

Phosphorus in Oxisols under agroforestry and conventional coffee systems in Brazil analysed by ^{31}P NMR

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Abstract

Phosphorus (P) is the primary limiting nutrient for crop production in highly weathered tropical soils. The deficiency is mainly caused by strong adsorption of H_2PO_4^- to Al- and Fe- (hydr)oxides, which turns large proportions of total P into a form that is unavailable to plants. Our hypotheses are that agroforestry coffee systems compared to conventional coffee fields have higher ratios of 1) organic P to total P, and 2) diester to monoester phosphates. The objective of this study is to test these hypotheses, using ^{31}P NMR for the analysis of different phosphate forms. Soils from on-farm experiments on Oxisols in Brazil were sampled at 2-3 cm, 10-15 cm and 40-60 cm. Two agroforestry coffee systems, medium aged (AM) and old (AO), and two conventional coffee systems, medium aged (CM) and old (CO) were compared. Based on literature and standards, ^{31}P NMR signals were interpreted as inorganic P (Pi), orthophosphate and pyrophosphate and as organic P (Po), orthophosphate monoester (inositol phosphates and mononucleotides) and orthophosphate diester (phospholipids, DNA, RNA and teichoic acid). The proportion of organic P (Po) found was on average 47 %. There were significant effects of system and depth on the ratio of organic P to total P as well as a significant interaction of system and depth. The fraction of Po in the deepest layers was higher in agroforestry systems than in the old conventional system, whereas that of the old agroforestry system was higher than of all other systems. These results are consistent with Hypothesis 1. The ratio of diester to monoester was on average 0.05. There were effects of systems and depth on the ratio diester/moanoester. The agroforestry fields showed a higher ratio in the deepest layer. These results are consistent with Hypothesis 2. The work presented here is in line with our previous work on differences between agroforestry and conventional systems coffee growing systems. We discuss the possible contribution of mycorrhiza to the phosphorus cycle, and conclude that the impacts of the differences in the mentioned P ratios on P cycling and efficiency of P use by the crops need further studies.

Keywords: organic phosphorus, agroforestry, coffee, Oxisol, Brazil, on-farm experiments, ³¹P NMR

Introduction

Phosphorus (P) is a major nutrient in relatively short supply in most natural ecosystems, and the primary limiting nutrient for crop production in highly weathered tropical soils (Linquist *et al.*, 1997). The deficiency is mainly caused by strong adsorption of H₂PO₄⁻ to Al- and Fe- (hydr)oxides, which turns large proportions of total P into a form that is unavailable to plants (Fontes and Weed, 1996). Trees are considered to increase nutrient availability and accelerate P cycling by (1) retrieving nutrients from lower soil horizons (Young, 1997), (2) enhancing the chemical and physical quality of soils, and (3) increasing microbial activity. Most tropical trees are arbuscular-mycorrhizal fungi (Carneiro *et al.*, 1998; Onguene and Kuyper, 2001). Some authors believe that arbuscular-mycorrhizal fungi can take P up only from the same pools as plants, but other authors have shown that arbuscular mycorrhizal plants can take P up also from organic P sources that are not available (Jayachandran *et al.*, 1996).

With the objective to use the potential of the trees to increase the availability of nutrients, especially of phosphorus (Cardoso *et al.*, 2001a), in 1993, several Rural Workers Unions, assisted by an agriculture-focused non-governmental organisation (NGO) and researchers from a Federal University started to implement agroforestry coffee systems in the Zona da Mata, where the present fieldwork was done. In the past the area has been under forest climax vegetation. Because in agriculture a major part of P, that has been absorbed by the crop, is removed from the fields and hence does not return to the soil, it is expected that in soils of cropped fields the ratio of organic P to total P is lower than e.g. in soils under forest. The most common forms of P found in soils are orthophosphate and pyrophosphate, as inorganic P, and polyphosphate, phosphanate, orthophosphate monoester and orthophosphate diester as organic P (Newman and Tate, 1980). It is expected that since the clearing of the forest for agricultural use the ratios of organic P to total P and of diester to monoester phosphates have decreased. Diester is considered more chemically labile than monoester phosphates (Tate and Newman, 1982). The objective of this paper is to test two hypotheses: (1) in agroforestry systems Po/total P is higher than in conventional coffee growing systems; (2) in agroforestry systems the ratio of diester to monoester phosphates is higher than in conventional coffee growing systems. For the analysis of the various P forms ³¹P NMR is used. ³¹P NMR spectroscopy is a direct technique, assessing inorganic P as well as organic P compounds in soil (Newman and Tate, 1980).

Material and Methods

Soils (Oxisols) were collected from two on-farm agroforestry systems of 15 and 19 years old with coffee (*Coffea arabica*) as a crop, further referred to as AM (medium-aged) and AO (old) respectively, and two on-farm conventional coffee systems of 15-20 and 20-24 years old, from now on called CM (medium-aged) and CO (old) respectively. The fields are located in the Zona da Mata, in the Atlantic Coastal Rainforest domain in Minas Gerais,

Brazil. Pedological, agricultural and sociological details are given by Cardoso *et al.* (2001a; 2002).

Samples were collected in June 1999 (dry season), in three profiles, approximately 10 m apart, in between the coffee rows, at depths of 2-3 cm, 10-15 cm and 40-60 cm. The coffee rows were, on average 3.0 m apart, and the space in between was used for agroforestry trees (AM and AO) or for annual crops and weeds (CM and CO). The extraction method was carried out according to the flow diagram presented in Figure 1. It was based on contributions of several authors (Cade-Menun and Preston, 1996; Robinson *et al.*, 1998) and was adapted to tropical soils by Rheinheimer *et al.* (2002). Phosphorus was extracted from three grams of soil with 30 mL NaOH 0.5 M + EDTA 0.1 M. The extract was shaken with chelex resin to remove extract paramagnetic ions, then freeze-dried and the product dissolved in 2 mL D₂O. ³¹P NMR spectra were recorded with a Bruker DPX-300 NMR spectrometer. Quantification was achieved by instrumental integration of the peaks. Spectra interpretations were based on literature assignments (Newman and Tate, 1980; Cade-Menun and Preston, 1996; Dai *et al.*, 1996; Rheinheimer *et al.*, 2002) and standards (orthophosphate, pyrophosphate and glucose-6-phosphate). These include: inorganic orthophosphate, at $\delta = 5.3-8$ mg kg⁻¹; orthophosphate monoesters (inositol phosphate, sugar phosphates, mononucleotides), $\delta = 3-6.2$ mg kg⁻¹; orthophosphate diesters (teichoic acid), $\delta = 0.36-3$ mg kg⁻¹; orthophosphate diesters (phospholipids, DNA and RNA), $\delta = 1.5-(-2.0)$ mg kg⁻¹; pyrophosphate, $\delta = -2.8-(-6.0)$ mg kg⁻¹. The total P was measured by ICP.

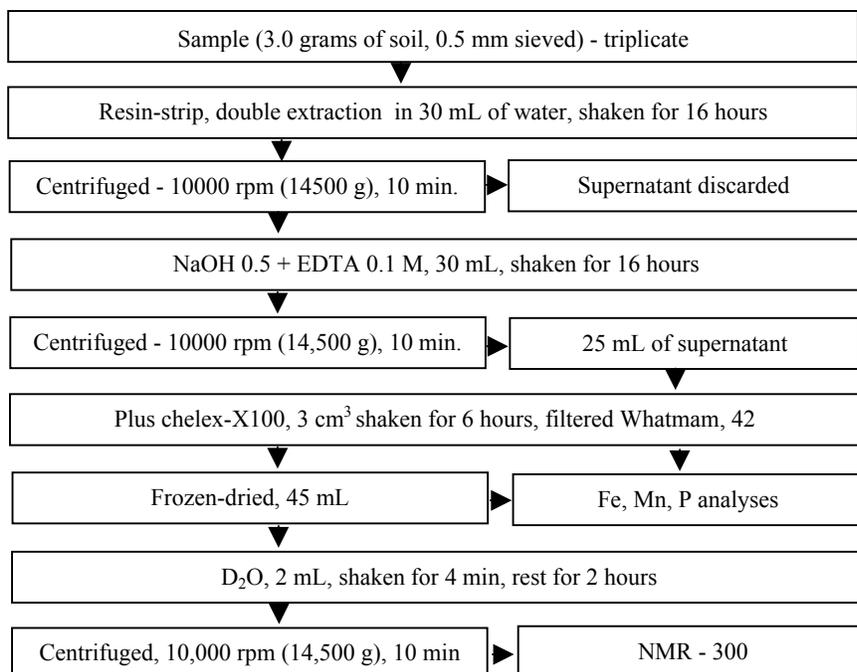


Figure 1 Flow diagram of the extraction method.

Results and Discussion

Figures 2 and 3 show representative ^{31}P NMR spectra of the alkali extracts of agroforestry (AM and AO) and conventional (CM and CO) coffee systems at three different depths (2-3 cm, 10-15 cm and 40-60 cm). Signals were interpreted as orthophosphate at $\delta = 6.8 \text{ mg kg}^{-1}$, as pyrophosphates at $\delta = -2.9 \text{ mg kg}^{-1}$, together forming inorganic P (Pi), and as orthophosphate monoester at $\delta = 5.1 \text{ mg kg}^{-1}$, as orthophosphate diester at $\delta = -0.4 \text{ mg kg}^{-1}$ and as teichoic acid at $\delta = 1.5 \text{ mg kg}^{-1}$, together forming organic P (Po). The sum of Pi and Po is denoted by total P in NaOH-EDTA extract.

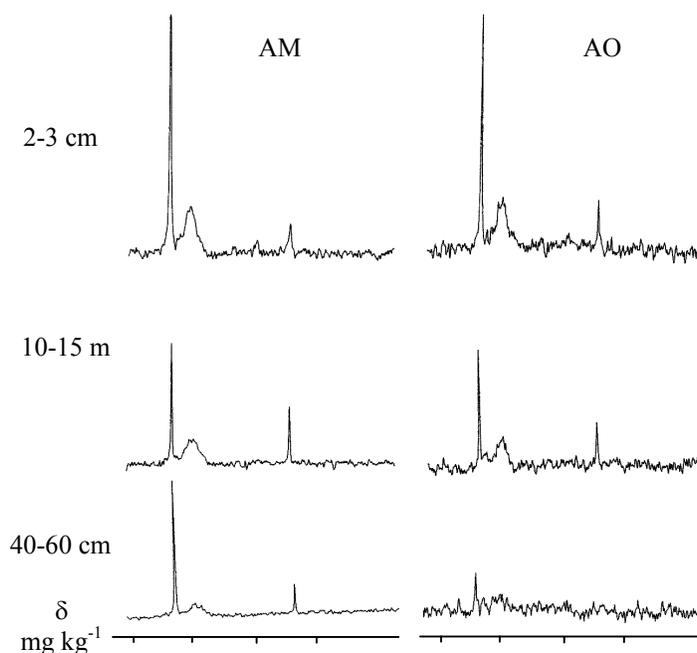


Figure 2 Representative ^{31}P NMR spectra of the alkali extracts of agroforestry (AM and AO) coffee systems, at three different depths (2-3 cm, 10-15 cm and 40-60 cm).

Table 1 shows the relative proportions of different P forms in NaOH 0.5 M + EDTA 0.1 M extract characterised by ^{31}P NMR spectroscopy and total P (mg Kg^{-1}) by chemical analysis. Organic P was on average 47 % of total P extracted, consisting of orthophosphate monoester (on average 95 %) and orthophosphate diester (on average 5 %). The dominance of monoesters within the organic P forms is consistent with most studies involving alkali extraction (Tate and Newman, 1982; Dai *et al.*, 1996; Rheinheimer *et al.*, 2002). Orthophosphate and pyrophosphate comprised 45.4 % and 7.6 % of the total P. There were significant effects of system and depth on the ratio of organic P to total P as well as a significant interactions of system and depth, and system and age (Table 2). The fraction of Po in the deepest layers was higher in agroforestry systems than in the old conventional system, whereas that of the old agroforestry system was higher than that of all other systems. These results are consistent with Hypothesis 1

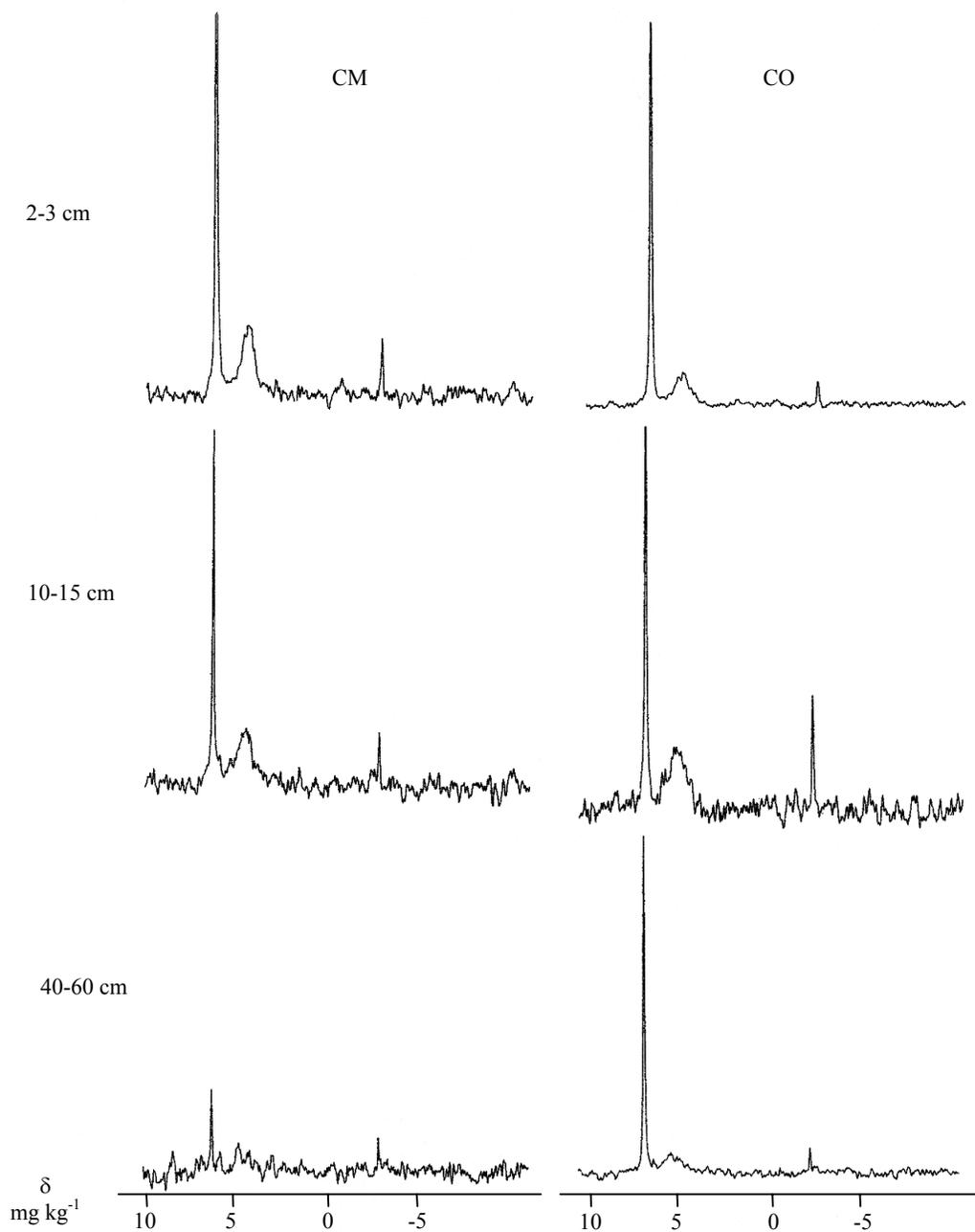


Figure 3 Representative ^{31}P NMR spectra of the alkali extracts of conventional (CM and CO) coffee systems, at three different depths (2-3 cm, 10-15 cm and 40-60 cm).

Table 1 Relative proportions (% and s.e. n = 3) of different P forms in NaOH 0.5 M + EDTA 0.1 M extract characterised by ³¹P NMR spectroscopy and total P (mg kg⁻¹) by chemical analysis.

Systems	Depths (cm)	Organic P				D/M ³	Inorganic P		Total P (chemical analyses) mg Kg ⁻¹
		Ortho- Phosphate Monoester (%)	Ortho- phosphate diester (%)	Teichoic Acid ¹ (%)	Organic P/ Total P (PNMR)		Ortho- phosphate (%)	Pyro- phosphate (%)	
AM	2-3	38.8(0.9)	3.5(1.0)	0.3(0.3)	0.43bcd ²	0.09ab ³	50.0(0.8)	7.4(0.6)	139.0(7.1)
	10-15	49.2(1.9)	3.2(0.3)	0.0	0.52abc	0.07abc	37.8(0.8)	9.8(2.8)	85.8(7.9)
	40-60	46.8(8.9)	1.8(1.1)	0.0	0.48bc	0.04c	45.7(8.8)	5.7(1.9)	94.3(5.3)
AO	2-3	47.3(3.3)	3.8(0.8)	0.0	0.51bc	0.08abc	39.8(2.8)	9.1(0.6)	97.7(2.7)
	10-15	51.3(0.6)	2.6(1.3)	0.0	0.54ab	0.05bc	31.9(1.9)	14.2(1.1)	62.3(3.5)
	40-60	60.4(7.8)	3.4(2.1)	0.0	0.64a	0.06bc	31.4(6.4)	4.8(2.0)	68.7(15.3)
CM	2-3	36.5(0.5)	4.2(0.4)	0.0	0.41cd	0.11a	54.5(1.2)	4.8(0.7)	140.7(3.8)
	10-15	50.8(2.2)	2.0(1.2)	0.5(0.5)	0.53abc	0.04bc	39.8(2.7)	6.9(0.6)	81.8(6.1)
	40-60	47.9(5.7)	0.0	0.0	0.48bc	0.00d	37.3(2.5)	14.8(3.3)	37.3(1.0)
CO	2-3	31.8(2.8)	2.3(0.9)	0.9(0.5)	0.35de	0.07abc	61.0(4.2)	4.0(0.8)	194.5(9.8)
	10-15	43.6(1.6)	1.3(0.9)	0.0	0.45bcd	0.03cd	48.0(1.1)	7.1(0.9)	121.7(2.6)
	40-60	28.6(1.6)	0.0	0.4(0.4)	0.29e	0.00d	65.6(1.7)	5.4(1.3)	175.7(2.5)

¹ Teichoic acid is also a orthophosphate diester but is shown separately here because it appeared in a different peak

² Numbers with the same letters are not significantly different at the 0.05 level

³ D/M = Diester Monoester ratio. It does not include diester monoester ratio to allow for comparison with literature data. Numbers with the same letters are not significantly different at the 0.1 level

Table 2 Statistical analysis of the effects of coffee growing systems (agroforestry and conventional), age (young and old) and soil depth (2-3, 10-15 and 40-60 cm) on the ratios of organic P to total P (Po/P) and of diester P to monoester P (D/M).

Factors	Po/P	D/M
System	***	**
Age	ns	ns
Depth	**	***
System x age	***	ns
Systems x depth	*	ns
Age x depth	ns	ns
Systems x age x depth	ns	ns

*Significance levels, * (P<0.1), ** (P<0.05) and

*** (P<0.001) or not significant (ns)

The fraction of diester phosphate was very low (on average 2.5% of total extracted P). Only traces of phospholipids, nucleic acids and teichoic acids were found (Table 1). This result differs from those reported elsewhere, but is consistent with those found by Rheinheimer *et al.* (2002) in Brazilian Oxisols. Table 2 shows significant effects of systems

and depth on the ratio diester/monoester. The agroforestry fields had a marginally significant higher ratio in the deepest layer. These results are consistent with Hypothesis 2. The ratio of diester to monoester was on average 0.05 (Table 1). The range is consistent with the literature (Dai *et al.*, 1996: 0.0 – 2.0; Rheinheimer *et al.*, 2002: 0.05 – 0.12). The phosphate diester is reported to be more chemically labile than monoester phosphorus (Tate and Newman, 1982), with higher accessibility to microbial or enzyme attack in the soil environment than monoester forms (Dai *et al.*, 1996).

The fraction of Pyrophosphate in total P was on average 8 % of total P (Table 1), higher than the average found by Rheinheimer *et al.* (2002) for Brazilian Oxisols (around 4 %). Pyrophosphate can originate from hydrolysis of organic esters during the alkali extraction (Condrón *et al.*, 1985). However, the extraction method we used is assumed not to cause such hydrolysis (Cade-Menun and Preston, 1996). This would mean that the soils studied here contain high amounts of pyrophosphates. Some authors claim that this is the result of microbial activity in the soil (Condrón *et al.*, 1985; Bedrock *et al.*, 1994), but Dai *et al.*, (1996) states that microbial activity could lead to rapid enzymatic hydrolysis of pyrophosphates, resulting in low concentrations in the soil. Hence, it is difficult to draw firm conclusions from the presence of high levels of pyrophosphate in our samples.

The generally higher percentage of organic P in agroforestry systems than in conventional systems, and the higher diester to monoester phosphate ratio in deeper soil layers confirm our two hypotheses and are in line with previous work in the same fields (Cardoso *et al.*, 2001b, 2002). In agroforestry systems a larger fraction of plant organic P might be recycled, and a larger part of the inorganic P (Pi) might be converted into organic P (Po), with or without the intervention of mycorrhiza. The fact that at the deeper soil layers a higher number of arbuscular mycorrhizal fungal spores were present in agroforestry than in conventional systems (Cardoso *et al.*, 2002) is an indication that mycorrhiza has played a role in the conversion of inorganic in the soils of our study. Arbuscular-mycorrhizal fungi could take up P from sources that are unavailable to plants either in short terms or in long terms or available sources that are in amounts inferior to the threshold for plant up take. In greenhouse experiment we found (data not published) that arbuscular-mycorrhizal plant took P up from low levels of ready (short term) and moderately (long term) available inorganic P sources, that roots could not take up.

P cycle in the soil with mycorrhiza contribution is schematically shown in Figure 4. For that diagram we used the P pools defined by Hedley *et al.* (1982): 1) Resine-Pi + NaHCO₃-Pi (IVL) and NaHCO₃-Po (OVL) considered as readily available to plants; 2) NaOH-Pi and NaOH-Po considered moderately available to plants; 3) HCl-Pi and residue-Pi (ISI) considered a stable pool, not available to plants. In the diagram shown part of the inorganic P that was taken up either by mycorrhizas or by plants is transformed in organic P through plant residue or dead mycorrhiza. Agroforestry is supposed to increase the plant residue production and microorganism activity and because the speed of recycling is higher for organic P than for inorganic P, agroforestry would maintain higher fractions of P available to agricultural crops while simultaneously reducing inputs (and hence reducing P losses to the unavailable pools).

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