by measured P_{Chl}, C_{Chl}, and N_{Chl} values, was significantly different between the soils (Table 5), despite the fact that the samples had been stored in an air-dried condition for more than 3 yr before being used in this study. The assumption that recovery of the label in organic fractions was actually due to active processes and not to any analytical artifact is supported by the observed increases of NaOH-$^{33}P_o$ and HCl-$^{33}P_o$ for all soils over time. The total recovery of 20% (SAV) or 14% (GL), respectively, of the label in organic fractions 2 wk after labeling shows that these compartments have to be taken into account to understand the fate of P in these very low-P soils (Tiessen et al., 1984; Beck and Sanchez, 1994; Linquist et al., 1997). Oberson et al. (2001) suggested that P_o mineralization significantly contributes to P availability in low input pastoral systems on these soils but that methods for quantification of mineralization remain to be developed.

The proportion of label in the hot HCl and residual P fractions increased significantly with incubation time in all soils. This contradicts the prevailing opinion of recalcitrance of P in these fractions (Guo and Yost, 1998; Neufeldt et al., 2000). While the total P content in the residual fraction varied significantly with time (Table 4), this was not the case for hot HCl extractable P_i, while hot HCl extractable P_o tended to decrease. This suggests that the movement of the label to these fractions was not due to net P movement but to exchange processes.

Total ^{33}P Label Recovery

At all sampling times during the incubation study, in total between 67 and 94% of the applied ^{33}P label could be recovered in the sum of all P fractions (Fig. 1). This total of label recovery was generally in the order SAV < GL < CR < RGM. Incomplete recoveries can be explained by the fact that the method used to assess total P or residual P was not efficient enough to extract all of the soil P. Comparative studies have shown that total P can only be extracted reliably by alkali fusion (Syers et al., 1967; Bowman, 1988), which could not be used in this work. The analysis of soil residues after the acid extraction of residual P (Table 6) indicated that significant amounts of the label remained unextracted, these being higher for SAV and GL than CR and RGM. Although counting of ^{33}P bound to solid phases is gener-

Table 5. Size of soil microbial biomass nutrient pool in different agricultural systems after 20 days of incubation of the formerly air-dried soils. Values are the averages of three replicates.

Treatment‡	C_{Chl}§	N_{Chl}§	P_{Chl}§
	mg kg^{-1}		
SAV	88.7a†	13.7a	1.6a
GL	80.8a	13.5a	1.2ab
CR	72.9a	8.5b	0.7b
RGM	48.2b	6.1b	0.5b
F-Test	**	***	***

** Indicates significance at $P = 0.01$.
*** Indicates significance at $P = 0.001$.
† Means within a column followed by the same letter within a column are not significantly different ($P = 0.05$) by Tukey's multiple range test.
‡ SAV: Native savanna, GL: Grass-legume pasture, CR: Continuous rice cropping, RGM: Rice-green manure rotation.
§ Microbially bound carbon, nitrogen and phosphorus, respectively, released by chloroform.

Table 6. Radioactivity measured in soil solid residues by scintillation counting after extraction of residual P by sequential P fractionation starting 1 week after soil labeling.

Treatment‡	Bq g^{-1} soil†	% of initial label
SAV	2251 (111)	4.4
GL	1843 (357)	3.6
CR	427 (215)	0.8
RGM	348 (140)	0.7

† Average of three replications, standard error in brackets, decay corrected to the day of soil labeling.
‡ SAV: Native savanna, GL: Grass-legume pasture, CR: Continuous rice cropping, RGM: Rice-green manure rotation.

ally possible, problems of phase, impurity, self absorption of scintillations by the soil particles, or color quenching effects (Gibson, 1980) are difficult to correct, as these influences might be highly variable between samples. However, the recovery of standard additions of ^{33}P to our soil residues was complete and the correlation of the measured radioactivity in the different soil treatment residues with the sample weight was linear (data not shown), thus confirming the qualitative information obtained from the counting of the soil residues.

Altogether the results suggest that the transfer of ^{33}P among the different fractions determined by the sequential extraction was strongly dependent on the degree of saturation of soil Al and Fe (oxy)hydroxides with P_i, and therefore on the bonding energy of P_i to the soil minerals. It is indeed known that a high P_i saturation of metal oxide surfaces causes a more negative charge on the surface and prevents the specific sorption of further P_i ions (Ryden et al., 1977; Bowden et al., 1980). In the low P treatments (SAV and GL), most P_i would be sorbed with such a high energy that its exchangeability would be very limited. Specific sorption of ^{33}P to the surface of Al and Fe (oxy)hydroxides of these soils, although unlikely (Frossard et al., 1994), cannot be excluded (Barrow, 1991). In contrast, in the P rich soils (CR and RGM), annual P additions may have resulted in the build up of larger quantities of P_i that was exchangeable with ^{33}P.

Specific Activities in the Fractions Determined by the Sequential Extraction

The highest specific activities observed in the incubation experiment were obtained in the resin extract after 4 h of incubation (Table 7). This is consistent with the assumption that the amount of P desorbed from the soil by resin is in very rapid exchange with P_i in the soil solution, as suggested by other studies (Amer et al., 1955; Bowman and Olsen, 1979; Tran et al., 1992; Schneider and Morel, 2000). The subsequent decrease in SA of resin-P_i reflected the process of isotopic exchange between ^{33}P and stable P_i located on the soil's solid phase (Fardeau, 1996). The order of the SAs in the P_i fractions after 4 h of incubation followed the extraction sequence (resin-P_i > Bic-P_i > NaOH-P_i > HCl-P_i > residual P), showing that the strongest reactants extracted either large quantities of slowly exchangeable P or a large quantity of P in which only a small part was rapidly exchangeable. After 2 wk the SAs of resin-P_i, Bic-P_i and NaOH-P_i became closer, suggesting that equilibrium with respect to P transfer between these fractions was being approached. The SAs of resin-P_i, Bic-P_i and NaOH-P_i were not significantly different in SAV while the SA of resin-P_i was still significantly higher than the SA of Bic-P_i in GL and higher than in Bic-P_i and NaOH-P_i in CR and RGM. These observations show that it is not possible to discuss the exchangeability of a certain P fraction without reference to a defined time of exchange (Fardeau, 1996).

Although the SAs of the NaOH-P_o and HCl-P_o fractions were relatively low, they showed that, depending on land use, these fractions were connected through active processes with the soil solution, most probably through microbial activity (Oehl et al., 2001). This indicates that the determination of plant available P with short-term isotopic exchange experiments might lead to errors since the dynamics of organic P forms are excluded.

CONCLUSIONS

The effect of contrasting land-use systems on soil P fractions extracted by a modified Hedley et al. (1982) P sequential fractionation procedure was assessed in an Oxisol during a 2-wk incubation on soils labeled with carrier free ^{33}P. The quantities of ^{31}P and ^{33}P recovered in the different fractions were strongly dependent on

Table 7. Specific activities (^{33}P/^{31}P) in extracts of the Hedley sequential fractionation in the different treatments of the labeled Oxisol at different times after labeling.†

Time	Treatment§	Resin P_i	Bic-P_i	NaOH-Pi	NaOH-Po	HCl-Pi	HCl-Po	Residual P
		kBq mg P^{-1}						
4 h	SAV	32.9aA	5.9aB	1.8aC	119 × 10^{-3}aD	180 × 10^{-3}aD	8 × 10^{-3}aE	3 × 10^{-3}aE
	GL	24.5bA	3.3bB	1.6aC	44 × 10^{-3}bE	138 × 10^{-3}bD	3 × 10^{-3}aF	3 × 10^{-3}aF
	CR	13.8cA	1.3cB	0.4bC	11 × 10^{-3}bE	54 × 10^{-3}cD	0aG	1 × 10^{-3}bF
	RGM	7.9dA	0.6cB	0.3bC	3 × 10^{-3}bE	33 × 10^{-3}dD	0aG	1 × 10^{-3}bF
	F-test:	***	***	***	***	***	ns	***
1 wk	SAV	5.1abA	2.7aA	1.9aB	480 × 10^{-3}aC	430 × 10^{-3}aD	280 × 10^{-3}aE	157 × 10^{-3}aF
	GL	6.4aA	2.2bB	1.3bC	293 × 10^{-3}bD	436 × 10^{-3}aD	497 × 10^{-3}aCD	140 × 10^{-3}aE
	CR	5.3abA	1.1cB	0.5cC	64 × 10^{-3}cD	138 × 10^{-3}bD	271 × 10^{-3}aCD	26 × 10^{-3}bD
	RGM	3.1bcA	0.6cB	0.4cC	35 × 10^{-3}cD	76 × 10^{-3}bD	159 × 10^{-3}aCD	18 × 10^{-3}bD
	F-test	*	***	***	***	***	ns	***
2 wk	SAV	2.1aAB	1.6aA	2.1aA	597 × 10^{-3}aB	290 × 10^{-3}aC	566 × 10^{-3}aB	154 × 10^{-3}aD
	GL	2.1aA	1.4aB	1.6aAB	357 × 10^{-3}bC	249 × 10^{-3}bC	741 × 10^{-3}aC	135 × 10^{-3}aD
	CR	2.6aA	1.1abB	0.7bB	70 × 10^{-3}cCD	99 × 10^{-3}cC	22 × 10^{-3}aD	43 × 10^{-3}bD
	RGM	1.9aA	0.8bBC	0.5bC	48 × 10^{-3}cDE	75 × 10^{-3}cD	56 × 10^{-3}aDE	26 × 10^{-3}bE
	F-test‡:	ns	*	***	***	***	ns	***

* Indicates significance at $P = 0.05$.
*** Indicates significance at $P = 0.001$.
† All values are the average of three replicates. Decay corrected to the day of soil labeling.
‡ As the effect of time × treatment was significant for all fractions, ANOVA was calculated separate for each time. Means followed by different lower case letters within one column at one time are significantly different ($P = 0.05$) by Tukey's test. The same is valid for means within one row followed by different upper case letters.
§ SAV: Native savanna, GL: Grass-legume pasture, CR: Continuous rice cropping, RGM: Rice-green manure rotation.

the total P content of the soil, which was affected by the amount of P added as fertilizer and removed by plant P uptake.

In the two treatments fertilized annually with P and with a large positive P input-output balance, most of the P_i was stored in the resin-P_i, Bic-P_i, and NaOH-P_i fractions. The use of carrier free ^{33}P confirmed that, under all land-use systems studied, these soil P fractions contained most of the exchangeable P and that ^{33}P was transferred from the soil solution first to the resin fraction and then to the Bic-P_i and NaOH-P_i fraction. This suggests that, when this Oxisol is regularly fertilized, P is stored in these three fractions while plants might take up P from the same fractions. In the two other treatments, which had either never been fertilized or had been fertilized only once at the beginning of the field trial, the transfer of ^{33}P in these three fractions (i.e. resin-P_i, Bic-P_i, and NaOH-P_i) was less clear, suggesting that the soil P_i was much less exchangeable. In these soils, however, the transfer of ^{33}P into organic P fractions was more important (up to 20% of the label was found in the organic P fractions 2 wk after labeling). As the pool sizes of these organic fractions did not change significantly over time of incubation, the label recovery indicates relatively quick cycling processes, probably as a result of microbial activity. In low P Oxisols, these processes are relevant and should be considered when estimating soil P availability for plants.

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