

Chemical nature of residual phosphorus in Andisols



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ABSTRACT

Sequential fractionation has been widely used to study the nature and dynamics of soil P. Residual P – the recalcitrant P fraction remaining after sequential extraction with alkali and acid reagents – often constitutes the majority of the soil P, yet its nature and bioavailability is poorly understood. The objective of this study was to isolate, quantify, and characterize residual P following Hedley fractionation in a range of Andisols under grazed pasture by ³¹P nuclear magnetic resonance (NMR) spectroscopy. Residual P accounted for 45–63% of the total soil P, of which 53–77% was inorganic orthophosphate. Organic P accounted for 21–42% of the residual P, the majority of which occurred as phosphomonoesters including *myo*- (16% of the residual P) and *scyllo*-inositol hexakisphosphate (10% of the residual P). No phosphodiester were detected in the residual fraction. We conclude that residual P in Andisols consists of a mixture of inorganic P and organic P. Our findings provide the basis for the development of new approaches to improve P use efficiency in agriculture.

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1. Introduction

Phosphorus (P) is the principle limiting nutrient in many agroecosystems and continued P inputs are required to increase and maintain production (Nash et al., 2014; Haygarth et al., 2013). However, most of the P applied to soils is not taken up by plants and accumulates in the soil as various forms of inorganic and organic P, which is commonly referred to as ‘legacy P’ (Stutter et al., 2012; Condron et al., 2005; Frossard et al., 2000). In many soils, a significant proportion of the P is accumulated in recalcitrant forms (Stutter et al., 2012; Gyaneshwar et al., 2002). Understanding the nature of recalcitrant P in soil could be helpful to develop new biological approaches and technologies designed to enhance the bioavailability and utilization of this valuable P resource in soil–plant systems, such as use of enzymes or P-solubilizing bacteria for mineralizing soil P (Calabi-Floody et al., 2012; Menezes-Blackburn et al., 2011).

Fractionation of soil P involving sequential extraction with a combination of neutral, alkali and acid reagents has been used extensively to investigate the dynamics and bioavailability of inorganic, organic and microbial P in soil–plant systems (Condron and Newman, 2011; Turner et al., 2005; Hedley et al., 1982). In particular, the fractionation developed by Hedley et al. (1982) has been widely used in its original

and modified versions, to study P cycling in a wide variety of managed and natural ecosystems (Negassa and Leinweber, 2009; Cross and Schlesinger, 1995; Tiessen and Moir, 1993; Sanyal and De Datta, 1991). A limitation of the Hedley P fractionation method is that the unextractable fraction remaining after sequential extraction (‘residual P’) constitutes a significant proportion of the total P, generally more than 30% in moderately weathered soils, but >80% in soils that are strongly weathered or derived from volcanic parent material (Condron and Newman, 2011; Turner et al., 2005).

The sparingly soluble nature of residual P is commonly assumed to indicate that it is recalcitrant and of limited availability to plants and microorganisms (Condron and Newman, 2011). However, there is some evidence that the residual P pool can be accessed by plants and/or their associated microflora. For example, significant long-term depletion of soil residual P was observed in response to plant uptake and removal in arable cropping and managed forest systems in the absence of P fertilizer inputs (Pierzynski and Gehl, 2005), while short-term depletion of residual P has also been observed in some rhizosphere soils (Chen et al., 2002; George et al., 2002). Also, Richter et al. (2006) showed depletion of residual pools over long-term plantation forest growth, and other studies have shown depletion of residual P close to roots (Gahoonia and Nielsen, 1992).

The limited information on the composition and bioavailability of residual P constrains our ability to fully understand P dynamics and manage soil P resources. To address this, we examined the detailed

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Table 1
Chemical properties determined in Andisols. F: fertilized soils; UF: Unfertilized soils. +ED. Upper case letters denotes significant differences ($p < 0.05$) between treatments for one soils. Lower case letters denotes significance differences ($p < 0.05$) between soils for one treatments.

Soils	pH	Total C	Total N	Total P	Organic P	Olsen P	C:N	Oxalate extractable			
								Al	Fe	P	
		(g kg ⁻¹)			(mg kg ⁻¹)			(g kg ⁻¹)	(mg kg ⁻¹)		
Puerto Fonck	F	5.3 ± 0.1 ^{Bb}	87.2 ± 0.4 ^{Bb}	6.3 ± 0.1 ^{Ab}	1755.1 ± 90 ^{Ab}	471.2 ± 38.3 ^{Ab}	9.0 ± 0.5 ^{Ab}	13.9	76.6	24.4	651.9
	UF	5.9 ± 0.2 ^{Aa}	101.5 ± 0.2 ^{Aa}	6.7 ± 0.2 ^{Aa}	1197.5 ± 73 ^{Bc}	487.2 ± 5.1 ^{Aa}	6.0 ± 0.2 ^{Bc}	15.3	37.6	15.1	528.6
Pemehue	F	5.6 ± 0.1 ^{Aa}	117.5 ± 2.0 ^{Aa}	8.6 ± 0.1 ^{Aa}	2473.3 ± 129 ^{Aa}	530.0 ± 4.6 ^{Ab}	15.0 ± 0.1 ^{Aa}	13.6	42.1	21.6	487.4
	UF	5.4 ± 0.2 ^{Ab}	74.7 ± 0.8 ^{Bb}	5.5 ± 0.3 ^{Bb}	1684.3 ± 131 ^{Bb}	337.3 ± 4.3 ^{Bb}	10.0 ± 0.2 ^{Bb}	13.5	53.8	20.6	1598.5
Piedras Negras	F	5.7 ± 0.1 ^{Aa}	90.2 ± 0.4 ^{Ab}	7.1 ± 0.2 ^{Ab}	2351.9 ± 150 ^{Aa}	739.3 ± 17.9 ^{Aa}	14.0 ± 0.1 ^{Aa}	12.7	38.9	16.3	503.5
	UF	4.9 ± 0.0 ^{Bc}	70.2 ± 0.2 ^{Bc}	4.8 ± 0.1 ^{Bc}	981.9 ± 17 ^{Ba}	477.8 ± 27.2 ^{Bb}	11.0 ± 0.3 ^{Ba}	14.7	42.0	19.4	719.8

chemical composition of residual P in a series of Andisols from Chile by introducing an additional extraction step with NaOH–EDTA solution coupled with ³¹P nuclear magnetic resonance (NMR) spectroscopy.

2. Materials and methods

2.1. Soils and chemical analysis

Six soil samples were collected from Andisols located in La Araucanía and Los Ríos regions in southern Chile belonging to the Piedras Negras (PN), Pemehue (PEH) and Puerto Fonck (PF) soil series (CIREN, 2003). These soils were developed originally under forest, but had been under grazed pasture for around 10 years. The agricultural management on the Pemehue soil was annual cropping (wheat) under conventional tillage, while the Piedras Negras and Puerto Fonck soils were under permanent grassland (white clover + ryegrass). At each site, three replicate samples were taken from the A horizon (0–20 cm) in fertilized and unfertilized plots. The fertilized plots were amended with an input of ranging from 90 to 150 kg P ha⁻¹ in the form of superphosphate (P₂O₅) for 8 years. Soil samples were air-dried, sieved <2 mm and stored in sealed plastic bags at ambient laboratory temperature prior to analyses.

Soil pH was measured in a 1:2.5 soil to deionized water ratio. Amorphous aluminum (Al) and iron (Fe) and associated P were determined by acidic ammonium oxalate extraction, with detection by inductively-coupled plasma optical-emission spectrometry (ICP–OES) (Shoumans, 2000). Total carbon (C) and nitrogen (N) were determined by dry combustion using a CHN auto-analyzer (CHN NA 1500, Carlo Erba). Readily-extractable phosphate, assumed to represent plant-available inorganic P, was determined by extraction in sodium bicarbonate (Olsen P) (Sadzawka et al., 2006). Total soil P was determined by alkaline oxidation (Dick and Tabatabai, 1977) with phosphate detection by molybdate colorimetry (Murphy and Riley, 1962).

2.2. Soil P fractionation

P fractionation was carried out according to a modified version of the sequential fractionation described by Hedley et al. (1982) involving

sequential extraction with deionized water, 0.5 M sodium bicarbonate (NaHCO₃), 0.1 M sodium hydroxide (NaOH), and 1 M hydrochloric acid (HCl). Inorganic P in each extract was determined by molybdate colorimetry, while total P was determined in the extracts by alkaline digestion with sodium hypobromite (NaBrO) (Dick and Tabatabai, 1977). The difference between total and inorganic P was assumed to represent organic P, although it can also include inorganic polyphosphates. After sequential fractionation, the residual soil was washed with deionized water, dialyzed (10,000 kDa) and freeze-dried prior to further analyses, which included total P, organic P, total C, total N, and oxalate-extractable Al, Fe and P as described above, together with ³¹P NMR spectroscopy (see below).

2.3. Solution ³¹P NMR spectroscopy

To study chemical P forms, the residual fractions were extracted with a solution containing 0.25 M NaOH and 50 mM disodium EDTA for 16 h in a 1:20 solid/solution ratio at 22 °C, and then centrifuged at 8000 × g for 30 min (Cade-Menun and Preston, 1996). An aliquot of each extract was taken for determination of total and inorganic P as described above. For solution ³¹P NMR spectroscopy, a 20 mL aliquot of soil extracts was spiked with 1 mL of methylene diphosphonic acid (MDP) as an internal standard. The extracts were freeze dried and ground to a fine powder. Spectra were acquired on a Bruker Avance 500 spectrometer using a 30° pulse, a 10 s delay, and a 0.8 s acquisition time. Up to 30,000 scans were run for soil extracts to ensure acceptable signal-to-noise ratios. Chemical shifts of signals were determined in parts per million (ppm) relative to an external orthophosphoric acid standard (85%). Signals were assigned to P compounds based on model compounds spiked in NaOH–EDTA soils extracts (Turner et al., 2012, 2003; Turner, 2007; Turner and Richardson, 2004). Signal areas were calculated by integration and P concentrations calculated from the integral value of the MDP internal standard at 17.63 ppm.

2.4. Statistical analyses

Statistical analyses were performed using Systat software version 3.5 (2007, USA). Normality and homogeneity of variance were

Table 2
Chemical properties determined for the residual soil fractions remaining after sequential extraction (fractionation).

Soils		Total C	Total N	Total P	NaOH–EDTA extracted		C:N	Oxalate extractable		
					Total P	Organic P		Al	Fe	P
		(g kg ⁻¹)			(mg kg ⁻¹)			(g kg ⁻¹)	(mg kg ⁻¹)	
Puerto Fonck	F	61.6 ± 2.8	7.0 ± 0.4	872.4	872.4	168.0	8.8	1.8	9.8	589.7
	UF	71.7 ± 1.1	7.9 ± 0.2	512.6	831.7	196.4	9.1	1.8	8.9	452.1
Pemehue	F	101.5 ± 12.3	6.8 ± 0.9	1454.5	872.1	155.4	14.9	3.3	10.7	983.6
	UF	58.5 ± 2.8	4.4 ± 0.9	1549.7	1446.3	303.9	13.4	4.0	11.9	784.0
Piedras Negras	F	58.3 ± 2.0	8.1 ± 0.7	1296.7	1089.9	330.7	7.2	1.9	1.7	171.4
	UF	62.7 ± 0.4	9.1 ± 0.1	386.7	240.8	94.0	6.9	1.9	3.8	22.6

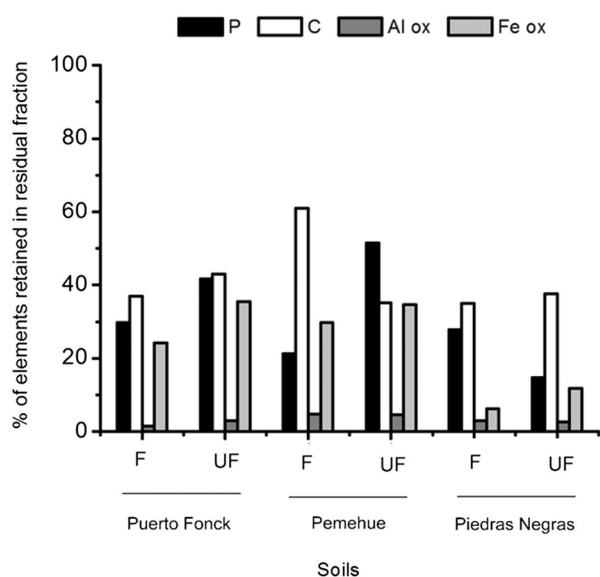


Fig. 1. Percentage (%) of phosphorus (P), carbon (C), aluminum oxalate (Al ox) and iron oxalate remaining in residual fraction after from Hedley procedure.

determined before analyses. Statistical differences of means (95% significance level) were analyzed using two way analyses of variance (two-way ANOVA) followed by Tukey–Kramer's LSD to identify significant differences among treatments. Linear correlations between selected chemical parameters were carried out using Pearson's correlation coefficient.

3. Results

3.1. Soil chemical properties of Andisols

Soil pH ranged between 4.9 and 5.9. Total P concentrations varied from 981 to 2473 mg P kg⁻¹, and all soils showed a significant increase in total P according with fertilization input. As expected, the Andisols contained significant quantities of total C (70–117 g C kg⁻¹) and total N (4.8–8.6 g N kg⁻¹) (Table 1), with C to N ratios between 13 and 15.

In Pemehue and Piedras Negras soils, total C and N were significantly higher in fertilized soils.

Organic P ranged from 337 and 739 mg P kg⁻¹ and accounted for between 20 and 49% of total P (Table 1). Organic P concentrations were significantly greater in fertilized Piedras Negras and Pemehue soils, although values were similar in both Pemehue soils (20–21%). Olsen P concentrations ranged from 3.8 to 14.5 mg P kg⁻¹ (Table 1), and were similar for unfertilized and fertilized soils. Oxalate-extractable Al was similar for all soils (38–54 g Al kg⁻¹) except for the fertilized Puerto Fonck soil (77 g Al kg⁻¹), while oxalate-extractable Fe was similar for all soils (15–24 g kg⁻¹). Oxalate-extractable P varied widely among soils, representing between 21 and 94% of total P (Table 1).

3.2. Residual fraction

The chemical composition of the residual fractions is shown in Table 2. Total P ranged between 241 and 1446 mg P kg⁻¹, which represented between 35 and 65% of total soil P (Fig. 1). In Puerto Fonck and Piedras Negras, both grassland soils, total P in the residual fraction was higher in fertilized soils. In contrast, total C ranged between 58.3 and 101.5 g C kg⁻¹ (15–52% of soil total C) and Puerto Fonck and Piedras Negras showed higher concentrations of total C in unfertilized soils (43 and 38% of total soil P). The C:N ratios of the residual soils were lower (7–15) than the original soils (13–15). Oxalate extractable Al was markedly lower in residual soils compared with the original soils (1.9–4.0 g Al kg⁻¹); values accounted for between 1.4 and 4.7% of oxalate extractable Al, and the concentrations were similar in all soils. Oxalate extractable Fe in the residual fraction ranged between 6.3 and 35.4% of the oxalate Fe in the original soils (Fig. 1), with values being greater in unfertilized soils.

3.3. ³¹P NMR analysis of residual P

Solution ³¹P NMR spectra of residual P extracts are presented in Fig. 2, while the corresponding data for quantities of different P species are shown in Table 3. Inorganic orthophosphate was the predominant P form detected in extracts of residual soil (125–1115 mg P kg⁻¹) and ranged between 52 and 77% of the total residual P. In both fertilized grassland soils, Puerto Fonck and Piedras Negras, orthophosphate was the highest P forms found in residual fraction (57 and 67% of total

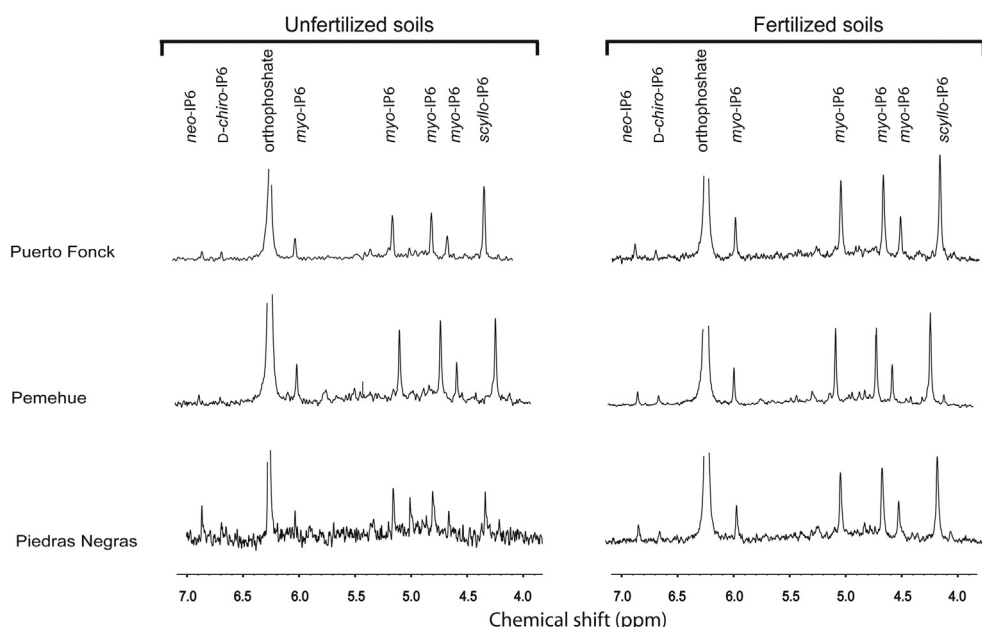


Fig. 2. ³¹P-NMR spectra of NaOH-EDTA extracts of residual fractions derived from Hedley fractionation in Chilean Andisols.

Table 3
Concentrations of different forms of P determined in NaOH-EDTA extracts of residual soils by ^{31}P NMR. F: fertilized soils; UF: unfertilized soils.

Soil		Inorganic phosphorus		Organic phosphorus			
		orthophosphate	pyrophosphate	<i>myo</i> -IP ₆	<i>scyllo</i> -IP ₆	<i>D-chiro</i> -IP ₆	<i>neo</i> -IP ₆
		mg P kg ⁻¹		mg P kg ⁻¹			
Puerto Fonck	F	500.4	30.7	173.3	120.1	29.3	18.6
	UF	439.0	39.9	156.4	139.0	33.1	24.3
Pemehue	F	593.5	17.2	106.0	72.0	54.3	29.1
	UF	1115.2	27.2	167.6	107.8	16.1	12.4
Piedras Negras	F	727.6	31.6	177.1	101.7	28.2	23.7
	UF	125.1	21.7	37.2	10.6	36.0	10.2

residual P, respectively). Inorganic pyrophosphate represented 2–7% of total residual P.

Concentrations of organic P in the residual fraction ranged between 94 and 353 mg P kg⁻¹, which represented between 21 and 42% of the total residual P (Fig. 2). In unfertilized Piedras Negras and Puerto Fonck soils, organic P was higher than in fertilized soils (39 and 42% of total residual P). Organic P in the residual fraction occurred as phosphomonoesters in the form of inositol hexakisphosphate (Fig. 2, Table 3). Of the inositol hexakisphosphate stereoisomers detected, *myo*-inositol hexakisphosphate was the most abundant (12–20% of total residual P), and concentrations were greatest in fertilized Piedras Negras and Puerto Fonck soils. We also detected *scyllo*, *neo*, and *D-chiro*-inositol hexakisphosphate stereoisomers in the residual fractions. These occurred in the order *scyllo* (10%), *D-chiro* (5%), and *neo* (3%).

4. Discussion

Our results highlight the quantitative importance of the pool of P in the residual fraction remaining after sequential fractionation in Andisols. This agrees with previous studies of soils derived from volcanic ash and other parent materials (Negassa and Leinweber, 2009; Cross and Schlesinger, 1995). The proportion of residual P found in Andisols is related to the abundance of amorphous Al and Fe oxides, which are known to stabilize P through polyvalent bridging cations (Frossard et al., 2000; Mora and Canales, 1995a). Prior studies have shown that much of the extractable P in agricultural soils occurs as orthophosphate and phosphomonoesters associated with Al and Fe soil surfaces (Stutter et al., 2015; Frossard et al., 2000; Mora and Canales, 1995b). However, the lower percentage of oxalate-extractable Al remaining in the residual soils compared with original soils suggests that P stabilization in the residual soil is not only related to interaction with oxide surfaces, but is also due to complexation with soil organic C.

Fertilizer application can play an important role in soil organic matter retention, and dynamics. As expected, the addition of inorganic fertilization significantly increased total P and plant available P in all three soils. For the Pemehue and Piedras Negras sites, fertilization significantly increased soil total C, N and organic P. The greater organic matter concentrations following P fertilization might be explained by greater inputs of roots and microbial biomass. Surprising, in the Puerto Fonck site, fertilized plot leads to a significant decrease of total C compared with the non-fertilized one while no significant difference on total N and organic P was observed. This contrasting effect observed for three soils could be due to different agronomic managements. In the Puerto Fonck site, it might not have above-ground crop residues returning into soil.

The residual soil was found to contain higher concentrations of total C, total P and organic P than the original soils, with an average 41% of C and 31% of P not extracted by sequential fractionation. These results indicate that organic matter in Andisols is stable against chemical attack. This is consistent with previous studies on Andisols, which determined

that P could be associated with stable organic macromolecules (Borie et al., 1989; Borie and Zunino, 1983). According to Calabi-Floody et al. (2011), the aggregates of allophane clay extracted from Chilean Volcanic soil retain a significant amount of soil organic matter (around 12%) against intensive peroxide treatment, leading to soil C sequestration and consequent soil stabilization. Nanoclay in Andisols sequesters around five times more C than in Cambisols (Calabi-Floody et al., 2015).

Most of P in the residual fraction was in inorganic forms as orthophosphate and pyrophosphate. The high proportions of inorganic P found in the residual soil fraction may be explained by stabilization as inner sphere complexes, which are known to occur in Andisols (Briceño et al., 2004; Mora and Canales, 1995a; Borie and Zunino, 1983). In addition, inorganic P can be associated strongly with oxyhydroxides in sediments and soils (Kopacek et al., 2005).

Organic P accounted from 21 to 42% of total residual P, which is consistent with proportions reported for bulk soil (Briceño et al., 2004; Borie and Zunino, 1983). Almost all the organic P determined in the residual soils occurred as stereoisomers of inositol hexakisphosphate, with no phosphodiester or phosphonates detected. This could be explained by the hydrolysis of labile organic P during several extractions. Inositol phosphates are considered to be the most stable form of soil organic P, which are synthesized by plants and entered to soil through direct deposition of plant material. Therefore, the predominance of inositol phosphates and absence of phosphodiester indicate the highly recalcitrant nature of residual organic P in Andisols. The residual fraction contained all four stereoisomers of inositol hexakisphosphate known to occur in soils (Turner, 2007; Turner et al., 2012). The persistence of the *D-chiro*, *neo* and *scyllo* stereoisomers might reflect their greater resistant to phytase hydrolysis compared with the *myo* stereoisomer (Crosgrave, 1972).

5. Conclusion

This work highlights that a significant proportion of the total P in Andisols is not extracted by sequential extraction using the Hedley fractionation procedure. These results provide the first direct determination of the chemical nature of residual P in soils. Between 21 and 42% of residual P was organic P in the form of inositol hexakisphosphate, which might have been stabilized in organic macromolecules. Further analyses of different soil types and different climate conditions are now needed to determine whether these results apply more broadly. While the relative solubility and chemical nature of organic P in Andisols suggest that it is recalcitrant, the actual bioavailability of the residual organic P remains unknown and needs to be assessed.

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