Abstract

The aim of this study was to compare P bioavailability in a Nothofagus rainforest Andisol (FS) and an adjacent clear-cut grassland soil (GS) in southern Chile to evaluate the effects of land use change on P chemical forms determined by chemical fractionation and $^{31}$P-NMR spectroscopy. Total phosphorus (P), Olsen P, microbial P, different soil P fractions (determined using a modified Hedley procedure), $^{31}$P-NMR spectroscopy results, acid phosphatase (P-ase) activity, pH and organic C were analyzed and compared. Forest samples were collected from the mineral soil at a depth of 2-20 cm and were compared with those collected from grassland soil at the same depth. Total P ranged from 2028 mg kg$^{-1}$ (FS) to 2157 mg kg$^{-1}$ (GL) and total organic P ranged from 829 mg kg$^{-1}$ (FS) to 1176 mg kg$^{-1}$ (GL). On the contrary, Olsen P, microbial P, labile P and P-ase activity were higher in the evergreen forest soil than in the grassland, with the predominance of the moderately labile (NaOH-P$_o$) fraction, which ranged from 668 to 720 mg kg$^{-1}$ in both soils. Phosphorus was mainly present in monoester-P form in the NMR extract in both soils (67 % on average). Other $^{31}$P-NMR signals were identified as C$_2$-myo-inositol phosphate and scyillo-inositol hexakisphosphate. The results suggest that land use change from forest to grassland will reduce P bioavailability and P-ase activity.

Keywords: Phosphorus fractions, forest soil, allophanic soil, organic P
1. Introduction

In the Cordillera de los Andes, *Nothofagus* forest ecosystems are located mainly on soils formed from deposits of volcanic ashes on the geological substratum of volcanic rocks. The native temperate forests of southern Chile are the largest forested area in South America and constitute more than half of the surface of temperate forests in the southern hemisphere (Donoso, 1993). However, most forest ecosystems have been modified from their original status by anthropogenic disturbance, including degradation practices such as poor logging, fire and clearing for agriculture and pasture.

The Hedley procedure, which has conventionally been used to study the dynamics of P forms in the soil, involves the use of chemical reactants such as NaHCO$_3$, NaOH and H$_2$SO$_4$ and assumes the sequential extracted fractions follow a decreasing gradient of plant bioavailability (Hedley et al., 1982). Following this understanding, NaHCO$_3$-extractable inorganic P (P$_i$) and organic P (P$_o$) fractions represent labile P pools, whereas P fractions extracted using 0.1 M NaOH are assumed to be moderately available (Hedley et al., 1982). H$_2$SO$_4$-extractable P mainly comprises primary Ca-bound P (Turner et al., 2005). According to Taranto et al. (2000), the principal benefit of the fractionation methods is to obtain a complete account or budget of the lability of P forms present in a soil. However, this methodology does not provide information about the chemical structure of the extracted phosphate compounds. This constraint may be overcome with the use of $^{31}$P-NMR spectroscopy. Volcanic ash-derived soils, mainly Andisols and Ultisols, are very common in the south of Chile. They are characterized by a high content of total P (PT), with (P$_o$) mainly present as inositol penta- and hexaphosphates (Borie et al., 1989; Borie and Rubio, 2003; Escudey et al., 2001). The P in these soils has been analyzed by chemical fractionation and $^{31}$P-NMR (Briceño et al., 2004; 2006; Redel et al., 2011). Borie and Zunino (1983) proposed that P fertilization enhanced the accumulation of organic P forms through the formation of iron (Fe)-organic matter-P complexes. These complexes can be formed due to the fast adsorption of P onto active allophanic surfaces and through the formation of Al bridges. We previously reported the effect of management systems on P fractions using $^{31}$P-NMR in a temperate cropped Ultisol (Redel et al., 2007, 2011), and the effects of management on evergreen and deciduous forests (Redel et al., 2008). Studying the different P chemical forms in volcanic soils and how they are affected by pristine forest or grassland inside the forest could increase our understanding of P cycling in native soils. Therefore, the objective of this study was to investigate the influence of soil use change on the P availability in a *Nothofagus* forest soil and an adjacent grassland using sequential chemical fractionation and $^{31}$P-NMR analysis. The phosphatase activity, measured as an approximation of microbial P demand, and further important soil parameters associated with P availability were also included.

2. Materials and Methods

The study was carried out at the Universidad Austral de Chile Experimental Station San Pablo de Tregua, Southern Chile, (39º30’-39º38’S, 72º02’-72º09’W), located in the Andes mountains, which lie at an altitude between 600 and 1600 m a.s.l. The climate is mountain rainy temperate, presenting a short and dry summer with a maximum average temperature of 20 ºC in February and a minimum average temperature of 5 ºC in August, even with snow events (Castillo et al., 2006). The mean annual temperature is 11 ºC, and annual rainfall ranges from 4,100 to 5,000 mm.
The land has a complex mountainous topography. The Andisol (Acrudoxic Hapludands, Liquiñe soil series) derived from recent volcanic ash is acidic (pH 4.7-5.4), with good water infiltration capacity and drainage. The soil is 0.7 to 1.5 m deep, with a high content of organic matter (15-25 %) (Castillo et al., 2006). Almost 90 % of the site is covered by pristine broad-leaf evergreen primary Nothofagus dombeyi forest. The other 10% is covered by deciduous forest and grassland (clear-cut areas). We selected the following sites:

a) Evergreen forest (FS). Pristine forest ecosystem, over 200 y old, containing N. dombeyi-Laurelia philipiana and Saxegothena conspicua and a rich community of epiphytes and climber plants. This forest has 450 stems ha⁻¹, a height of 45 m, a basal area of 91.3 m² ha⁻¹, a litter layer of 23.900 kg ha⁻¹, litterfall of 4.775 kg ha⁻¹ y⁻¹ (the litterfall contains 3.44 kg P ha⁻¹), and the soil has a bulk density of 350 kg m⁻³ (Godoy, personal communication).

b) Grassland (GL): The grassland is a result of clear cutting the forest 100 years ago and is a site without forest species. It consists of a mixture of Holcus lanatus, Poa annua, Plantago lanceolata, Taraxacum officinale, Trifolium pratense and other species. The soil has a bulk density of 530 kg m⁻³ (Godoy, personal communication). Further details are described by Castillo et al. (2006).

The FS and GL sites were divided into 5 plots (each measuring 1000 m²). Fifteen soil samples were collected from all plots at each site with a tube sampler (20-cm length and 5-cm diameter), using a diagonal sampling method across the plot, and bulked to form a composite sample per plot. The mineral horizon (2-20-cm depth) was collected from the forest site; the organic layer (0-2 cm) was discarded. At the grassland site, we collected the mineral horizon (2-20-cm depth), and roots and leaf material (0-2 cm) were discarded. The soil samples were transported to the laboratory, sieved (< 2 mm), and visible leaves, branches and roots were removed.

2.1. Soil analysis

The soils were sieved (< 2 mm) and stored at 4°C in plastic containers until analysis. Soil pH was determined in a 1: 2.5 soil/ water (w/v) suspension using a glass electrode. Acid phosphatase (E.C.3.1.3.2 orthophosphoric-monoester phosphomonoesterase) activity was determined using the p-nitrophenyl phosphate method with modifications described by Rubio et al. (1990) for volcanic soils with high organic matter. Briefly, soil samples (1 g) were incubated with 1 mL 50 mM p-nitrophenol phosphate and 4 mL 0.1 M tris buffer (pH 5.5) for 1 h at 20 ºC and corrected with a soil sample incubated with p-nitrophenol and buffer to determine p-nitrophenol adsorption onto soil to avoid underestimation of the final amount of free p-nitrophenol. Another sample was incubated with soil and buffer only (control). At the end of the incubation period, 1 mL 0.5 M CaCl₂ was added, and the solution was rapidly filtered; the filtrate was treated with

organic carbon (C₀) was determined by dry combustion using a VARIO-EL Analyzer. Olsen P was measured by extraction with 0.5 M NaHCO₃ (pH 8.5) as described by Olsen and Sommers (1982). Total P was determined using the alkaline oxidation method (Dick and Tabatabai, 1977) with NaOBr digestion. Organic P (Pₒ) was extracted once using 0.1 M HCl and three times using 0.5 NaOH and ultrasound (Borie and Zunino, 1983) instead of 0.1 M NaOH, proposed in the original method of Stewart and Oades (1972), to remove the high organic matter content of these soils. Soil phosphorus was characterized according to a modified Hedley fractionation method with sequential extraction using different chemicals (Hedley et al., 1982; Tiessen and Moir, 1993). Briefly, the soil was...
Redel et al.

air dried, sieved and crushed to 106 µm using a mortar; 0.5 g was extracted with deionized water, and with anion and cation exchange resin membranes (BDH # 55164 2S and BDH # 55165 2U) in HCO₃⁻ and H⁺ forms, respectively (Hedley et al., 1994). The soils, resin membranes and water (as an extracting solution) were shaken for 16 h to determine resin extractable P. The soil residue was further extracted using 0.5 M NaHCO₃ for determination of the NaHCO₃-Pi fraction, followed by 0.1 M NaOH extraction for determination of the NaOH-Pi fraction. The extracts (containing Pi and Pₐ) were further digested with NaOBr to determine total P. Pₐ values were calculated as the difference between the P extracted in each alkaline extraction and the Pi fraction. Finally, the soil residue was extracted with 0.5 M H₂SO₄ for determination of the H₂SO₄-Pi fraction. The Pi of all extracts was measured at 700 nm and pH 5.0 following the method of Murphy and Riley (1952), whereas Pₒ was estimated as the difference between Pₐ and Pi following Dick and Tabatabai (1977).

Microbial biomass P was analyzed within one week of sampling using simultaneous liquid fumigation with 1-hexanol and extraction with anion exchange resin membranes (Bünnemann et al., 2004). Briefly, moist soil, equivalent to 2 g dry weight, was shaken with H₂O and two resin membranes strips (BDH # 55164, in HCO₃⁻ form) with 1 mL 1-hexanol for 16 h (Bünnemann et al., 2004). Hexanol fumigation has been shown to be as effective as chloroform fumigation to release microbial biomass P (McLaughlin et al., 1986). Microbial biomass P was calculated as the difference in the P content of the fumigated and non-fumigated soil samples, using a P recovery factor that accounted for the P fixation during extraction (determined for each soil according to Bünnemann et al., (2004)) and an extractability factor kEP = 0.4 (Khan and Joergensen, 2009). The inorganic phosphate in the extracts was measured at pH 5.0 using a UV-VIS spectrophotometer at 700 nm (Murphy and Riley, 1962).

2.2. Extraction procedure for ³¹P-NMR spectroscopy and measurement conditions

Soil samples (10 g dry weight equivalent) collected from a depth of 2-5 cm were extracted using 75 mL of 1 M HCl by shaking the mixture for 1 h at room temperature to improve the signal/noise ratio of the soils with high organic C levels (Briceño et al., 2006) in the ³¹P-NMR measurements. The suspension was separated by centrifugation and the supernatant was discarded. The solid residue was sequentially treated with 125, 75 and 75 mL of 0.5 M NaOH and sonicated for 3, 1 and 1 min, respectively. The suspensions were separated by centrifugation and combined. Sodium-saturated Chelex 100 resin (30 mg) was added to the alkaline extract, shaken for 17 h on a reciprocal shaker, and filtered through 0.45-µm pore size micro filters. The filtrate was freeze dried, redissolved in 3.0 mL of D₂O, shaken for 2 h, centrifuged, and transferred to 5-mm NMR tubes. The Chelex 100 resin was used instead of the common EDTA procedure because it was found that this chelant enhanced the signal/noise ratio whereas the NaOH-EDTA extraction procedure is only efficient for samples with low organic C contents (Briceño et al., 2006).

2.3. ³¹P-NMR analysis

The ³¹P-NMR spectra of the soil extracts were obtained using a Bruker Avance 400 MHz spectrometer at 162 MHz using a 70° pulse, 0.506-s acquisition time, and 1-s delay, with 4000 to 15000 scans. Chemical shifts were measured relative to 85% orthophosphoric acid. The proton decoupler was gated on during the acquisition time, using the standard Waltz decoupling scheme. Peak areas were determined by integration over predetermined spectral regions using Mestre-C software processed with a Lorentzian line shape of 5 Hz (Gómez and López, 2004).
The spectral assignment followed that of Turner et al. (2003): δ 6 to 8 ppm, ortho-P; δ 4 to 6 ppm, monooester-P; δ 2 to 1 ppm, phospholipids; δ 1 to -1 ppm, DNA (DNA-P); and δ -3 to -5 ppm, pyrophosphates and polyphosphates (Pyr- and Poly-P, respectively). The spectral assignments of δ 4.2 ppm, Scyillo-inositol hexakisphosphates, and δ 5.6 ppm, C₂-myo-inositol phosphates, followed Turner et al. (2003) and Turner and Richardson (2004).

2.4. Statistical analyses

The results of the soil analyses represent the mean value of five replicates. The data obtained were subjected to a t-test (using SPSS 12) to check for differences between sites. The soil analysis data were arcsine transformed to meet the statistical requirements of normality. Statistical significance was determined at $P \leq 0.05$.

Table 1. Selected properties and P forms of the mineral soil (2-20 cm) in the forest (FS) and grassland (GL). Data are presented as the mean ± S.E. Different letters within a column indicate significant differences ($P \leq 0.05$).

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Organic C</th>
<th>P-ase</th>
<th>Olsen P</th>
<th>Microbial P</th>
<th>Organic P</th>
<th>Total P</th>
<th>Orthophosphate-P</th>
<th>Monooester-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS</td>
<td>4.7 ± 0.4 b</td>
<td>120 ± 29 a</td>
<td>6.1 ± 1.3 a</td>
<td>4.1 ± 0.9 a</td>
<td>230 ± 15 a</td>
<td>829 ± 25 b</td>
<td>2628 ± 111 a</td>
<td>36</td>
<td>64</td>
</tr>
<tr>
<td>GL</td>
<td>5.3 ± 0.1 a</td>
<td>120 ± 8 a</td>
<td>3.3 ± 0.9 b</td>
<td>1.4 ± 0.1 b</td>
<td>38 ± 2 b</td>
<td>1176 ± 62 a</td>
<td>2157 ± 120 a</td>
<td>31</td>
<td>69</td>
</tr>
</tbody>
</table>

3. Results

The evergreen forest soil (FS) was more acidic (0.6 pH units) than that collected from the grassland (GL). Soil acid phosphatase (soil P-ase) activity, Olsen P and microbial P were approximately two to six times higher in FS than in GL. However, the $P_0$ concentration was 42 % higher in GL than in FS, but $P_T$ was not significantly different (Table 1). Microbial P in FS was equivalent to approximately 28 % of total $P_0$, but only accounted for slightly more than 3 % in GL. With respect to P fractionation, we found that the bicarbonate + NaOH-P fraction represented 96 % of total $P_0$ in GL versus 77 % in FS. More specifically, P was found mainly in the moderately labile NaOH-$P_0$ fraction (Figure 1), representing 42 % of $P_T$. The NaOH-$P_0$ fraction was higher in GL than in FS. There was a greater amount of $H_2SO_4$-extractable P in GL than in FS (Figure 1). The most available resin-P fraction represented only approximately 1 % of the amount of $P_T$, with higher contents in FS than in GL (Figure 1).

Analysis of the NMR spectra (Figure 2) showed that monoester- P signals (δ 4.0 to 6.0 ppm) were predominant in FS and GL. This result was confirmed by spectra area integration (64 % with FS and 69 % with GL). Furthermore, only traces of diester, pyrophosphate and polyphosphate-P signals were found for FS and GL. More $C_2$-myo-inositol phosphate was found in FS (3 % vs. 1 % in GL), but the scyillo-inositol hexakisphosphate amounts were quite similar (14 vs. 12 % for FS and GL, respectively). Furthermore, unidentified inositol phosphate signals were found at δ 6.5-6.7 ppm (2 %), and an unidentified signal was found at δ 3.3-3.5 ppm in GL (1 %).
**Figure 1.** Phosphorus fractionation in forest (FS) and grassland (GS) soils. Different letters for the same P fraction indicate significant differences ($P \leq 0.05$).

**Figure 2.** Analysis of the NaOH-Chelex extract from the forest and grasslands soils determined using $^{31}$P-NMR spectroscopy. In-P: inositol phosphate
4. Discussion

The selection of the FS site was based on the fact that this forest is almost pristine, in contrast to other sites close to the study area. The selected sites can be compared the original forest in the grassland site was cut at the end of the XIX century, whereas the forest site remained unaltered before and after this time period. Therefore, the P cycle was altered at this time in GL, but not in FS, and a transient equilibrium between the P pools in the soil and aboveground biomass was reached in the GL site. Various facts influencing the new P equilibrium have to be considered, e.g., (i) exportation of P in wood, the P remaining in the residues and the P in the residues incorporated by GL, (ii) eventual cattle grazing and manure, and (iii) enhanced leaching of P in GL during the disturbance process. There are no data on the P transfers of these processes, and data do not exist for the P pools in the above and belowground biomass of GL and FS. Regardless, the $C_o$, $P_T$ and the sum of the P-resin, P-NaHCO$_3$, and P-NaOH fractions were quite similar for both soils, but a relatively higher amount of the poorly available P fractions and NaOH-P$_o$ was found in GL compared with FS, in agreement with an enrichment of Po fractions under meadows and pastures compared with forest based on a review conducted by Negassa and Leinweber (2009).

The results show that these Andisols, which are covered by forests, have higher amounts of $P_T$ and NaOH-P$_o$ compared with other volcanic soil ecosystems (Cross and Schlesinger, 1995, Thomas et al. 1999; Liu et al. 2004; Murphy et al., 2009) and Nothofagus forests (Satti et al., 2007). However, these $P_T$ and NaOH-P$_o$ amounts are similar to those reported for Chilean soils (Thomas et al., 1999; De Brouwere et al. 2003; Borie and Rubio, 2003). In this study, soil acid phosphatase activities in FS and GL were several fold higher than in agricultural Ultisols of southern Chile (Rubio et al., 2002; Redel et al., 2007; Redel et al., 2011) and other soil-forest ecosystems around the world, including allophanic forest soil and Andisols (Liu et al., 2004; Satti et al., 2007). This high soil P-ase activity may result from microbes investing more resources in P-ase production under conditions of low soil P availability (Cheesman et al., 2012, DeForest et al., 2012; Kitamaya, 2013). In this regard, P-ase activity may be used as an indicator of microbial activity to identify enhanced $C_o$ availability in FS, although GL and FS presented similar soil $C_o$ contents.

The higher microbial activity and P-ase production in FS site was reflected in changes in the most available P fractions, such as the resin P and NaHCO$_3$-Pi fractions, which enhance the P bioavailability in the forest soil. This fact was also corroborated by the NMR spectrum, which showed more orthophosphate-P in FS but more inositol P in GL. Although the values of the most available P fractions (resin and bicarbonate P) were lower in FS than in other similar broadleaf south Chilean forests (Thomas et al., 1999), the P concentrations were sufficiently high to supply the annual P requirements of the forest.

In relatively young soils (formed ca. 10,000 years ago), such as the ones used in this experiment, a large proportion of the organic phosphorus often occurs as inositol phosphates (Turner et al., 2007). Scyillo-inositol hexakisphosphate can accumulate in the early stages of soil development due to the greater apparent recalcitrance of the scylo structure (Turner and Richardson, 2004). Myo-inositol phosphates are mainly of plant origin (Turner et al., 2007); therefore, leaf litter recycling may contribute to $C_o$-myo-inositol phosphate in FS soil. Further investigations are needed to determine the importance of both P-NMR signals in soil and their relationship with soil/plant management. It was expected that FS would have more diester P than GL (McDowell and Stewart, 2006); however, no diester P-NMR signals were found in our soil spectra. Diester P is more
labile and is more accessible to microbial or enzymatic attack compared with monoester P (Tate and Newman, 1982; Zhu et al., 2013); thus, it can be rapidly transformed into monoester P (Chiu et al., 2005).

The results presented in this paper demonstrate the impact of land use change on soil P cycling and fertility, a fact that can be important for soil management because of the considerable extent of native forest areas that have been clear-cut in southern Chile, and the consequences for soil quality degradation reflected by a reduced P availability in GL compared with FS soil.

5. Conclusions

Comparisons between the evergreen forest and the clear-cut grassland clearly suggest that soil use change influences soil P bioavailability; there was a lower amount of phosphorus in the resin and bicarbonate fractions and in the microbial and Olsen-P in the grassland soil. Furthermore, $^{31}$P-NMR analysis indicated that the P form, i.e., C$_2$-myo-inositol phosphate and scyllo-inositol hexakisphosphate, was affected. Higher P availability in forest soils can be related to higher soil phosphatase activity, suggesting the importance of this enzyme in maintaining P availability through Po mineralization.

Acknowledgements

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References


