8 Sequential P Fractionation of Relict Anthropogenic Dark Earths of Amazonia

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8.1 Introduction

Dark earths which are rich in small artifacts can be found in a wide range of environmental settings in the Amazon Basin. While various conflicting theories have been proposed about the origin of these soils (Smith 1980), it is now widely accepted that they formed under anthropogenic influence (Sombroek 1966; Smith 1980; Woods et al. 2000). These relict Anthrosols are often the only testimony of pre-Columbian settlements and can provide important information about the former inhabitants (Vacher et al. 1998). Amazonian dark earths have high soil organic matter and nutrient contents such as P (Sombroek 1966; Smith 1980; Kern and Kämpf 1989), and are therefore highly fertile. Phosphorus availability is the most important constraint to crop production in central Amazonia (Lehmann et al. 2001a) and high P contents are the primary reason for the high production potential of these Anthrosols. Farmers value these soils in many areas of Amazonia, and some authors claim that continuous cropping is possible for 40 years or more without fallowing (Petersen et al. 2001). Whether the so-called *terra preta do índio* soils were intentionally created for agricultural purposes or whether they formed as a result of habitation is still under debate (McCann et al. 2001). In order to understand the origin of these soils, it is important to know what type of organic input caused the high organic matter and P contents, and under which conditions. More information is needed about the properties of these soils to answer such questions. This would provide clues about the livelihood of the former inhabitants as well as strategies for future soil use.

Readily extractable P contents have been widely used for the identification and study of anthropogenic soils (Arrhenius 1931; Eidt 1977; Woods 1977; Wells et al. 2000). The spatial extent of anthropogenic activities can be determined without large excavations and provide important information even in the absence of artifacts. A sequential fractionation scheme that distinguishes between different soil P pools has been applied to archaeological sites, yield-

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ing more reliable information about settlement history than total or readily available P contents (Eidt 1977; Woods 1977; Lillios 1992). Increasing use of a fractionation procedure developed by Hedley et al. (1982) and modified by Tiessen and Moir (1993) for use in agricultural research has prompted the present study. The procedure addresses the need to explain the high fertility of *terra preta* soils from the viewpoint of current soil use as well as the origins of P deposited in these soils. The Hedley fractionation technique distinguishes between several inorganic and organic soil P pools of increasing recalcitrance and has frequently been used to quantify biochemical cycling of P in agricultural (Friesen et al. 1997) and natural (Cross and Schlesinger 1995) ecosystems. This fractionation procedure was developed for mineral soils and has rarely been applied to soils with high organic matter contents. Since the relict Anthrosols additionally have very high P contents (Sombroek 1966; Kern and Kämpf 1989), the extraction efficiency may be insufficient using the procedure recommended for the Hedley fractionation. In this experiment, we studied the applicability and usefulness of the sequential fractionation modified by Tiessen and Moir (1993) for *terra preta* soils of Amazonia. Different ratios of soil-to-extractant were compared between two Anthrosols and two fertilized Ferralsols from central Amazonia.

8.2 Materials and Methods

8.2.1 Study Sites

This study compares different P pools of two anthropogenic dark earths with unfertilized and fertilized *terra firme* soils from central Amazonia. One Anthrosol was collected near Rio Preto da Eva, 60 km north of Manaus, Brazil. The second Anthrosol was obtained from the lower Rio Negro in the community of Marajá. The Ferralsols were sampled at the station of the Embrapa Amazonia Ocidental, 29 km north of Manaus. The average temperature at the Embrapa is 26 °C and average precipitation is 2,503 mm $year^{-1}$ (1971–1993) with a maximum between December and May. The natural vegetation is a tropical rainforest. The Xanthic Ferralsols (FAO 1990) are clayey, strongly aggregated, with medium organic C contents, and low pH values (Table 8.1). The Fimic Anthrosols are sandy loams to sands, with high organic C contents, and moderately acid pH.

Samples with both high and low available P contents were selected from the Ferralsols and the Anthrosols (Table 8.1). Composite soil samples from the two Anthrosols were randomly collected from forest sites. The fields have never received commercial fertilizers. Soils from the fertilized Ferralsols were obtained from a mixed tree crop plantation with *Bixa orellana* L. (urucum; annatto). These samples were collected at two points in 0.5 m distance from two trees (four subsamples). The soil representing low additions of inorganic

Table 8.1. Characterization of a fimic Anthrosol ("*terra preta do índio*") and xanthic Ferralsol from central Amazonia. *n.d.* Not determined

Soils	Sampling depth (m)	pH (H_2O)	Sand (%)	Silt (%)	Clay (%)	TOC ($g\,kg^{-1}$)	$P_{available}$ ($mg\,kg^{-1}$)
Anthrosol Marajá	0–0.1	5.7	58.7	17.0	24.3	n.d.	25.4[a]
Anthrosol Rio Preto da Eva	0–0.1	5.7	71.0		29.0	84.7	6.5[b]
Ferralsol – high fertilization	0–0.1	4.7	21.4[c]	19.6	59.0	36.0	142.6[b]
Ferralsol – low fertilization	0.1–0.2	4.2	27.2	14.9	57.9	21.2	4.3[b]
Ferralsol – unfertilized	0–0.05	4.1	21.4	19.6	59.0	40.6	4.2[b]

[a] Mehlich-1 extraction (Soil and Plant Analysis Council 1999).
[b] Mehlich-3 extraction (Mehlich 1984).
[c] Particle size distribution determined at a nearby soil pit (J. Marques, unpubl. data).

P was collected at a depth of 0.1–0.2 m from trees receiving an application of 11.9 g P $tree^{-1}\,year^{-1}$, whereas the soil representing high additions of inorganic P was obtained from 0–0.1 m depth with an application of 59.4 g P $tree^{-1}\,year^{-1}$. Phosphorus was applied as triple super phosphate and split between a December and a May application. Data for unfertilized soils were taken from a replicated assessment of soils under primary forest presented earlier (n=3; Lehmann et al. 2001b). The soils show a wide range of available P contents (Table 8.1).

8.2.2 Soil P Fractionation and Analysis

The soils were air-dried and sieved to 2 mm. Samples were sequentially extracted according to a modified Hedley procedure (Fig. 8.1; Hedley et al. 1982; Tiessen and Moir 1993). The different fractions have been assigned to different soil P pools by various authors (Hedley et al. 1982; Tiessen and Moir 1993; Cross and Schlesinger 1995), but uncertainty about whether these pools can be clearly identified and quantified through sequential extraction prevails. In this publication, we will therefore interpret trends between soils rather than compare different soil P pools. For this reason, Fig. 8.1 gives only the most robust differentiations between pools, which can be unambiguously interpreted.

Four different ratios of soil to extractant were used, 1:1,000, 1:200, 1:100, and 1:40 (10, 50, 100, or 250 mg soil with 10 ml of solution). Tiessen and Moir (1993) recommended 1:60. Two resin strips (each 6×15 mm; Prod 55164 2S, BDH Laboratory Supplies, Poole, UK) were added to 10 ml of distilled water and shaken for 16 h. After retrieving the strips and washing adhering soil with distilled water into the centrifuge tube, P was extracted from the resin with 10 ml 0.5 M HCl for 16 h. The soil suspension was centrifuged at

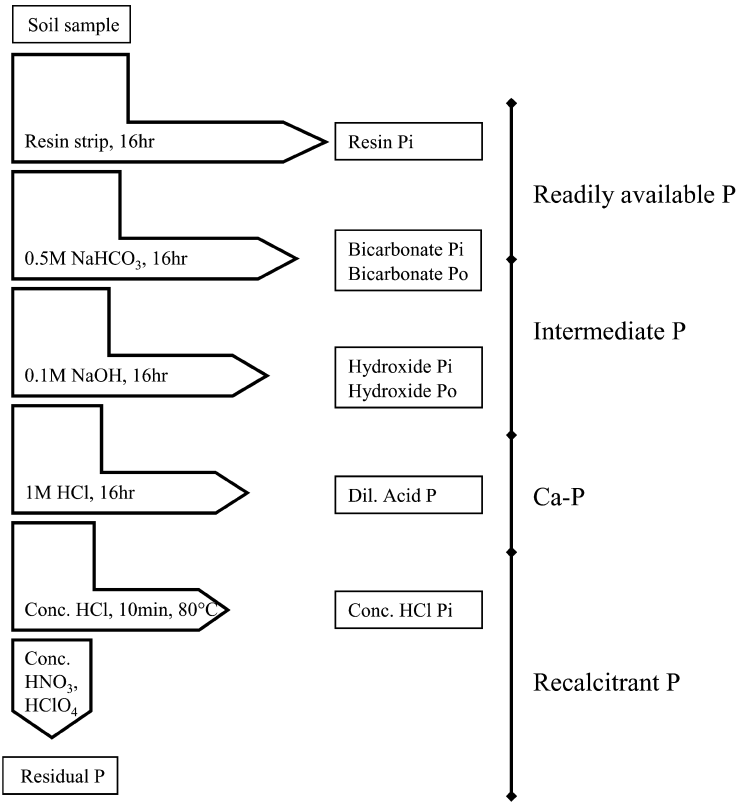

Fig. 8.1. Outline of the sequential fractionation of P forms. (Modified from Tiessen and Moir 1993)

2,000 rpm for 10 min and the supernatant was discarded. Consecutively, extractions were made with 10 ml of 0.5 M $NaHCO_3$ (adjusted to pH 8.5), 0.1 M NaOH, and 1 M HCl, each separately shaken for 16 h and centrifuged at 2,000 rpm (Fig. 8.1). The supernatant was carefully decanted and stored in the refrigerator for analysis. Two milliliters of concentrated HCl was added to the soil and the tubes were placed in a water bath at 80 °C for 10 min. After removal, the suspension was allowed to cool down for 1 h, while shaking the tubes every 15 min by hand. After adding 1 ml of concentrated HCl to the same tubes, they were centrifuged at 2,000 rpm for 10 min and the supernatant was poured into a 10-ml volumetric flask. This was repeated with 2 ml of distilled water. The volumetric flask was then made up to volume with distilled water. After adding 3 ml of concentrated HNO_3 and 1 ml of concentrated $HClO_4$ the soil was transferred to a crucible and placed in a sand bath at 200 °C for 16 h. After the soil cooled down, 2 ml of 5 M HNO_3 was added to the crucible, filtered into a 10-ml volumetric flask and made up to volume with HNO_3.

Inorganic P in the resin-, dilute-acid-, acid-, and residual-P extracts was measured directly using the molybdate ascorbic acid method (Murphy and Riley 1962) on a spectrophotometer at 712 nm. For the alkaline extracts, 1.2 and 0.3 ml of 0.9 M H_2SO_4 was added to 2 ml of bicarbonate and hydroxide extract, respectively. After centrifugation at 4,000 rpm for 20 min and cooling in a refrigerator for 30 min, inorganic P was measured as described above. This procedure separates any organic precipitates from the solution (Tiessen and Moir 1993). Organic P in the bicarbonate and hydroxide extracts was calculated as the difference between total and inorganic P, since organic P cannot be determined directly. Total P was analyzed as inorganic P after digestion with ammonium persulfate and sulfuric acid, followed by autoclavation (Tiessen and Moir 1993). An aliquot of 1 ml bicarbonate or hydroxide extract was digested with 0.1 g ammonium persulfate and 2 ml 0.9 M H_2SO_4 in an autoclave for 2 h. Inorganic P was determined as described above for the resin and acid extracts.

8.3 Results and Discussion

8.3.1 Extraction Efficiency by Sequential Fractionation

The yield of total P and the distribution between pools in the Anthrosol did not change significantly over a wide range of soil-to-extractant ratios, considering the large amounts of P present in this soil (Fig. 8.2). With high ratios, readily extractable P in the resin-P and bicarbonate-Pi fractions of the

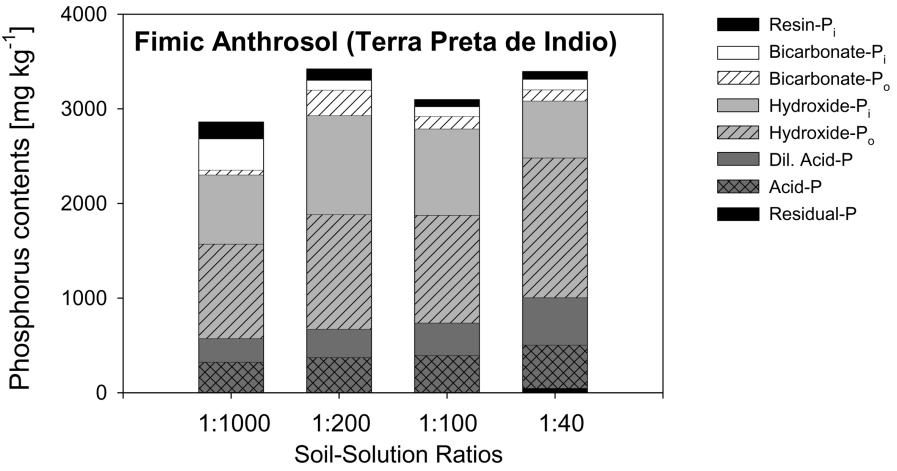

Fig. 8.2. Phosphorus contents in P pools of a Fimic Anthrosol ("*terra preta de indio*"; Amazonian Dark Earth) from central Amazonia with different soil-to-extractant ratios (n=3; for 1:40 n=1; CV<10 %)

Anthrosol may have been overestimated. It can be concluded, however, that the fractionation procedure applied is efficiently extracting the targeted P pools despite the high P and organic matter contents. The proportion of soil to extractant of 1:60 recommended in the standard procedure (Tiessen and Moir 1993) is suitable for the studied Anthrosols. The amount of soil for the extraction should not be less than 0.25 g as the heterogeneity of the soil sample will make it difficult to obtain a representative subsample.

In the Ferralsol, a higher proportion of extractant increased the total P extracted and changed the distribution of P pools. More readily available P (resin- and bicarbonate-P) as well as recalcitrant P (dilute-acid-, acid-, and residual-P) were obtained with these higher ratios. With higher proportions of the extractant, the initial steps of the sequential fractionation yielded more P at the expense of intermediate fractions. Most likely, inorganic P from interlattice layers of clay minerals or from strongly adsorbed P forming bridging ligands with Fe- and Al-oxides may have been extracted at an earlier step in the fractionation. This process was not important for the Anthrosols, which contain very low amounts of clay-sized particles. However, it shows clearly that sequential extraction methods have to be evaluated with caution, as earlier extraction steps have important effects on fractions extracted later in the procedure. Independent analyses of the fractions and parallel extractions are needed to verify the results of sequential fractionations.

8.3.2 Phosphorus Distribution in Soil Pools of Anthropogenic Dark Earths in Central Amazonia

Total P contents of the four soils varied widely between 193 and 3,097 mg kg^{-1} (Table 8.2). These values are far above the ones that are typically found in acid upland soils of central Amazonia, which range from 40–100 mg P kg^{-1} from several different sites (Lehmann et al. 2001a). The Anthrosols must have received large amounts of P during pre-Colombian occupation, whereas the Ferralsols studied here received large and sustained fertilization of 12–59 g P $tree^{-1}$ $year^{-1}$ for 7 years. Glaser (1999) reported total P values of 980–2,170 mg kg^{-1} for five different Anthrosols from central Amazonia.

While increasing total P content, the P added to the Anthrosols by pre-Columbian Indians increased not only readily available P pools but also more stable P pools at least in the Anthrosol with the high total P content. A higher proportion of the inorganic P (triple super phosphate) applied to the Ferralsol remained in readily available forms and was extracted by resin and bicarbonate. An important difference between the pool distribution of the Anthrosol and the fertilized Ferralsol was the higher proportion of hydroxide- and dilute-acid-P in the Anthrosol, as well as the virtual absence of residual P in these soils.

A comparison of the Anthrosol with an unfertilized Ferralsol from a primary forest site (Fig. 8.3) demonstrates this pattern more clearly. The pool

Table 8.2. Inorganic and organic P pools in a fimic Anthrosol ("*terra preta do índio*") and a fertilized xanthic Ferralsol from the central Amazon (only soil-to-extract ratio of 1:100; n=3; means ± standard errors; in mg kg^{-1}). *Sum inorganic* Bicarbonate-P_i+hydroxide-P_i+dil. Acid-P+acid-P+residual-P; *Sum organic* bicarbonate-P_o+hydroxide-P_o

Soils	Resin-P	Bicarb-P_i	Bicarb-P_o	Hydr-P_i	Hydr-P_o	Dil. Acid-P	Acid-P	Res-P	Sum inorganic	Sum organic	Total P
Anthrosol Marajá	73.5±4.9	102.4±2.2	131.4±1.7	914.2±12.5	1137.4±29.4	343.4±0.7	394.7±0.8	0.0±0.0	1754.6±13.9	1342.2±29.5	3096.5±13.9
Anthrosol Rio Preto da Eva	6.4±0.4	27.5±1.9	42.5±6.0	74.0±2.6	24.9±2.8	5.5±0.7	11.8±0.7	0.0±0.0	118.8±4.2	73.8±8.1	192.5±7.0
Ferralsol – high fertilization	40.5±2.1	55.0±2.5	144.1±1.3	351.4±2.6	283.7±5.1	29.6±1.7	64.0±2.1	9.3±0.4	509.4±5.0	468.3±6.9	977.7±5.7
Ferralsol – low fertilization	4.5±0.4	12.1±1.0	83.4±2.7	59.3±2.0	47.5±4.2	1.2±0.4	20.4±0.8	7.2±0.4	100.2±1.4	119.5±19.0	219.7±17.7

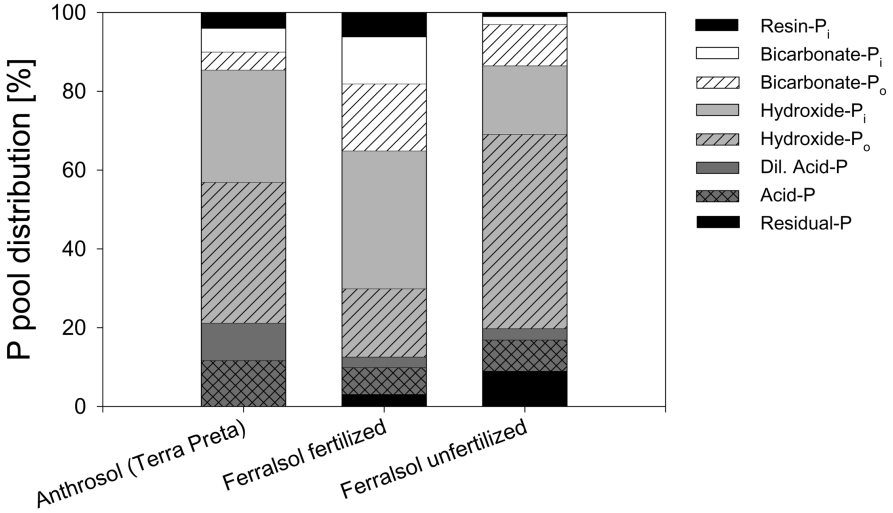

Fig. 8.3. Proportion of P pools to total P in a Fimic Anthrosol ("*terra preta de indio*"; Amazonian Dark Earth) and a fertilized Ferralsol (tree crop plantation – high fertilization) (means of three soil-solution ratios), compared to an unfertilized Ferralsol (mean of forest sites from Lehmann et al. 2001b) in the central Amazon

distribution shifts from plant-available P in the Anthrosol to highly occluded P in the unfertilized Ferralsol. Phosphorus availability seemed to be controlled to a greater extent by intermediate P pools in the Anthrosol and by more recalcitrant P pools in the Ferralsol. This led to a higher proportion of readily plant-available P in the Anthrosol relative to the unfertilized Ferralsol. More recalcitrant P (in the residual-P fraction) can be explained by a higher amount of clay minerals and Fe- and Al-oxides in the Ferralsols, which is confirmed by the particle size distribution (Table 8.1). In support of this explanation, higher proportions of P in coarse particle size fractions such as sand were found in Anthrosols than in Ferralsols (Glaser 1999).

8.3.3 Sources of P in Pre-Colombian Anthrosols

What can the P pool distribution tell us about the types of inputs that led to high levels of P in these Anthrosols? Eidt (1977) and Woods (1977) introduced a sequential fractionation technique that was successfully used to estimate settlement history and soil use. Equal proportions of available P (estimated as hydroxide- and citrate-extractable), occluded P (dithionite-extractable), and Ca-P (HCl-extractable) were shown to be indicative of archaeological soils and intensive human occupation in the past. The modified Hedley fractionation employed in the present study did not yield suitable analoga to the fractions described above. Some patterns of P distribution

nevertheless show clear indications of prior occupation: (1) the dominant proportion (65 %) and high amounts (>2,000 mg kg^{-1}) of P in inorganic and organic hydroxide fractions indicate large inputs of stable organic P compounds; (2) the low importance of residual-P suggests little geogenic or occluded P; and (3) the relatively low proportion of available P (resin- and bicarbonate-Pi) compared to the fertilized Ferralsol, demonstrating the absence of recent inorganic fertilizer inputs.

Judging from the P distribution as well as from the known resources available in the region, it is unlikely that mineral amendments were used by Amerindian inhabitants prior to European contact. More likely is the use of organic P sources as soil amendments, which is supported by the large amount of organic P in these soils. Significant amounts of dilute-acid-P indicate that Ca-phosphates (Ca-P) are present in the Anthrosols. Since pH values of below 6 (Table 8.1) do not promote the formation of Ca-phosphate, P was most likely added in the form of Ca-P. Livestock manure was shown to increase total P as well as available and Ca-P pools (Solomon and Lehmann 2000), but livestock were not present in the Amazon during pre-Columbian times (Gilmore 1963). Human excreta are a possible source of manure with high P contents, and turtle farming has been mentioned as a way of generating manure among early riverine inhabitants (W. Sombroek, pers. comm..). Fish manure in trout farms has been shown to contain high P concentrations of 25.4 g kg^{-1} with N-to-P ratios close to unity, as well as high Ca concentrations of 69.9 g kg^{-1} (Naylor et al. 1999). Based on the current scholarship, it seems unlikely that Amerindian groups alternatively raised fish in ponds.

A more probable source of organic P is from application of kitchen residues containing high proportions of fish. Since these Anthrosols are usually situated on bluffs near rivers (Denevan 1996), fish may have constituted a significant part of the diet. Juvenile tilapia in an aquarium study contained between 20 and 30 g P kg^{-1} (Mbahinzireki et al. 2001). Most of the P (85 %) in fish is found in the skeleton (Lall 1991 and Persson 1987 cited in Rønsholdt 1995), which can contain as much as 50 g P kg^{-1} as reported for mackerel (Shimosaka 1999). Fish wastes were shown to contain high P and Ca contents, which were greater in skin and bone fractions (61 and 106 g kg^{-1}) than in total fish processing waste (23 and 45 g kg^{-1}) or in deboned waste (12 and 19 g kg^{-1} for P and Ca, respectively; Rathbone et al. 2001). Thus, inedible parts of fish, such as the bones, had especially high P and Ca concentrations. After cooking the fish, the relative content of P in bones increases even more and is present largely as hydroxo-apatite (Shimosaka 1999). This apatite or similar Ca-phosphates may be the source of Ca-P and the large amounts of P found in *terra preta* soils. Since amounts of P input into villages were as high or higher in fish bones than fish meat, a direct application of fish residues to soil is more probable than through human excreta. Therefore, much of the P in the relict Anthrosols was most likely derived from fish residues and transformed from Ca-P to organic P through microbial activity and simultaneous additions of organic matter.

8.4 Conclusions

The sequential extraction method described in this chapter is suitable for analysis of anthropogenic dark earths of the Amazon, despite the large amounts of P and organic matter in these soils. In comparison to a Ferralsol, the Anthrosols contain much more P which is less abundant in highly recalcitrant pools. However, in comparison to a highly fertilized Ferralsol, the Anthrosols also contained less P in easily extractable and plant-available forms. The dark earths were shown to have the highest fractions of P in intermediate soil P pools. A high amount and proportion of dilute-acid-P in the Anthrosols is indicative of P associated with Ca, suggesting that the high quantities of P could have derived from fish residues. Most of the P in fish is found in the bones, which have high concentrations of P mainly present in the form of Ca-P. The large amounts of P found in these relict Anthrosols from pre-Columbian occupation are likely to result from an application of fish waste. Further studies should include electron microscopy to identify Ca-P compounds in soil. Relative abundance of Ca-P may then be used to examine local diets, market strategies, and long-term turnover of soil P. With sufficient information about settlement history, questions may be addressed about the type of contact between riverine and upland settlements.

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