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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 ^{31}P 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 phosphodiesters 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.

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 ^{31}P 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 Araucania and Los Rios 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 (P_2O_5) 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 ($NaHCO_3$), 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 ^{31}P NMR spectroscopy (see below).

2.3. Solution ^{31}P 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 ^{31}P 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		
								Al	Fe	P
					Total P	Organic P				
		(g kg^{-1})		(mg 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