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Inorganic and organic phosphorus pools in earthworm casts (Glossoscolecidae) and a Brazilian rainforest Oxisol

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Abstract

We compared differences in soil phosphorus fractions between large earthworm casts (Family Glossoscolecidae) and surrounding soils, i.e., Oxisols in 10 year-old upland agroforestry system (AGR), pasture (PAS), and secondary forest (SEC) in the Central Brazilian Amazon. AGR and PAS both received low-input fertilization and SEC received no fertilization. We found that earthworm casts had higher levels of organic hydroxide P than surrounding soils, whereas fertilization increased inorganic hydroxide P. Inorganic P was increased by fertilization, and organic P was increased by earthworm gut passage and/or selection of ingested materials, which increased available P (sum of resin and bicarbonate fractions) and moderately available P (sum of hydroxide and dilute acid fractions), and P fertilizer application and land-use increased available P. The use of a modified sequential P fractionation produced fewer differences between earthworm casts and soils than were expected. We suggest the use of a condensed extraction procedure with three fractions (Available P, Moderately Available P, and Resistant P) that provide an ecologically based understanding of the P availability in soil. Earthworm casts were estimated to constitute 41.0, 38.2, and 26.0 kg ha^{-1} of total available P stocks (sum of resin and bicarbonate fractions) in the agroforestry system, pasture, and secondary forest, respectively.

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Keywords: Agroforestry; Amazonia; *Bactris gasipaes*; *Bertholletia excelsa*; Oxisol; Pasture; Secondary forest; Sequential phosphorus extraction

1. Introduction

Low soil fertility of the *terra firme* (upland) soils is a major constraint for sustaining the productivity of agroecosystems in the Amazon (Sanchez et al., 1982), especially because 96% of Amazon soils are phosphorus (P) deficient for agricultural productivity (Dematê and Dematê, 1997). Where P inputs are not available or locally affordable, tree-based cropping systems can be a sustainable alternative to annual cropping systems (Lehmann et al., 2001). However, research has focused little on the soil biological component of these systems, which has the potential for compensating some of the soil deficiencies, either of chemical or physical nature.

'The plow is one of the most ancient and most valuable of man's inventions, but long before he existed the land was in fact regularly plowed, and still continues to be thus plowed by earthworms (Darwin, 1881).' Not only have earthworms been recognized to improve soil physical characteristics, but they also impact soil chemical characteristics (Barois et al., 1999). Earthworms are among the most important soil macrofauna (>2 mm) influencing the soil, and may be highly influential in increasing soil P availability. Earthworms are abundant and are concentrated in the top 10 cm of soil, however they often occur in the top 40 cm of the soil profile (Fragoso and Lavelle, 1992). They can ingest 4–10% of the A horizon annually, depending on soil type (James, 1991). In a forest soil in central Amazonia, north of Manaus, Brazil, soil macroinvertebrate biomass was 54 g m^{-2}, which included large earthworms

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(Oligochaeta) measuring more than 1 m in length and accounting for more than 80% of the earthworm biomass (Barros et al., 2000). The Families Glossoscolecidae and Ocnerodrilidae dominate South American tropical forest earthworm communities (Fragoso and Lavelle, 1992). In an abandoned rubber tree system north of Manaus, Brazil, eight earthworm species were observed. They were mostly from the Family Glossoscolecidae, including earthworms of the genus *Rhinodrilus*, measuring up to 1.1 m long (Römbke et al., 1999).

Although the activities within the gut of an earthworm are not entirely understood, it is clear that the ingested soil and/or litter is broken down, rearranged, and re-aggregated (Barois and Lavelle, 1986). This restructuring of soil after its passage through the earthworm gut has been called 'regeneration' (Barois et al., 1993), which has been recognized to influence soil N availability (Barois et al., 1987; Pashanasi et al., 1992; Araujo et al., 2004), as well as soil P availability (Guerra, 1982). In a glasshouse experiment, Guerra (1982) found that plant P uptake was up to three times higher in the presence of *Pontoscolex corethrurus,* either from the labeled P added to the soil or stable soil P. Earthworms may affect P cycling in soils by concentrating P in their casts through ingestion of P-rich particles, modifying the relative proportions of different P forms, and modifying stability of P forms (Brossard et al., 1996).

The Hedley fractionation is a sequential P extraction used to quantify plant-available inorganic P, Ca-associated inorganic P, Fe- and Al-associated inorganic P and labile and more stable forms of organic P (Hedley et al., 1982). This approach is thought to be the only sequential fractionation that can evaluate available organic P with moderate success (Tiessen and Moir, 1993). We designed this study to compare the Hedley P fractions between earthworm casts of the large earthworms (Family Glossoscolecidae) and surrounding soils.

2. Materials and methods

2.1. Site description

The study area is located in central Amazonia at the EMBRAPA-CPAA field station located along highway BR-174, 53 km north of Manaus (2°30′36″ S and 2°30′42″ S and 60°01′29″ W and 60°01′46″ W (Coolman, 1994)), in the State of Amazonas, Brazil. The natural vegetation is upland Evergreen Tropical Rainforest and the soil is a fine, isohyperthermic, Xanthic Hapludox (US Soil Taxonomy) with a pH of 4.3, available P of 2.5 mg P kg^{-1} soil (Mehlich-1), organic C of 26 mg g^{-1}, total N of 2.0 mg g^{-1}, and 356.7, 77.8, and 35.2 mg of Ca, Mg and K, respectively, kg^{-1} soil in the top 15 cm (McKerrow, 1992). Soil bulk density is 0.96 g cm^{-3} (Coolman, 1994). The climate is humid tropical, the mean annual rainfall is 2500 mm, and mean annual temperature is 26.2 °C. The mean relative air humidity is 83.9% (Tapia-Coral et al., 1999).

2.2. Site history

The site is a former *Brachiaria decumbens* pasture that was grazed for 4–8 years prior to being abandoned. The scrub forest that regenerated on the abandoned pasture was slashed and burned in 1991, 3–5 years post-abandonment and four agroforestry prototype systems were established (Fernandes et al., 1995). Of the four systems, three systems were selected, with sampling focused on soils beneath specific trees and grasses, chosen to represent useful and profitable species for local economies. The species sampled in the agroforestry system (AGR) were *Theobroma grandiflorum* Schumann, *Bactris gasipaes* Kunth, *Bertholletia excelsa* Humb. and Bonpl., and *Eugenia stipitata* McVaugh. The pasture (PAS) was sampled beneath *Brachiaria humidicola* Rendle, and the secondary forest (SEC) was sampled around the base of *Vismia* and *Cecropia* trees.

AGR was fertilized with Triple Super Phosphate (20.1% P), KCl (49.8% K), lime (40.0% Ca), and urea (45% N) to provide 15.8 kg N ha^{-1} y^{-1}, 23.7 kg P ha^{-1} y^{-1}, 12.3 kg K ha^{-1} y^{-1}, and 4.7 kg Ca ha^{-1} y^{-1} for the period from 1991, when the systems were established, to 2001, when sampling was conducted. PAS received fertilization over the same time period with the same products, in addition to ammonium sulfate (21% N; 23% S), at the rate of 4.6 kg N ha^{-1} y^{-1}, 5.7 kg P ha^{-1} y^{-1}, 0.5 kg K ha^{-1} y^{-1}, 1.6 kg S ha^{-1} y^{-1}, and 80.0 kg Ca ha^{-1} y^{-1}. PAS was originally grazed for 7 days with a 21-day rest period. Stocking rates and grazing periods were adjusted with varying plant-growing conditions. SEC was left to grow post-burn since 1991, without chemical amendments.

2.3. Sample collection

Earthworm cast and soil samples were taken within a 1 m radius from the base of tree and grass species. Each soil sample was a composite of five soil samples (0–15 cm depth) ('soil' is used to distinguish earthworm casts from adjacent soil substrate that did not visibly constitute earthworm casts). Earthworm casts from large earthworms, approximately 1–1.5 m long (personal observation), of the Family Glossoscolecidae, locally referred to as 'minhocuçus', were taken from the soil and litter surface. Beneath each of the tree species in AGR and SEC, nine earthworm cast samples and nine soil samples were taken from the entire sampling area, with three earthworm cast samples and three soil samples per block. In PAS, 18 earthworm cast samples and 18 soil samples were taken from the entire sampling area, with six earthworm cast samples and six soil samples per block. The sampling of earthworm casts and soils was random by tree species in each land-use system. The representative species in AGR sampled were

Theobroma grandiflorum Schumann, *Bactris gasipaes* Kunth, *Bertholletia excelsa* Humb. and Bonpl., and *Eugenia stipitata* McVaugh. PAS was sampled beneath *Brachiaria humidicola* Rendle, and SEC was sampled around the base of *Vismia* and *Cecropia* trees.

Only intact, semi-fresh casts (5–15 cm diameter) were collected for analysis. Semi-fresh casts were moist, but not wet, and resistant to moderate tactile pressure. Casts of all colors present were collected to represent the observed range of casts.

We chose to study the casts of these large earthworms because the earthworm casts (1) cover a large area on the soil surface, 90–96% of soil surface, and are found to depths of 0–20 cm, with mean depths of 6.4 cm ± 0.3 (data not shown), (2) are easily identified as earthworm cast material, and (3) provided sufficient sample material to run numerous nutrient analyses.

2.4. Analyses

2.4.1. Sequential phosphorus extraction

Samples were sequentially extracted and analyzed according to a modified Hedley fractionation (Table 1) (Hedley et al., 1982; Tiessen and Moir, 1993), closely following the extraction procedure detailed by Tiessen and Moir (1993). However, residual P was extracted using concentrated HNO_3 and $HClO_4$ at 200 °C in a sand bath until dry, because locally available H_2SO_4 was found to contain P impurities.

Phosphorus fractions were grouped into inorganic/organic fractions and by availability representing biological functions in order to better understand P dynamics in earthworm casts and soils. First, we grouped fractions as organic (Po) and inorganic (Pi) (Table 2). When doing this, earthworm casts had more Pi than soils in AGR; and earthworm casts had more Po than soils in all land use systems. Second, we grouped P by availability. The groups of P arranged by availability included available P (AP), moderately available P (MAP), and resistant P (RP) (Table 3). We have defined AP as the sum of P in the resin, inorganic bicarbonate, and organic bicarbonate fractions. Resin and bicarbonate extractions desorb phosphates from mineral surfaces, and the sum of these extracts is a measure of potential exchangeable phosphates present in the soil and/or cast (Brossard et al., 1996). Tiessen and Moir (1993) stated that labile forms of inorganic P are extracted with resin and bicarbonate, and bicarbonate extractable organic P is easily mineralized and contributes to plant-available P (Bowman and Cole, 1978). We defined MAP as the sum of P in the inorganic hydroxide, organic hydroxide, and the dilute acid fractions. Inorganic P in the hydroxide fraction is thought to be less plant available than P in resin and bicarbonate fractions, and organic P in the hydroxide fraction represents more stable forms of P involved in longer-term transformations (Tiessen and Moir, 1993). MAP represents fractions believed to be Fe-, Al- and Ca-associated P (Tiessen and Moir, 1993). We defined RP as the sum of P in the inorganic acid, organic acid, and the residual fractions. Due to the acidic nature of the extractants of RP, it is thought that RP is highly occluded or bound to recalcitrant organic matter.

2.4.2. Nutrient analysis

Exchangeable Al, Ca, Mg, K, total N and soil acidity were measured in earthworm casts and soils. Exchangeable K was analyzed using a double acid extraction (0.05 M HCl and 0.0125 H_2SO_4) and exchangeable Ca, Mg, and Al with

Table 1
Phosphorus pools, extractions procedures, and P pool properties

Pool	Extraction procedure or fractions represented[a]	Pool properties[b]
Resin—Pi	Resin strips, distilled water, 16 h	Freely exchangeable Pi
Bicarbonate—P	0.5 M NaHCO3, 16 h	Immediately plant available P; bound to mineral surfaces
Hydroxide—P	0.1 M NaOH, 16 h	Successively available P; bound to oxides; amorphous phosphates
Dil. acid—Pi	1 M HCl, 16 h	Successively available Pi; Ca-bound
Acid—P	Concentrated HCl, 10 min., 80 °C	Long-term available P; Ca-bound and occluded P
Residual—Pi	Concentrated HNO3 and concentrated HClO4, 200 °C until dry	Highly resistant and occluded P
Available P (AP)	Sum of P in resin and bicarbonate fractions	Plant available P, immediately available and/or bound to mineral surfaces
Moderately available P (MAP)	Sum of P in hydroxide and dil. acid fractions	Successively available P; bound to oxides, amorphous phosphates, and Ca-bound
Resistant P (RP)	Sum of P in acid and residual fractions	Highly resistant and occluded P
Inorganic P (Pi)	Sum of P in resin, bicarbonate Pi, hydroxide Pi, dil. Acid, acid Pi, and residual fractions	
Organic P (Po)	Sum of P in bicarbonate Po, hydroxide Po, and acid Po fractions	
Total P	Sum of P in all Hedley fractions	

[a] Based on Lehmann et al. (2001) and Tiessen and Moir (1993).

[b] Based on Lehmann et al. (2001), Cross and Schlesinger (1995), and Tiessen and Moir (1993).

Table 2
Phosphorus fractions in earthworm casts and soils by land-use system

Land-use System	Resin Pi ($g\ m^{-3}$)	Bicarb. Pi ($g\ m^{-3}$)	Bicarb. Po ($g\ m^{-3}$)	Hydrox. Pi ($g\ m^{-3}$)	Hydrox. Po ($g\ m^{-3}$)	Dil. acid Pi ($g\ m^{-3}$)	Acid Pi ($g\ m^{-3}$)	Acid Po ($g\ m^{-3}$)	Residual Pi ($g\ m^{-3}$)	Total Pi ($g\ m^{-3}$)	Total Po ($g\ m^{-3}$)	Total P
AGR, Cast	18.8 a	6.0	11.8	47.6 a	63.7 a	9.1	8.8	8.8	15.6	106.0 a	84.3 a	190.0 a
AGR, Soil	11.3 ab	2.3	6.5	15.2 b	28.8 d	1.7	4.1	5.4	5.2	39.8 b	40.7 b	80.5 cd
PAS, Cast	7.9 ab	4.4	10.5	32.7 ac	55.4 ab	2.7	7.9	5.9	12.0	67.6 ab	71.8 a	139.0 ab
PAS, Soil	3.7 b	2.9	3.3	17.0 bc	21.6 d	1.8	2.9	2.7	9.9	38.1 b	27.6 bc	65.7 d
SEC, Cast	7.7 ab	6.7	7.8	22.0 bc	46.0 bc	1.6	5.8	3.6	9.3	53.0 ab	57.4 a	110.0 bc
SEC, Soil	2.2 b	2.3	1.9	10.2 b	13.0 e	0.6	3.4	1.3	6.1	24.8 b	16.2 c	40.9 d

AGR, agroforestry system; PAS, pasture; SEC, secondary forest. Bicarb., bicarbonate; Hydrox., hydroxide; Dil. acid, dilute acid; Pi, inorganic P; Po, organic P; Total Pi, sum of inorganic fractions; Total Po, sum of organic fractions; Total P, sum of all fractions. Letters indicate significant differences within each column ($P<0.05$). Columns without letters indicate the main effects were not significantly different.

Table 3
Phosphorus pools in earthworm casts and soils by land-use system

Land-use, System	AP ($g\ m^{-3}$)	MAP ($g\ m^{-3}$)	RP ($g\ m^{-3}$)
AGR, Cast	36.9 a	121.0 a	34.0 a
AGR, Soil	20.1 bd	45.8 cd	14.7 bc
PAS, Cast	22.8 b	91.5 ab	25.8 ac
PAS, Soil	9.9 ce	40.7 cd	15.8 bc
SEC, Cast	21.6 bc	68.6 bc	18.8 bc
SEC, Soil	6.4 e	23.8 d	10.7 b

Letters indicate statistical differences ($P<0.05$) within P pools.

1 M KCl (EMBRAPA, 1999). Total soil N was determined by the Kjeldahl technique (EMBRAPA, 1999).

2.4.3. Coverage of earthworm casts

Earthworm cast coverage was estimated using a 0.5 m^2 frame on the soil surface. Sixty sample points were selected randomly within each land use system. Two trained individuals estimated coverage by sight and the average of their estimates was used to represent earthworm cast coverage. A larger sampling size was utilized in systems with higher cast cover variation to minimize standard error, i.e., $n=119$ in AGR, $n=60$ in PAS; and $n=46$ in SEC. Earthworm cast depth was estimated by viewing to what depth earthworm casts occurred through investigation of a small trench approximately 10 cm long by 10 cm wide up to the depth earthworm casts were found occurring at concentrations similar to the soil surface. Bulk density of earthworm casts was determined by dry weight of casts divided by cast volume ($n=9$). Earthworm cast volume was determined by displacement of water, with water absorbed by casts added to the water displaced. P ($kg\ ha^{-1}$) sequestered in earthworm casts by land-use system was calculated from their surface area coverage, depth, bulk density, and amount of P.

2.4.4. Statistical analysis

The results were compared by a one-way analysis of variance (repeated measures, general linear model) using a completely randomized design. The SAS System and Minitab computer software were used to determine statistical significance (SAS Institute, Inc., Cary, NC). The repeated measures tool within The SAS System was used to account for multiple values (nine extracts per sample) of the dependent variable for each sample. Where the variances were not homogeneous, a logarithmic transformation was applied. Differences were considered significant by LSD, using the Tukey test, at $P<0.05$.

3. Results

3.1. Phosphorus pools in earthworm casts

Earthworm casts had more total P (sum of Hedley fractions) than soils in any of the land-use systems. Total P

Table 4
Earthworm cast surface area coverage, depth, and P availabilities by land-use system

Land-use, System	Area covered (%)	Depth (cm)	AP (kg ha^{-1})	MAP (kg ha^{-1})	RP (kg ha^{-1})	TP (kg ha^{-1})
AGR	90 c	5.7 b	41.0	135.0	37.8	214
PAS	93 b	8.3 a	38.2	153.0	43.2	235
SEC	96 a	5.8 b	26.1	82.8	22.7	132

AGR, agroforestry system; PAS, pasture; SEC, secondary forest. Letters indicate statistical differences ($P<0.05$) within columns. Statistical differences for P fractions were not available, being a result of calculations of means.

in earthworm casts was 238, 211, and 266% greater than AGR, PAS, and SEC soils, respectively (Table 2). Earthworm casts in AGR contained more total P than earthworm casts in SEC. Earthworm casts additionally contained more P than soils in the organic hydroxide fraction in all land-use systems, and the earthworm casts of AGR contained more P in the inorganic hydroxide fraction than soils of AGR. Earthworm casts of AGR contained more P in the organic hydroxide fraction than earthworm casts of SEC.

In all land-use systems, earthworm casts contained more AP and MAP than soils, and AGR earthworm casts contained more RP than AGR soils ($P<0.05$) (Table 3). Earthworm casts in SEC had 376% more AP than SEC soils, and earthworm cast in AGR had 206% more AP than AGR soils. MAP was the largest pool of P in both earthworm casts and soils. MAP in earthworm casts was 264, 225, and 288% greater than AGR, PAS, and SEC soils, respectively. MAP in earthworm casts accounted for 63, 65, and 63% of total P in AGR, PAS, and SEC, respectively.

AGR earthworm casts contained 1.6 and 1.7 times more AP than PAS and SEC earthworm casts, respectively. AGR earthworm casts contained more MAP and RP than SEC earthworm casts.

3.2. Calculation of P contribution by earthworm casts

Earthworms deposited casts that concentrated P in and on soil of all land-use systems studied (Table 4). Earthworm casts covered a greater soil surface area in SEC (96%) than in PAS (93%), which was greater than earthworm cast coverage in AGR (90%). Earthworm cast depth varied by land-use system: it was significantly greater in PAS (8.3 ± 0.6 cm) than in both AGR (5.7 ± 0.4 cm) and SEC (5.8 ± 1.0 cm). However, occurrence of earthworm casts was fairly consistent throughout the soil profile in each land-use system. Earthworm cast bulk density did not differ between land use systems or species: it was 1.47 ± 0.20 g cm^{-3}.

3.3. Nutrient availability in earthworm casts versus soil

SEC earthworm casts contained more total N than soils in all land-use systems, and SEC earthworm casts contained more exchangeable Ca and Mg than SEC soil (Table 5). The difference between Ca in SEC soils and casts was about 15-fold. AGR earthworm casts contained more exchangeable K than AGR soil and AGR soil contained more exchangeable K than SEC soil. AGR earthworm casts contained less exchangeable Al than AGR soil. Soil acidity was greater in SEC earthworm casts than AGR and PAS earthworm casts.

4. Discussion

4.1. Phosphorus pools

Earthworms studied increased organic hydroxide P, whereas fertilization increased inorganic hydroxide P. The hydroxide organic P represents a more stable form of P that is involved in longer-term P transformations, whereas hydroxide inorganic P is associated with amorphous and some crystalline Fe and Al phosphates and is moderately plant available (Tiessen and Moir, 1993). In a study in the Peruvian Amazon, however, earthworm casts of *P. corethrurus* contained more inorganic hydroxide P but less organic hydroxide P than surrounding soils (Chapuis-Lardy et al., 1998). Similarly, inorganic P was influenced by P fertilization in our study, whereas organic P was influenced by earthworm gut passage and/or selection of consumed

Table 5
Chemical properties of earthworm casts and soils by land-use system

Land-use, System	Total N (g kg^{-1})	Exch. Ca (cmol kg^{-1})	Exch. K (cmol kg^{-1})	Exch. Mg (cmol kg^{-1})	Exch. Al (cmol kg^{-1})	pH H_2O
AGR, Cast	2.2 b	0.87 b	0.08 a	0.30 a	0.49 c	5.0 a
AGR, Soil	1.7 b	0.42 c	0.05 b	0.22 ab	1.20 bc	4.8 ab
PAS, Cast	2.5 b	0.88 abc	0.08 abc	0.42 a	0.24 abcd	5.2 a
PAS, Soil	1.7 b	0.62 abc	0.05 bc	0.21 ab	0.72 cd	4.9 ab
SEC, Cast	4.1 a	1.16 ab	0.05 abc	0.31 a	2.12 a	4.5 b
SEC, Soil	1.8 b	0.07 c	0.03 c	0.07 b	1.53 ab	4.7 ab

Exch., exchangable; AGR, agroforestry system; PAS, pasture; SEC, secondary forest. Letters indicate significant difference ($P<0.05$) within column.

materials. These data also contradict the results of the aforementioned study in the Peruvian Amazon, where casts of *P. corethrurus* contained more inorganic P and less organic P after passage through the gut (Chapuis-Lardy et al., 1998). The observed differences could be due to the different earthworm genera and their study site likely having soils containing higher Fe- and Al-oxides. Strong sorption of PO_4^{3-} to Fe- and Al-oxides can fix large proportions of total soil P into 'unavailable forms' thought to be a medium term sink for P (Solomon et al., 2002).

Earthworm gut passage and/or selective ingestion influenced AP and MAP while application of P fertilizer and influence of land-use system influenced AP. Exchangeable P has been shown to increase 2 days after and again 4 days post-cast deposition in earthworm casts of *P. corethrurus* (Lopez-Hernandez et al., 1993). It was suggested that the P increase in casts was due to (1) a significantly greater pH of the gut contents along the earthworm intestinal tract (6.8 and 6.0 for the anterior and posterior parts and 4.6 for soil, respectively) (Barois and Lavelle, 1986); (2) the large amounts of mucus in the earthworm gut, that is rich in carbohydrate compounds, which contain carboxyl groups that can block and compete for orthophosphate sorbing places, and in turn, increase soluble P (Lopez-Hernandez et al., 1993); and (3) an increase in microbial activity during digestion (Lopez-Hernandez et al., 1993). The activity of different earthworm species may additionally be the reason for differing AP and MAP in earthworm casts between land-use systems, because gut microflora and digestive abilities have been shown to differ (Lattaud et al., 1998), suggesting different earthworm species have different effects on nutrients in their casts.

A negative impact of *P. corethrurus* on soil physical properties has been shown in Amazonian pastures (Chauvel et al., 1999). Our results showed that the large earthworms (Family Glossoscolecidae) are not as efficient in PAS as in SEC to increase AP, which could prove another less than positive impact of earthworms in non-tree based land-use systems. This may be related to different earthworm species inhabiting the different land-use systems, but may also be an effect of the nutrient concentration of materials consumed in PAS and SEC.

In all land-use systems earthworm casts contained more AP and MAP than soils, and MAP accounted for a majority of the total P. This confirms earlier findings that earthworms play a role in Fe-, Al-, and Ca-associated P and more stable forms of organic P involved in longer-term transformations. Earthworms deposit large quantities of casts, approximately 400 Mg soil (dry wt) ha^{-1} y^{-1} (Barois and Lavelle, 1986) in the humid tropics, which is continuously recycling P and adding to soil AP and MAP and sustaining soil RP over time. The fact that MAP accounts for the majority of total P confirms the importance of this pool in maintaining AP on a long-term basis.

Earthworm casts contained more total P (TP) (sum of modified Hedley fractions) than soils in all land-use system. Earthworms were found mostly at depths of 0–15 cm. It is likely that they were ingesting soil at this depth and plant residues that would be incorporated into the soil at 0–15 cm. TP was at least twice as much in earthworm casts as soils, and foliar nutrient concentrations were 0.1 mg P g^{-1} in all species, with the exception of pupunha, with 0.2 mg P g^{-1} (McCaffery, 2002). Earthworms may have consumed tree leaf litter, increasing the total P concentration in earthworm casts over soils. Earthworm casts in AGR had more TP than SEC casts. Therefore, AGR earthworms ingested more P than SEC earthworms. This was probably due to the higher level of fertilizer applied to AGR compared with lower P fertilizer in PAS and no fertilizer in SEC, in addition to higher P concentrations in AGR litter than in SEC litter (Gallardo-Ordinola, 1999).

4.2. Fractionation critique

The use of a modified Hedley fractionation alone produced fewer differences than were expected being that earthworm cast material had been significantly processed by the earthworms in comparison to soil. Phosphorus in earthworm casts differed from soil only in the hydroxide fractions, which did not fully represent the difference of P concentrations between earthworm casts and soils in the land-use systems studied. Because the difference was not fully represented by the modified Hedley fractionation, we grouped fractions to represent biological functions of a greater scale than the modified Hedley fractionation, i.e., AP, MAP, and RP. Applying these groups to our results provided a better method to understand differences between land-use systems within each P availability group. Thus, we suggest grouping Hedley fractions as an alternative to reporting and/or analyzing individual P fractions in soils.

Using the availability groups (AP, MAP, and RP) suggested in this study provides the reader with an ecologically-based understanding of the P availability in the soil tested, in contrast to an understanding of numerous chemical P attributes. Recent evidence suggests that the sequential fractionation scheme may not yield P pools that are sufficiently chemically distinct to allow a mechanistic understanding of P transformations in soil, since the transfer of ^{33}P among different fractions of an Oxisol in Colombia was strongly dependent on the degree of saturation of soil Al and Fe (oxy)hydroxides with Pi (Buehler et al., 2002). Although the availability groups provide less specific information on soil chemical and biological properties of P fractions than the complete Hedley fractionation, they can better illustrate relevant P differences in pools that control P availability. In some instances, this procedure highlighted differences between land-use systems that were not apparent otherwise, e.g., AP in all land-use systems was greater in earthworm casts than in soils. Grouping P into AP and MAP provides a distinction between shorter- and longer-term transformations.

If the use of grouping P by availability is a better method to understand P dynamics, is it worth extracting all P fractions and analyzing the data both ways? Depending on the information one is interested in obtaining, it may be more economical, time-efficient, and simpler to do three extractions for the soils in this study: (1) 0.5 M $NaHCO_3$ for 16 h; (2) 1 M NaOH for 16 h; and (3) concentrated H_2SO_4 or concentrated HNO_3 and $HClO_4$ (depending on quality of available solutions). The HCl extractions may be excluded when working with tropical soils that have had little or no fertilization. The HCl fractions may represent Ca-bound P, and in most instances no P is Ca-bound in soils with pH less than 5. Using three extractions would provide less error between extractions, while the $NaHCO_3$ fraction could also be compared with a common P availability test (Olsen et al., 1954). Additionally, extraction time would be diminished by two-thirds, costs cut by at least one half, and results and analyses simplified.

A downside of soil phosphorus fractionation is that one cannot learn about phosphorus transformations between fractions. Phosphorus availability and pool transformations are influenced by organic P mineralization and mycorrhyizal associations. P pool transformations are best measured using radioactive P isotopes (^{32}P or ^{33}P) as tracers (Frossard et al., 1999), or through control of select P transformations, e.g., irradiation (Zou et al., 1992).

4.3. Phosphorus contributions by earthworm casts

Earthworm casts concentrated significant amounts of P in the soil environment. Casts in PAS concentrated the most P because the earthworm casts occupied more volume than in the other land-use systems. SEC casts concentrated the least P of the land-use systems studied, however, they still contributed 26.1 kg AP ha^{-1}, which is greater than the mean annual contribution of P fertilizer to AGR, 23.7 kg ha^{-1}. Therefore, fertilizer application increases AP, but without fertilizer, the contribution of earthworm casts to AP is still considerable in the systems studied. Other long-term studies in the Amazon showed that P additions above 16 kg P ha^{-1} are sufficient for a variety of annual crops (Smyth and Cassel, 1995) and even lower additions of P may remain in available form in tree cropping systems due to efficient P recycling by some tree crops (Lehmann et al., 2001).

4.4. Soil nutrients in earthworm casts

Total N in earthworm casts of AGR was expected to be greater than in AGR soil because mean foliar N was greatest in AGR (1.84 mg N g^{-1}) of the three land-use systems studied (1.11 mg N g^{-1} in PAS; 1.16 mg N g^{-1} in SEC) (McCaffery, 2002). However, mixed results of total N after earthworm inoculation have also been shown in various low-input agricultural systems (Villenave et al., 1999). Additionally, earthworm casts did not exhibit K concentrations similar to that of mean foliar K concentration in AGR, PAS, and SEC, with 0.54, 1.08, and 0.50 mg K g^{-1}, respectively (McCaffery, 2002), which begins to suggest that the diet of these earthworms may be non-foliar.

Earthworm casts of SEC more consistently exhibited differences of nutrient concentrations, specifically Ca, N, and Mg, over SEC soils, than any other land-use system. Suggestions for differences of nutrient concentrations between earthworm casts and soils have been attributed to microbial activity and earthworm gut pH (Barois et al., 1993). However, it is likely that some factor distinguishing SEC from the other land-use systems is causing these differences. Having had no fertilization in SEC, the earthworms may have a digestion mechanism to extract a base minimum of certain nutrients used for their own structural maintenance.

4.5. Conclusions

Earthworm casts contained more total P and a higher percentage of more labile P pools (AP and MAP) than soils in all land-use systems studied. It is suggested that grouping of P fractions by availability into three main groups may better represent the biological and functional relevance of the P pools, thus implying a simpler condensed extraction procedure. Such modified groupings of P pools suggest that earthworm casts of the large earthworms (Family Glossoscolecidae) are a significant source of P for plant growth in the systems we studied.

It is suggested that more controlled laboratory studies are conducted to better understand the impact of gut passage through these earthworms and the microbial populations associated with their guts and casts. Such experimentation should be accompanied by field studies to better understand what these earthworms consume, where they reside, and the volume of soil they impact on a landscape scale.

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