RESEARCH ARTICLE

Assessment of phosphorus status influenced by Al and Fe compounds in volcanic grassland soils

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Abstract

Volcanic ash derived soils represent between 50-60% of the total arable land area of southern of Chile, and they are the most important soils for pasture production. In these soils, high phosphorus (P) fixation and, in turn, low P availability and high aluminium (Al) soluble concentrations (at low pH) are the most limiting factors for pasture production. At the same time, the complexes between Al-or iron- (Fe) and organic matter as well as short-range order alumino-silicates (allophane) allow the retention of huge quantities of soil P. The aim of this work was to assess the status of P by both sequential extraction procedure (Hedley) and ^{31}P-NMR analysis as influenced by Al and Fe in volcanic grasslands Andisols (Pemehue, Gorbea, Piedras Negras and Llastuco Soil Series) from Southern Chile. We applied Hedley chemical sequential fractionation to soils in order to examine the potential differences in extractable soil inorganic P (P_i) and organic P (P_o) fractions. We also determined total P and Olsen P in these grassland Andisols. Oxalate and pyrophosphate were employed to determine the active and organic matter complexed Al and Fe, respectively. Furthermore, we quantified Al and Fe in extracts of the Hedley P fractions. We found that Al extracted in oxalate was correlated positively with labile P_o concentration, specifically with both the NaHCO$_3$–P_o (r=0.45, $P \leq 0.01$), and the NaOH–P_o (r=0.43, $P \leq 0.01$) fractions. This observation was reinforced by ^{31}P-NMR analysis that showed higher monoester P and *myo*-IP$_6$ content in soils with higher amounts of oxalate Al. Hedley sequential fractionation procedure confirmed the role of Al in the NaOH-P_o fraction for promoting P_o storage, as both fractions were correlated (r=0.33, $P \leq 0.05$). In addition, Fe plays a substantial role in recalcitrant P accumulation as we found a high correlation between residual P and oxalate Fe (r=0.55, $P \leq 0.01$).

Keywords: Aluminum, phosphorus, availability, iron, andisol

1. Introduction

Acid soils comprise about 30% of the world's arable land, and Al toxicity and low P availability are major constraints for crop production on these soils (Lynch, 2007). This is particular the case for Andisols and Ultisols that comprise 5.4 million hectares of soils (Soil Survey Staff, 1996), and represent between 50 and 60% of Chile total arable land area. About 50% of the Chilean Andisols present a high soil acidity level, limiting agricultural production (Mora *et al*., 1999).

In volcanic soils, the formation of complexes between Al- or Fe- and soil organic matter (SOM) as well as the short-range order alumino-silicates (allophane and imogolite) allow retention of huge quantities of soil organic P (P_o). This P_o is mainly present as inositol penta-and hexaphosphates that comprises between 30-82% of total P (P_T) in topsoils (Borie and Barea, 1985; Redel *et al*., 2007; 2008; 2011; 2015). Specifically, P retention is due to the adsorption by minerals like allophane, imogolite and Fe- or Al- oxides associated with humic substances and Al present in the interlayers of expandable phyllosilicates (Gunjigake and Wada, 1980; Vistoso *et al*., 2012). Phosphorus sorption to these minerals can protect it against microbial and enzymatic decomposition (Stutter *et al*., 2015). This protected P can represent more than 90% of any added P (Borie and Zunino, 1983), leaving little P available for plants in spite of high P_T and P_o contents in volcanic soils (Borie and Rubio 2003).

In the case of Chilean Andisols, dairy and meat production in Southern Chile is based on the use of highly productive forage species, especially ryegrass (*Lolium perenne*) alone or mixed with clover (*Trifolium repens*) Demanet *et al*. (2015). The productivity of these grass species has been studied showing the negative effects of high Al saturation on P availability in soils (Mora *et al*., 2002), thus reducing pasture production and quality (Mora *et al*., 2006).

Chemical sequential extraction procedures are widely used for determining extractable soil P into inorganic P (P_i) and organic P (P_o) fractions differing in extractability (Hedley *et al*., 1982; Cross and Schlesinger, 1995; Condronand Newman, 2011). Hedley's procedure has conventionally been used for studying soil P fractions involving the use of chemical reactants like $NaHCO_3$ and NaOH, assuming that the sequential extracted fractions follow a decreasing gradient of plant availability (Hedley *et al*., 1982). In this procedure the inorganic pool extracted with 0.5 M sodium bicarbonate is attributed mainly to labile P_i in the solid soil phase linked by P-ester bounds to Al and Fe surfaces (Cross and Schlesinger, 1995); moderately labile P_i extracted with 0.1M NaOH comprises chemisorbed P_i of lower plant availability associated with amorphous and crystalline Fe and Al oxide/hydroxides (Hedley *et al*., 1982); non labile primary Ca-bound extractable P is identified through 0.5 M HCl or H_2SO_4 (Cross and Schlesinger, 1995). The P_o fractions are determined by difference between P in digested and P_i fractions, although there exist some controversy about the digestion methodology as described by Do Nascimento *et al*. (2015). Easily labile $NaHCO_3$-P_o and less labile NaOH-P_o comprises P linked to SOM, and they have greater relevance in unfertilized or low-P soils and more highly weathered soils (Cross and Schlesinger, 1995). The non-extractable recalcitrant P_i and P_o comprise residual P of very low availability (Herlihy and McGrath, 2007).

On the other hand, it is well known that solution ^{31}P-NMR is useful for the identification of specific P forms. In this sense, this method has allowed to establish that temperate soils with intensive management of grasslands have similar proportion of inorganic orthophosphate and monoester P concentrations (Stutter *et al*., 2015), and the long-term addition of organic

fertilizers has little effect on soil P_o (Annaheim *et al.*, 2015). Furthermore, in Chilean volcanic soils, it is reported a greater proportion of monoester P under a lupin-wheat cropping system compared to oat-wheat (Redel *et al.*, 2011), and Briceño *et al.* (2004) reported a wide range of monoester P and orthophosphate concentrations for diverse natural Chilean soil Series.

For temperate grasslands (including Gleysol and other soil types), Herlihy and McGrath (2007) found that P sorption in soil was mainly correlated with oxalate Al, clay content, SOM and oxalate Fe. In volcanic soils Broquen *et al.* (2005) and Satti *et al.* (2007) investigated acidification processes in a forest Andisol climatic sequence; they found that in drier climates a less strong Al and P retention occurred. In Chilean Andisols, there are several reports on P fractionation; some of them include[31]P-NMR analysis (Borie and Zunino, 1983; Borie *et al.*, 2002; Redel *et al.*, 2007; 2008; 2011; 2015; Velásquez *et al.*, 2016a, b) and pyrophosphate/ oxalate Al and Fe determinations (Mora and Canales, 1995; Matus *et al.*, 2006; Panichini *et al.*, 2012). However, simultaneous determinations of these parameters in the same extract are still lacking. This is important in order to know the precise amounts of Al and Fe that are co-extracted by each Hedley´s fraction in the soil. Therefore, the aim of this work was to assess the status of P by both sequential extraction procedure (Hedley) and ^{31}P-NMR analysis as influenced by Al and Fe in volcanic grasslands Andisols.

2. Material and Methods

Andisol samples (0-20 cm) were collected at winter from eight different grassland sites, belonging to four different Soil Series (Figure 1). For each soil Series we collected samples from two different sites, located in a ratio within 5 to 15 km, with similar management characteristics. All sites were ryegrass (*Lolium perenne*) pastures, mixed with other *Poaceae* species,

less than 10% of legumes (principally *Trifolium repens*) and weeds (principally *Hypochaeris radicata*). Climate is temperate humid (12 °C-14 °C average temperature, 1,500-2,500 mm average annual precipitation) as described by Mella and Kühne (2005). All sites presented comparable topography (< 1-3% steep) and good drainage, being exclusively dairy cattle grazed or left for grass cut and fertilized. The soils Series were: a) Gorbea (GB): Typic Hapludands with silt loam texture, located near Gorbea at the Central Valley (site 1 at 39º06'12"S, 72º37'42"W and 106 meters about the sea level (m.a.s.l.); site 2 at 39º05'30"S, 72º34'20"W and 110 m.a.s.l.); b) Piedras Negras (PN): Acrudoxic Hydric Melanudands with silt loam texture, located near Río Bueno at the Andes foothill, (site 1 at 40º23'47"S, 72º32'35"W and 331 m.a.s.l.; site 2 at 40º21'06"S, 72º31'16"W and 341 m.a.s.l.); c) Pemehue (PH): Eutric Pachic Fulvudands with silt loam texture, located near Los Laureles at the Andes foothill, (site 1 at 39º00'49"S, 72º10'23"W and 324 m.a.s.l.; site 2 at 39º02'58"S, 72º08'58"W and 333 m.a.s.l.); and d) Llastuco (LL): Acrudoxic Hapludands with silt loam texture, located near Máfil at the Central Valley, (site 1 at 39º41'32"S, 72º56'45"W and 34 m.a.s.l.; site 2 at 39º36'30"S, 73º00'57"W and 38 m.a.s.l.) (Mella and Kühne, 1985).

Pemehue and Gorbea pastures were established between 3 and 5 years before sampling, after cereal crop production. These pastures were used under non-intensive grazing system and for silage production. Piedras Negras pastures were established 15 years ago after forest; they were used under infrequently but intensively grazing management. Llastuco pastures have around 10 years-old; they were intensively grazed and have the highest dung input due to night confined cattle. The soils of all sites were limed every 2 years with 1 or 2 Mg ha^{-1} and fertilized with P at doses ranging from 50 to 100 kg ha^{-1} applied as triple superphosphate. Nitrogen was supplied as urea (100

kg ha^{-1} year^{-1}). In each site, sampling was performed crossing the plots (3-5 hectares) by diagonal way, and five samples composed of four subsamples were taken per plot. Soils were air dried, sieved (< 2 mm) and stored until analysis. Soil chemical characterization is shown in Table 1.Soil bulk density of the sites was similar (around 1 Mg m^{-3}); therefore, soils were compared on a dry weight basis.
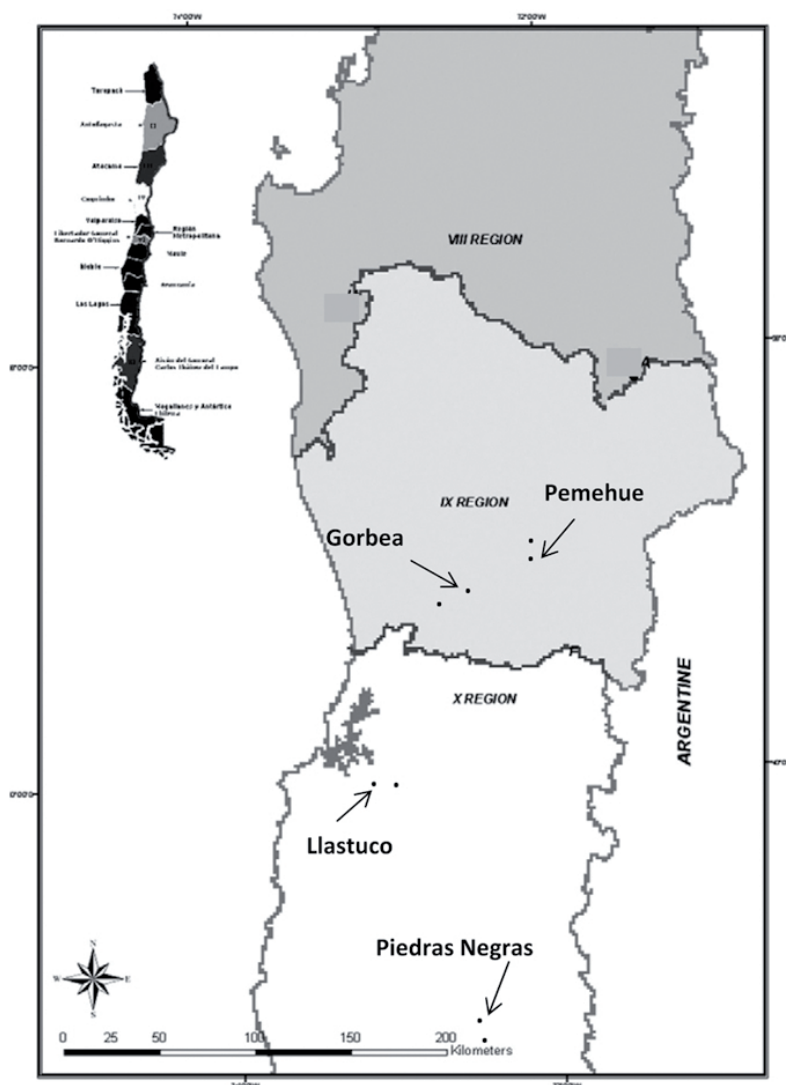

Figure 1. Localization of the sampling sites in volcanic grasslands Andisols belonging to four soil Series of southern Chile.

Table 1. Chemical characterization in volcanic grasslands Andisols belonging to four soil Series in southern Chile. Different letters for soil Series denote significant differences according to Duncan tests ($P \leq 0.05$).

	Gorbea	Piedras Negras	Pemehue	Llastuco
Chilean region	IX	X	IX	XIV
pH (1:2.5 H_2O)	5.71 (5.82-5.51)[a]	5.42 (5.96-5.28)[b]	5.62 (5.70-5.50)[a]	5.45 (5.70-4.77)[b]
Total C/ g kg^{-1}	136 (179-96)[a]	140 (172-108)[a]	126 (140-102)[a]	104 (122-90)[b]
Total P / mg kg^{-1}	1609 (1831-1254)[d]	2166 (2875-1623)[c]	2498 (3395-1799)[b]	3036 (3388-2807)[a]
Olsen P / mg kg^{-1}	12 (20-4)[c]	20 (25-12)[b]	20 (30-16)[b]	45 (60-27)[a]
Organic P / mg kg^{-1}	848 (1160-458)[b]	1065 (1210-945)[a]	1015 (1212-748)[a]	1006 (1560-623)[a]
Inorganic P/ mg kg^{-1}	930 (1225-788)[c]	802 (1315-495)[c]	1198 (1642-839)[b]	1823 (2275-1520)[a]
Microbial P/ mg kg^{-1}	28 (40-17)[b]	46 (65-25)[b]	29 (63-13)[b]	159 (221-99)[a]
Total C:organic P (NMR)[(1)]	444 (584-312)[a]	379 (464-291)[b]	284 (316-229)[c]	295 (346-255)[c]

[(1)] Organic P was calculated as the sum of monoester P and diester P from ^{31}P-NMR spectra.

2.1. Soil general characterization, P parameters and fractions

Total P (P_T) in soil samples was extracted with NaOBr (Dick and Tabatabai, 1977) and determined by the method of Murphy and Riley (1962). Total C (C_T) was determined with elemental analyzer (CHN NA1500; Carlo Erba Elemental Analyzer, Stanford, CA, USA). Organic P (P_o) was determined by the calcination method (Saunders and Williams, 1955). Olsen-P was determined by soil extraction with 0.5 M NaHCO$_3$ adjusted to pH 8.5, according to the Olsen and Sommers (1982) method. Inorganic P (P_i) was determined as the difference between P_T and P_o. Soil pH was determined in H_2O in 1:2.5 soil: water solution relation. Microbial biomass P (microbial P) was estimated by chloroform fumigation extraction with ethanol-free CHCl$_3$ (Brookes *et al.*, 1982), and P was analyzed in aliquots of the extracts by the ammonium molybdate–ascorbic acid method described by Murphy and Riley (1962). Microbial P was calculated as: microbial P =$EP/(k_{EP}*recovery)$, where *EP* is the difference between NaHCO$_3$-P_i extracted from fumigated and non-fumigated soil, and *recovery* is the recovering factor obtained for correcting P_i fixation and k_{EP} is 0.4 (Khan and Joergensen, 2012). Phosphorus

fractions (NaHCO$_3$-P_i, NaHCO$_3$-P_o, NaOH-P_i, NaOH-P_o, H_2SO_4-P_i) in soil were determined according to a modified Hedley's fractionation procedure (Hedley et al., 1982). Briefly, 0.5 g soil was extracted with 0.5 M NaHCO$_3$ pH 8.5 during 16 h followed by extraction with 0.1 M NaOH and further with 0.5 M H_2SO_4. The extracts were centrifuged and filtered. In all extracts P_i was measured spectrophotometrically by the method of Murphy and Riley (1962) at 820 nm and pH 5.0, whereas P_o was calculated as the difference between P_T determined for each fraction by alkaline oxidation with NaOBr (Dick and Tabatabai, 1977) and the P_i determined in the extracts. Thus, this fractionation procedure yielded five different analytical fractions: NaHCO$_3$-P_i, NaHCO$_3$-P_o, NaOH-P_i, NaOH-P_o, and H_2SO_4-P_i. Residual P was calculated as the difference between P_T and P fractions.

2.2. Aluminum and Fe in soil

Pyrophosphate Al and Fe (Al_{py} and Fe_{py}) were extracted by 0.1 M sodium pyrophosphate diphosphate (van Reeuwijk, 2002). Oxalate Al and Fe (Al_{ox} and Fe_{ox}) were determined by extraction with 0.2 M ammonium oxalate pH 3.0 (van Reeuwijk, 2002). Al and Fe associated with P fractions were determined

in $NaHCO_3$, NaOH and H_2SO_4 extracts obtained by the P fractionation procedure. Thus, Al and Fe associated to the different P_o fractions were calculated as the difference between the total and inorganic pools as described for P.

2.3. ^{31}P-NMR analysis

^{31}P-NMR analysis was performed for a mixed sample with soil of both sites for each one of the four soil Series. The samples were extracted with a solution containing 0.25 M NaOH and 50 mM disodium EDTA (ethylenediaminetetra acetate) for 16 h in a 1:20 solid: solution ratio, at 22 °C, and centrifuged at 8,000 g for 30 min (Cade-Menun and Preston, 1996). Approximately 20 mL aliquot of soil extract was spiked with 1 mL of methylene diphosphoric acid (MDPA) as an internal standard. The extracts were lyophilized and ground to a fine powder. Freeze dried extracts were solubilized with 3.0 mL of D_2O, shaked for 2 h, centrifuged and transferred to 5 mm NMR tubes. Spectra were acquired on a Bruker Avance 500 spectrometer using a 30° pulse delay and 0.58 s acquisition time. The number of scans varied from ~1,000 to ~30,000 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%). Peak areas were determined by integration over predetermined spectral regions using Mestre-C software processed with Lorentzian line shape of 5 Hz. Signals were assigned to P compounds spiked in NaOH-EDTA soils extracts (Turner *et al*., 2003; Turner and Richardson, 2004). Signals areas were calculated by integration, whereas P compounds concentration was calculated from integral value of MDPA internal standard of 66.7 mg P kg^{-1}, with a signal at 17.4 ppm. Spectra assignment were according to Turner *et al.* (2003): pyrophosphate at -4.3 to- 4.6 ppm, with a peak at -4.4 ppm;

P associated to DNA (diester) at -0.4 to 0.1 ppm with a peak at 0.11 ppm, inorganic orthophosphate at 5.87 to 6.25 ppm with a peak at 6.15 ppm, *scyllo*-inositol phosphates (*scyllo*-IP_6) (monoester P) at 4.00 to 4.35 ppm with a peak at 4.22 ppm, mononucleotides of RNA (monoester) at 4.59 to 5.00 ppm with a peak at 4.70 and 4.90 ppm, *myo*-inositol phosphates (*myo*-IP_6) and *β*-glycerolphosphates (monoester P) at 5.00 to 5.8 ppm with a peak at 5.24 and 5.05 ppm; specific peaks in soils were also identified and compared with Annaheim *et al*., (2015) and Stutter *et al.* (2015).

2.4. Statistics

Data were analyzed using analysis of variance (ANOVA) followed by the Duncan test to identify significant differences among treatments. Normality and homogeneity of variance were tested before analysis. Statistical differences between means were tested at 95% significance level. Correlations between selected parameters were carried out using Pearson`s correlation coefficient ($P \leq 0.05$ or $P \leq 0.01$). Principal component analysis (PCA) was performed over selected parameters; varimax rotation was applied to achieve the maximum variance and the two principal components (PC's) with the highest eigenvalues were selected for the plot performing.

3. Results

Table 1 shows the chemical characterization of the studied soils. Gorbea and Pemehue soils had higher pH values than the other soils. Llastuco soil had the lowest C_T content, but this soil exhibited the highest total P (P_T), Olsen P, microbial P and inorganic P (P_i) contents .Organic P (P_o) represented on average 42% of P_T in all soils and it was the lowest at Gorbea soil. Total C to organic P relation was the highest in Gorbea soil.

Piedras Negras showed the greatest Al_{py} and Fe_{py} and the lowest Al_{ox} as shown in Table 2. For Gorbea, Pemehue and Llastuco soils, Al_{py} and Fe_{py} were between 75 and 85 % lower than Al_{ox} and Fe_{ox}, whereas for Piedras Negras these differences were around 60%. Interestingly, when the arithmetic difference between Al_{ox} and Al_{py} was calculated, we found that Piedras Negras soil showed the lowest value; the corresponding difference for Fe_{ox} and Fe_{py} was the highest in Llastuco soil. Estimated allophane content did not show differences among soils.

Table 2. Al and Fe extracted with pyrophosphate and oxalate and allophane content in volcanic grasslands Andisols belonging to four soil Series of southern Chile. Different letters for soil Series denote significant differences according to Duncan tests ($P \leq 0.05$). ns: not significant.

	Gorbea	Piedras Negras	Pemehue	Llastuco
Pyrophosphate Al / g kg^{-1}	12 (15-9)^b	14 (15-13)^a	12 (15-10)^b	10 (12-9)^c
Pyrophosphate Fe / g kg^{-1}	3 (4-3)^b	10 (12-8)^a	4 (5-3)^b	4 (6-4)^b
Oxalate Al / g kg^{-1}	46 (52-42)^a	32 (41-28)^c	48 (51-45)^a	41 (44-38)^b
Oxalate Fe / g kg^{-1}	18 (25-13)^b	26 (41-18)^a	23 (30-17)^a	26 (33-17)^a
Oxalate Al -pyrophosphate Al / g kg^{-1}	34 (27-40)^a	18 (14-28)^c	35 (33-39)^a	31 (26-34)^b
Oxalate Fe -pyrophosphate Fe / g kg^{-1}	14 (10-22)^c	16 (8-32)^c	19 (14-22)^b	22 (14-29)^a
Allophane$^{(1)}$ / g kg^{-1}	182 (266-56)^{ns}	145(344-70)^{ns}	160 (248-109)^{ns}	146 (206-68)^{ns}

(1) Allophane content was estimated through Parfitt and Wilson (1985) modified by Mizota and Van Reeuwijk (1989) formula: allophane = 100*oxalate Si* [23.4-5.1*((oxalate Al-pyrophosphate Al)/oxalate Si)]%,with oxalate Si average contents in soil of 15, 10, 15 and 13 g kg^{-1} for Gorbea, Piedras Negras, Pemehue and Llastuco soil, respectively

According to the sequential Hedley P fractionation, Llastuco soil presented the highest P_i contents in the $NaHCO_3$, NaOH, H_2SO_4 and residual P fractions compared to the other soils (Table 3). Piedras Negras and Llastuco presented the lowest NaOH-P_o content; Piedras Negras also exhibited a reduced accumulation of $NaHCO_3$-P_o compared to the other soils. In terms of the labile P_o/P_i relation, Llastuco exhibited the lowest value. Furthermore, Gorbea soil had the greatest labile P_o (i.e. NaOH-P_o and $NaHCO_3$-P_o) fractions relative to P_T, and Piedras Negras the lowest P_i fractions compared to the other soils (Figure 2). Piedras Negras also showed the highest residual P proportion.

Table 4 shows the contents of Al and Fe in the P extracts obtained by Hedley's fractionation procedure. In general, extractable Al in P_i fractions ($NaHCO_3$ and NaOH) was the highest at Gorbea soil, whereas Al in P_o fractions ($NaHCO_3$ and NaOH) was the greatest at Pemehue soil. Extracted Al in P fractions was highest for Gorbea and lowest for Piedras Negras soil. Iron concentration in the different P fractions did not show a clear trend among soils. Whereas no Fe was detected in the NaOH-P_o extract, almost all Fe was associated to the H_2SO_4-P_i fraction irrespective to the soil Series. Llastuco presented the broadest orthophosphate-P_i as demonstrated by its^{31}P-NMR spectra (Figure 3) and the respective peak integration (Table 5). Pemehue presented the highest total monoester P (Table 5), and Gorbea showed the greatest *ß*-glycerophosphate peak (Figure 3). In addition, Piedras Negras presented the highest *scyllo*-IP_6 signals. In general, Llastuco accumulated the lowest proportion of organic P forms (monoester and diester) and pyrophosphate P as compared to the other soils (Figure 3). Recovery rates

from ^{31}P-NMR in relation to total P (extracted with NaOBr) were ranged between 37-49% (Table 5).

Correlations were performed in order to determine the different relationships between selected parameters (Table 6). Pyrophosphate extractable Al presented negative correlations with all P_i fractions, whereas Al_{ox} as well as the difference between Al_{ox} and $Al_{py}(Al_{ox}-Al_{py})$ presented positive correlations with P_o fractions. Pyrophosphate extractable Fe presented negative correlations with all P_o fractions, whereas residual P was correlated positively with Fe_{ox} and $Fe_{ox}-Fe_{py}$ (Table 6). We also found that C_T correlated negatively with residual P, $Fe_{ox}-Fe_{py}$ and with almost all P_i fractions, but positively with Al_{py} and Fe_{py}. Allophane was positively correlated with NaOH-P_o.

Figure 4 show the principal component (PC) analysis of the selected parameters. Briefly, Al_{py} and Fe_{py} were positively related with PC1, whereas labile P_i, no labile P and Fe_{ox} were positively related with PC2. Both, labile P_o and Al_{ox} were negative lyrelated with PC1 and PC2.
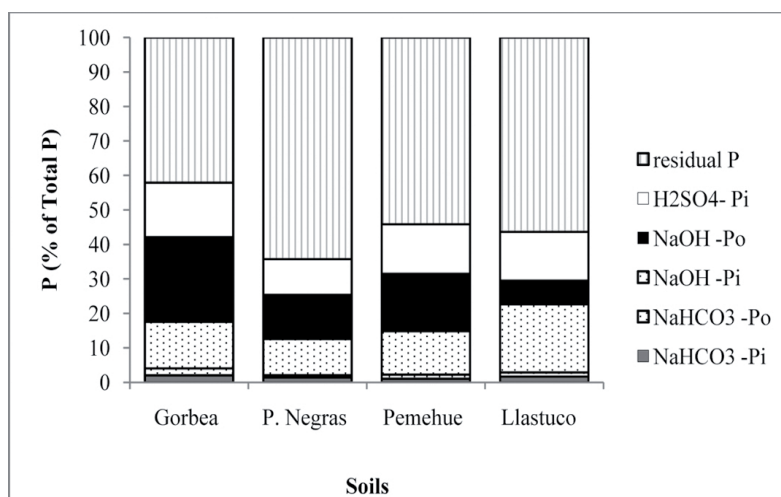

Figure 2. P concentration in Hedley extracts of volcanic grassland Andisols belonging to four soil Series of southern Chile
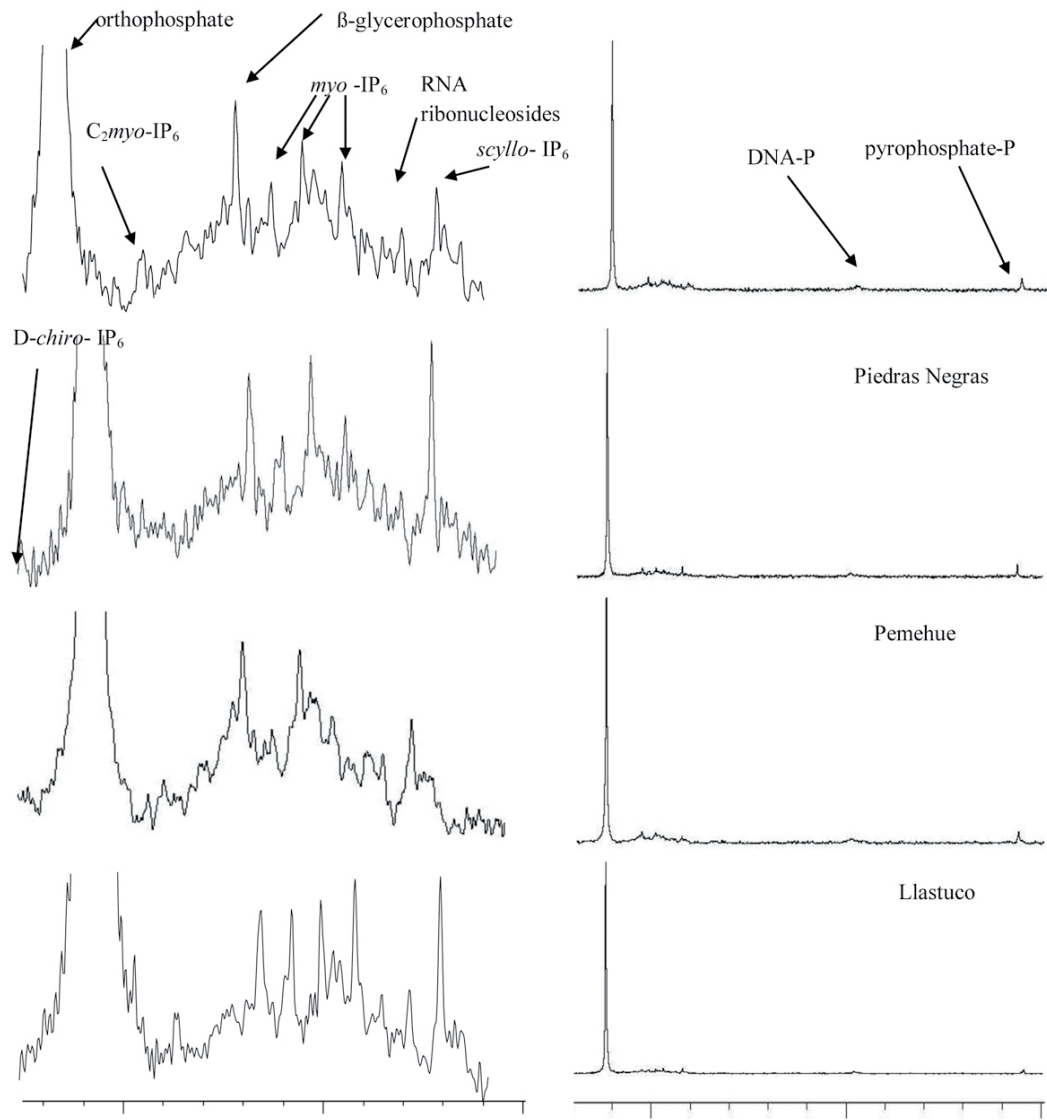
Figure 3. ^{31}P NMR spectra of the NaOH- EDTA extracts determined in volcanic grassland Andisol belonging to four soil Series of southern Chile
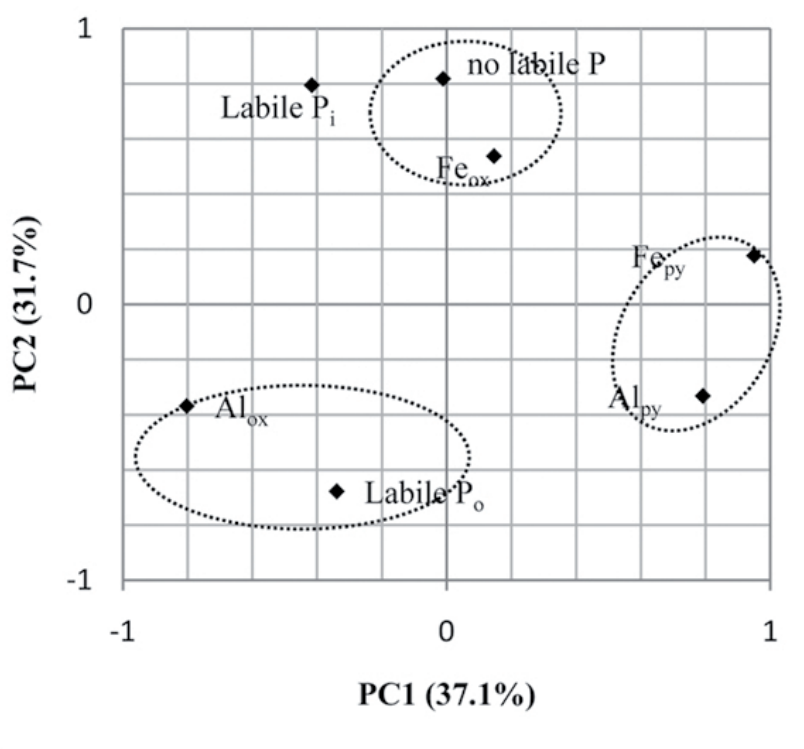
Figure 4. Principal component analysis determined in volcanic grassland Andisol of southern Chile. PC1, principal component 1, PC2, principal component 2. Labile-P_i: $NaHCO_3$-P_i+ NaOH-P_i, labile-P_o : $NaHCO_3$-P_o+ NaOH-P_o, no labile P: H_2SO_4-P + residual P

Table 3. Sequential Hedley P fractionation extracts in volcanic grasslands Andisols belonging to four soil Series of southern Chile. Different letters for soil Series denote significant differences according to Duncan tests ($P \leq 0.05$).

Extracts	Soil Series			
(mg kg^{-1})	Gorbea	Piedras Negras	Pemehue	Llastuco
$NaHCO_3$–P_i	33 (52-10)[b]	31 (59-10)[b]	27 (53-15)[b]	52 (77-29)[a]
$NaHCO_3$–P_o	32 (50-20)[a]	14 (23-7)[b]	30 (54-15)[a]	36 (61-13)[a]
NaOH–P_i	217 (402-112)[c]	227 (407-60)[c]	314 (552-164)[b]	599 (697-518)[a]
NaOH–P_o	395 (651-147)[a]	277 (523-78)[b]	415 (608-157)[a]	208 (309-143)[b]
H_2SO_4–P_i	254 (468-151)[bc]	224 (527-63)[c]	359 (571-246)[ab]	431 (643-233)[a]
Residual P[(1)]	677 (954-289)[c]	1392 (2075-554)[b]	1352 (2102-570)[b]	1710 (2205-1434)[a]
Labile P_o/P_i[(2)]	1.8 (3.3-0.8)[a]	1.7 (4.9-0.2)[a]	1.4 (2.4-0.5)[a]	0.4 (0.5-0.2)[b]

[(1)] Calculated as the difference between PT and P fractions.   [(2)]Determined as Labile Po/Pi= ($NaHCO_3$-Po + NaOH-Po)/ ($NaHCO_3$-Pi + NaOH-Pi)

(Table 4. Al and Fe in Hedley P fractionation extracts in volcanic grasslands Andisols belonging to four soil Series of southern Chile. Different letters for soil Series denote significant differences according to Duncan tests ($P \leq 0.05$). ns: not significant.

P-Extracts		Soil Series			
		Gorbea	Piedras Negras	Pemehue	Llastuco
Al in P extracts (g kg^{-1})	NaHCO$_3$–P$_i$	0.12 (0.31-0.02)^a	0.05 (0.07-0.03)^b	0.02 (0.04-0.01)^c	0.03 (0.04-0.01)^c
	NaHCO$_3$–P$_o$	0.16 (0.34-0.08)^b	0.32 (0.64-0.19)^a	0.36 (0.68-0.17)^a	0.13 (0.18-0.09)^b
	NaOH–P$_i$	16 (24-6)^a	12 (18-3)^b	8 (14-4)^c	12 (14-10)^b
	NaOH–P$_o$	12 (18-7)^b	9 (21-4)^{bc}	16 (24-11)^a	10 (14-4)^{bc}
	H$_2$SO$_4$–P$_i$	17 (25-12)^a	8 (14-5)^b	15 (21-8)^a	17 (22-14)^a
	Total	46 (66-36)^a	30 (35-26)^c	40 (47-36)^b	39 (48-31)^b
Fe in P extracts (g kg^{-1})	NaHCO$_3$–P$_i$	0.033 (0.045-0.023)^{ns}	0.038 (0.072-0.020)^{ns}	0.035 (0.053-0.019)^{ns}	0.035 (0.041-0.025)^{ns}
	NaHCO$_3$–P$_o$	0.144 (0.282-0.082)^{ab}	0.125 (0.171-0.088)^b	0.167 (0.257-0.074)^a	0.119 (0.170-0.071)^b
	NaOH–P$_i$	0.337 (0.536-0.235)^{ab}	0.437 (0.834-0.107)^a	0.231 (0.698-0.040)^b	0.288 (0.595-0.040)^{ab}
	H$_2$SO$_4$–P$_i$	17 (22-13)^a	11 (15-10)^b	13 (19-10)^b	16 (21-11)^a
	Total	18 (22-13)^a	12 (15-11)^b	14 (19-10)^b	16 (21-12)^a

Table 5. ^{31}P-NMR different species concentration determined by peak integration and standard addition (MDPA) in volcanic grassland Andisol belonging to four soil Series of southern Chile

P form (mg kg^{-1})	Soil Series			
	Gorbea	Piedras Negras	Pemehue	Llastuco
Pyrophosphate [1]	51.56	74.57	80.40	54.96
Orthophosphate P$_i$ [2]	214.83	299.53	630.26	967.17
Diesters (DNA-P) [3]	38.63	61.82	102.46	86.42
Monoesters				
Scyllo [4]	34.58	40.59	25.36	29.25
RNA [5]	106.89	132.31	152.17	104.92
Myo [6]	125.75	133.93	164.85	132.90
Total	267.22	308.46	342.38	267.07
Sum of ^{31}P-NMR species [7]	638.95	809.45	1222.16	1442.32
Recovery of total P (%) [8]	40	37	49	47
^{31}P-NMR P$_o$/P$_i$ [9]	1.2	1.0	0.5	0.3
Monoesters/ Sum of ^{31}P-NMR species	0.41	0.38	0.28	0.19

[1] Pyrophosphate as detected between δ= -4.3 and -4.6 ppm. [2] Inorganic orthophosphate as detected between δ= 5.87 and 6.25 ppm

[3] DNA-P as detected between δ= -0.40 and -0.10 ppm

[4] *scyllo*-IP$_6$ as detected between δ= 4.00 and 4.35 ppm, include also ribonucleotides

[5] RNA-P as detected between δ= 4.59 and 5.00 ppm

[6] *myo*-IP$_6$ as detected between δ= 5.00 and 5.87 ppm, include also glycerophosphates

[7] Content of all P$_i$ and P$_o$ species as determined by standard addition (MDPA)

[8] Recovery calculated as Total P (^{31}P-NMR)/ Total P (Table 1)

[9] Determined as ^{31}P-NMR P$_o$/P$_i$ = (monoester+DNA-P) / (orthophosphate+pyrophosphate)

Table 6. Pearson correlations coefficients of P fractions in Hedley's extracts with Al or Fe (extracted with pyrophosphate and oxalate) and C in volcanic grassland Andisol of southern Chile.

		$NaHCO_3–P_i$	$NaHCO_3–P_o$	$NaOH–P_i$	$NaOH–P_o$	$H_2SO_4–P_i$	Residual P	Total C
Al	Pyrophosphate	**-0.44****	**-0.33**	**-0.56****	-0.01	**-0.32***	-0.05	**0.45****
	Oxalate	-0.07	**0.45****	-0.03	**0.43****	0.18	-0.25	-0.14
	Oxalate-pyrophosphate	0.04	0.49**	0.12	0.40**	0.23	-0.20	-0.27
Fe	Pyrophosphate	-0.16	**-0.65****	-0.24	**-0.38***	-0.20	0.15	**0.35***
	Oxalate	0.22	-0.13	0.17	-0.06	0.07	**0.55****	-0.30
	Oxalate-pyrophosphate	0.30	0.18	0.28	0.14	0.15	0.47**	-0.45**
	Allophane	-0.05	0.25	-0.15	**0.35***	-0.24	-0.23	-0.20
C	Total C	-0.27	**-0.49****	**-0.44****	-0.13	-0.09	**-0.35***	1.00

P_T: Total P; P_i: inorganic P; P_o: organic P. *,** and bold numbers denotes significant correlations ($P \leq 0.05$ and $P \leq 0.01$, respectively)

4. Discussion

Soil P status in grassland soil is one of the main concern for meat and milk production because the high P retention in volcanic soils of southern Chile limits the dry matter yield. The high P retention as P_i is mainly a consequence of adsorption processes on the clay fraction (i.e. allophane, Al and Fe oxides). Nowadays, we have focused our attention on soil P_o because in Andisols between 40 and 60 % of the total P is allocated inorganic forms, mainly in the recalcitrant fraction (Velásquez *et al*., 2016 a,b). The knowledge of the P input fate ingrassland soils is very important to design fertilization management strategies. The annual dry matter production in permanent pastures like those studied here range from 9.80 Mg ha^{-1} year^{-1} to 17 Mg ha^{-1} year^{-1}, with an average of 13.5 Mg ha^{-1} year^{-1} (Demanet *et al*., 2015). Phosphorus fertilization has been similar in our experimental sites (50 to 100 kgha^{-1} year^{-1}), but comparatively Llastuco showed the higher contents of Olsen P and microbial P (Table 1) because the high dung input as a consequence of night confined cattle. We have also determined that Olsen P is increased as pH is raised in the rhizosphere of Andisols (Paredes *et al*., 2011). In our study, we found that P behavior was strongly regulated by Al; thus, Al-clay fraction (oxides/hydroxides or allophane) was highly correlated with labile P fractions ($NaHCO_3$ and NaOH) as shown in Table 6. The high amounts of Al_{ox} and amorphous compounds (Al_{ox}-Al_{py}) indicate a high P fixation capacity in the clay fraction of soils (Reddy *et al*., 1996). In this sense, our results suggest that active Al_{ox} is the main factor that governs P_o storage in volcanic Andisols since Al is regulating $NaHCO_3$-P_o and NaOH-P_o accumulation (Table 6). Thus, P mobilization is affected by sorption sites of Al and Fe (Giesler *et al*., 2004) that are protecting P against microbial and enzymatic decomposition as showed by Stutter *et al*. (2015). In addition, the soil with the highest Al_{ox} and amorphous Al contents (Pemehue) presented the highest monoester P, *myo*-IP_6 and NaOH-P_o contents. Thus, Al_{ox} might promote monoester P retention in soil (Murphy *et al*., 2009). Furthermore, allophane plays a fundamental role in P_o accumulation, as we found that NaOH-P_o and allophane were positively correlated (r=0.35, $P \leq 0.01$). In general, the highest content of Al in P_i fractions together with low microbial P in Gorbea soils (Tables 1 and 3) suggest that soil microorganisms might beinhibited by Al toxicity, thus reducing phosphatase activity (Kunito *et al*., 2016. Aluminium and Fe in SOM complexes might also inhibit microbial activity and P

mineralization as we found that Al_{py} and P_i fractions were inversely correlated (Table 6).

Interestingly iron oxides (Fe_{ox}) seem to be associated to residual P (r=0.55, $P \leq 0.01$, Table 6), which is the most recalcitrant P fraction. Therefore, both the residual P and the Fe_{ox} were closely related in the PCA plot (Figure 4). Nevertheless, Fe_{ox} did not correlate with labile P_i ($NaHCO_3$-P_i and NaOH-P_i; Table 6). The relationship between Fe_{ox} and residual P has been previously reported by Jiang *et al*. (2015); they found that the majority of P in the residual fraction was occluded within Fe oxides, which implies that this P may be released and become available for plants and microbial communities following the dissolution of Fe oxides in soil. On other hand, Fe oxides/hydroxides and Fe- substituted allophane may contribute to the formation of highly resistant organo-mineral associations, contributing to high resistance of SOM against oxidation in allophanic Andisols, as showed recently by Filimova *et al*. (2016). In this sense, our previous research indicates that aggregates of allophane in Piedras Negras can retain 12% of C against intensive peroxide treatment (Calabi-Floody *et al*., 2012). However, in our study allophane and C_T were not correlated (Table 6); possible explanations for this finding require further studies.Otherwise, Piedras Negras showed the highest residual P relative to P_T, which can be attributed to its high Fe_{ox} content and the positive correlation between both parameters as discussed earlier. Recently, Velásquez *et al*. (2016a) showed that Chilean Andisols have a high content of residual P, which is formed by monoester P, including *myo*- and *scyllo* -IP_6, as we also found in our experiment (Figure 3).

In this study, all soils presented C_T:P_o ratio over or near to 300 (Table 1); such values are considered as indicators for net P immobilization according to Dalal (1977) and Stutter *et al*. (2015). The C_T:P_o ratio was higher in Gorbea soil, which presented both the highest labile P_o relative to P_T in the sequential fractionation (Table 3)

and the greatest P_o species (monoester and diester) relative to all P species as determined by ^{31}P-NMR (Table 5). Contrastingly, Llastuco soil presented the highest microbial P, Olsen P and total P_i content (Table 1) as well as the highest amounts of NaOH-P_i and $NaHCO_3$-P_i and the lowest labile P_o/P_i relation (Table 3 and Figure 2). Furthermore, Llastuco had the highest orthophosphate ^{31}P-RMN peak (Figure 3), but the lowest monoester P and ^{31}P-NMR P_o/P_i relations (Table 5). High orthophosphate P peaks are most likely a consequence of either the P addition in cattle manure as reported by Fuentes *et al*. (2009), or the heavy P_i fertilization inputs to cropped soils previous to the establishment of these relatively new pastures, which were sown between 5 and 15 years ago. Moreover, in soils under intensive pasture management, orthophosphate P could be equivalent or even higher than P_o forms (Stutter *et al*., 2015). In terms of the contribution of monoester-P to total ^{31}P-NMR, our results were lower than those reported for other grassland and forestry Chilean soils (Redel *et al*., 2015), but within a range of natural, unfertilized soils given by Briceño *et al*. (2004).

Additionally, this experiment had the singular uniqueness that Al and Fe were determined in the P extracts obtained by sequential Hedley fractionation. Aluminium and Fe amounts extracted with oxalate were comparable those found by sequential extraction ($NaHCO_3$+NaOH+H_2SO_4, Tables 2 and 4). Briefly, Fe was almost entirely extracted in the H_2SO_4 fraction, whereas comparable proportions of Al were determined in NaOH-P_i, NaOH-P_o and H_2SO_4-P_i Hedley's extracts (Table 4). Thus, our findings suggest that Fe is mainly bound into more stabilized mineral fraction than Al, since Fe was only accessible after NaOH remotion of SOM. However, for the interpretation of these results it must considered that our fractionation scheme did not included a sonication step in the NaOH-P extraction as originally proposed by Hedley

et al. (1982), since sonication negatively affect due the weak aggregate stability of our soils. Therefore, in our work, H_2SO_4 might be extracting higher P amounts than those reported with ultrasound treatment (Baeza, 2002), due to sonication release P bound to Fe oxides/hydroxides.

The comparison among the methodologies used here allowed to establish a correspondence between Al_{ox} and Al content in the NaOH-P_o fraction (r=0.41, $P\leq0.01$) and between the Fe_{py} and Fe content in the NaOH-P_i fraction content(r=0.35, $P\leq0.05$). Furthermore, we found that Al and P extracted in the NaOH-P_o fraction were correlated (r=0.33, $P\leq0.05$), confirming the role of Al in P_o storage.

In summary, in our study P availability was influenced by the P_o retention due active Al (Al_{ox}), whereas Fe played a substantial role in residual P accumulation. These findings were also supported by the principal component analysis that showed Al_{ox} and P_o in the same group, whereas Fe_{ox} and residual P were together in the other group (Figure 4). Finally, concentration of P_T and in general P_i fractions followed the order of gradient: Gorbea < Piedras Negras < Pemehue < Llastuco soil Series, according with Al, Fe and C contents and pasture management.

5. Conclusions

Pasture growing in volcanic soils of southern Chile presented high P_o storage (848-1065 mg kg^{-1}) and Al extracted by oxalate (Al_{ox}). Phosphorus availability was mainly regulated by the formation of amorphous Al-P_o complexes, being Al_{ox} the main factor that governs P_o storage. Furthermore, Al_{ox} was related with the Al determined in Hedley's NaOH-P_o fraction and with the monoester P and *myo*-IP$_6$ content as determined by ^{31}P-NMR. Otherwise, Fe_{ox} plays the main role in residual P storage in the more recalcitrant fraction.

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