Journal of Soil Science and Plant Nutrition, 2015, 15 (4) , 1061-1070
RESEARCH ARTICLE

Effects of land use change on P bioavailability determined by chemical fractionation and ^{31}P-NMR spectroscopy in a *Nothofagus* forest and adjacent grassland

Y. D. Redel[1*], M. Escudey[2], M. Alvear[3], J. Conrad[4], F. Borie[3]

*[1]Scientific and Technological Bioresource Nucleus (Bioren-UFRO), Universidad de La Frontera,Temuco, Chile. [2]Center for the Development of Nanoscience and Nanotechnology, CEDENNA, 9170124, Universidad de Santiago de Chile, Santiago, Chile, [3]Departamento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, Temuco, Chile, [4]Hohenheim University, Institute of Chemistry, Garbenstrasse 30, 70599, Stuttgart, Germany. *Corresponding author: yonathan.redel@ufronter*a.cl

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 *scyllo*-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_2SO_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_2SO_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 phillipiana* and *Saxegothaea 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 m^2). 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 4ºC 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 $CaCl_2$ was added, and the solution was rapidly filtered; the filtrate was treated with 4 mL 0.5 M NaOH. The samples were homogenized and centrifuged at 2.500 x g for 10 min, and the p-nitrophenol released was determined spectrophotometrically by measuring the absorbance of the supernatant at 400 nm. Organic carbon (C_o) was determined by dry combustion using a VARIO-EL Analyzer. Olsen P was measured by extraction with 0.5 M $NaHCO_3$ (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 (P_o) 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

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 HCO_3- 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 $NaHCO_3$ for determination of the $NaHCO_3$-P*i* fraction, followed by 0.1 M NaOH extraction for determination of the NaOH-P*i* fraction. The extracts (containing P*i* and P_o) were further digested with NaOBr to determine total P. P_o values were calculated as the difference between the P extracted in each alkaline extraction and the P*i* fraction. Finally, the soil residue was extracted with 0.5 M H_2SO_4 for determination of the H_2SO_4-P*i* fraction. The P*i* 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 P_T and P*i* 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 (Bünemann *et al*., 2004). Briefly, moist soil, equivalent to 2 g dry weight, was shaken with H_2O and two resin membranes strips (BDH # 55164, in HCO_3- form) with 1 mL 1-hexanol for 16 h (Bünemann *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 Bünemann *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 ^{31}P-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 ^{31}P-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 D_2O, 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. ^{31}P-NMR analysis

The ^{31}P-NMR spectra of the soil extracts were obtained using a Bruker Avance 400 MHz spectrometer at 162 MHz using a 70º 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, monoester-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, *Scyllo*-inositol hexakisphosphates, and δ 5.6 ppm, C_2–*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.

Stand	pH	Organic C	P-ase	Olsen P	Microbial P	Organic P	Total P	Orthophosphate-P	Monoester-P
		(g kg^{-1})	(mg PNF g^{-1} h^{-1})		----------(mg kg^{-1})----------			-----(% of the spectra)-----	
FS	4.7 ± 0.4 b	120 ± 29 a	6.1 ±1.3 a	4.1 ± 0.9 a	230 ± 15 a	829 ± 25 b	2028 ± 111 a	36	64
GL	5.3 ± 0.1 a	120 ± 8 a	3.3 ± 0.9 b	1.4 ± 0.1 b	38 ± 2 b	1176 ± 62 a	2157 ± 120 a	31	69

Different letters within a column indicate significant differences ($P \leq 0.05$).

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_o 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_o, 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_o in GL versus 77 % in FS. More specifically, P was found mainly in the moderately labile NaOH-P_o fraction (Figure 1), representing 42 % of P_T. The NaOH-P_o 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 *scyllo*-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 3-3.5 ppm in GL (1 %).

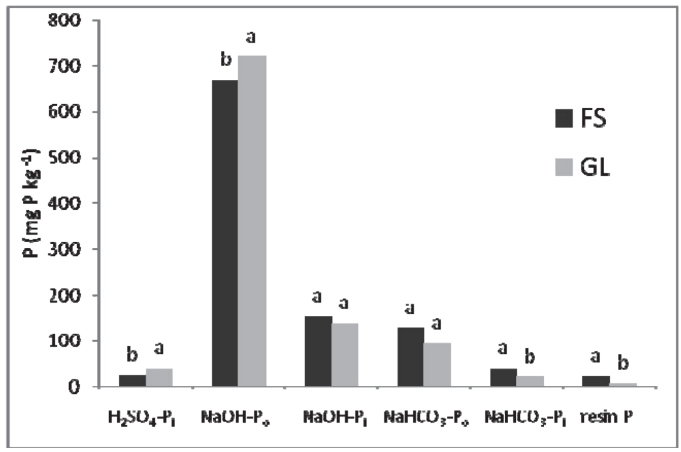

Figure 1. Phosphorus fractionation in forest (FS) and grassland (GS) soils. Different letters for the same P fraction indicate significant differences ($P \leq 0.05$).

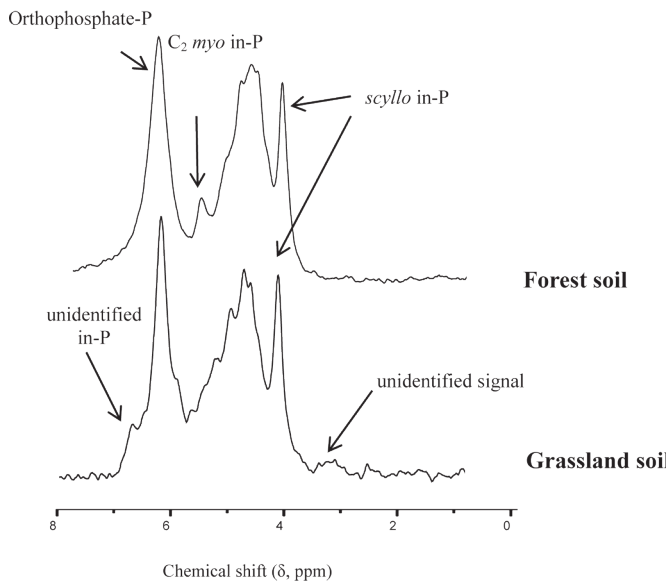

Figure 2. Analysis of the NaOH-Chelex extract from the forest and grasslands soils determined using ^{31}P-NMR spectroscopy. In-P: inositol phosphate

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 P_o 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 $NaHCO_3$-P*i* 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). *Scyllo*-inositol hexakisphosphate can accumulate in the early stages of soil development due to the greater apparent recalcitrance of the scyllo 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_2-*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

This work was supported by Fondecyt 11121619 grants.

References

Borie, F., Zunino, H. 1983. Organic matter P associations as a sink in P-fixation processes in allophanic soils of Chile. Soil Biol. Biochem. 15, 599–603.

Borie, F., Rubio, R. 2003. Total and organic phosphorus in chilean volcanic soils. Gayana Bot. (Chile), 60, 69–78.

Borie, F., Zunino, H., Martínez, L. 1989. Macromolecule- P associations and inositol phosphates in some chilean volcanic soils of temperate regions. Commun. Soil Sci. Plant Anal. 20, 1881–1884.

Briceño, M., Escudey, M., Galindo, G., Borchardt, D., Chang, A. 2004. Characterization of chemical phosphorus forms in volcanic soils using ^{31}P-NMR spectroscopy. Commun. Soil Sci. Plant Anal. 35, 1323–1337.

Briceño, M., Escudey, M., Galindo, G., Borchardt, D., Chang, A. 2006. Comparison of extraction procedures used in the determination of phosphorus species by ^{31}P-NMR in Chilean volcanic soils. Commun. Soil Sci. Plant Anal. 37, 1–17.

Bünemann, E.K., Steinebrunner, F., Smithson, P.C., Frossard, E., Oberson, A. 2004. Phosphorus dynamics in a highly weathered soil as revealed by isotopic labeling techniques. Soil Sci. Soc. Am. J. 68, 1645–1655.

Castillo, G.C., Borie, F., Godoy, R., Rubio, R., Sieverding, E. 2006. Diversity of mycorrhizal plant species and arbuscular mycorrhizal fungi in evergreen forest, deciduous forest and grassland ecosystems of Southern Chile. Journal of Applied Botany and Food Quality, 80, 40–47.

Cheesman, A., Turner, B.L., Reddy, K. R. 2012. Soil phosphorus forms along a strong nutrient gradient in a tropical ombrotrophic wetland. Soil Sci. Soc. Am. J. 76, 1496–1506.

Chiu, C.Y., Pai, C.W., Yang, K.L. 2005. Characterization of phosphorus in sub-alpine forest and adjacent grassland soils by chemical extraction and phosphorus-31 nuclear magnetic resonance spectroscopy. International Symposium on Impacts of Soil Biodiversity on Biogeochemical Processes in Ecosystems, Taipei, Taiwan, 2004. Pedobiologia, 49, 655–663.

Cross, A., Schlesinger, W. 1995. A literature review and evaluation of the Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. Geoderma, 64, 197–214.

De Brouwere, K., Thijs, A., Maarten, H., Merckx, R. 2003. Forms and availability of soil phosphorus in temperate forests in southern Chile and Flanders. Gayana Botánica (Chile) 60, 17–23.

DeForest, J., Smemo, K., Burke, D., Elliott, H. & Becker, J. 2012. Soil microbial responses to elevated phosphorus and pH in acidic temperate deciduous forests. Biogeochemistry, 109, 189–202.

Dick, W.A., Tabatabai, M.A. 1977. Determination of orthophosphate in aqueous solutions containing labile organic and inorganic phosphorus compounds. J. Environ. Qual. 6, 82–85.

Donoso, C. 1993. Bosques templados de Chile y Argentina: variación, estructura y dinámica. Librería Universitaria, Santiago, Chile.

Escudey, M., Galindo, G., Förster, J., Briceño, M., Díaz, P., Chang, A. 2001. Chemical forms of phosphorus of volcanic ash-derived soils in Chile. Commun. Soil Sci. Plant Anal., 32, 601–616.

Gómez, J.C.C., López, F.J.C. 2004. Mestre-C, nuclear magnetic resonance companion v.3.5.1. Available at http://www.metrec.com (verified Feb 2009).

Hedley, H., Steward, J., Chauhuan, B. 1982. Changes in organic and inorganic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci. Soc. Am. J. 46, 970–976.

Hedley, H., Kirk, G., Santos, M. 1994. Phosphorus efficiency and the forms of soil phosphorus utilized by upland rice cultivars. Plant Soil, 158, 53–62.

Khan, K. S., Joergensen, R. G. 2009. Changes in microbial biomass and P fractions in biogenic household waste compost amended with inorganic P fertilizers. Biores. Techn. 100, 303–309.

Kitayama, K. 2013. The activities of soil and root acid phosphatase in the nine tropical rain forests that differ in phosphorus availability on Mount Kinabalu, Borneo. Plant Soil, 367, 215–224.

Liu, Q., Loganathan, P., Hedley, M.J., Skinner, M.F. 2004. The mobilization and fate of soil and rock phosphate in the rhizosphere of ectomycorrhizal Pinus radiata seedlings in an allophanic soil. Plant Soil, 264, 219–229.

McDowell, R.W., Stewart, I. 2006. The phosphorus composition of contrasting soils in pastoral, native and forest management in Otago, New Zealand: Sequential extraction and 31P NMR. Geoderma, 130, 176–189.

McLaughlin, M.J., Alston, A.M., Martin, J.K. 1986. Measurement of phosphorus in the soil microbial biomass: a modified procedure for field soils. Soil Biol. Biochem. 18, 437–443.

Murphy, P.N.C., Bell, A., Turner, B.L. 2009. Phosphorus speciation in temperate basaltic grassland soils by solution 31P NMR spectroscopy. Eur. J. Soil Sci., 60, 638–651.

Murphy, J., Riley, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27, 31–36.

Negassa, W., Leinweber, P. 2009. How does the Hedley sequential phosphorus fractionation reflect impacts of land use and management on soil phosphorus: A review. J. Plant Nutr. Soil Sci. 172, 305–325.

Olsen, S.R., Sommers, L.E. 1982. Phosphorus. In: Page AL, Miller RH, Deeney DR. (eds.) Methods of Soil Analysis. Part 2. ASA, Madison, Wisconsin, USA, pp. 403-430.

Redel, Y., Rubio, R., Rouanet, J., Borie, F. 2007. Phosphorus bioavailability affected by tillage and crop rotation on a Chilean volcanic derived Ultisol. Geoderma, 139, 388–396.

Redel, Y., Rubio, R., Godoy, R., Borie, F. 2008. Phosphorus fractions and phosphatase activity in an Andisol sampled under different forest ecosystems. Geoderma, 145, 216–221

Redel, Y., Escudey, M., Alvear, M., Conrad, J., Borie, F. 2011. Effects of tillage and crop rotation on chemical phosphorus forms and some related biological activities in a Chilean Ultisol. Soil Use Manage. 27, 221–228.

Rubio, R., Borie, F., Schalchli, C., Castillo, C, Azcon, R. 2002. Plant growth responses in natural acidic soil as affected by arbuscular mycorrhizal inoculation and phosphorus sources. J. Plant Nutr. 25, 1389-1405.

Rubio, R., Moraga, E., Borie, F. 1990. Acid phosphatase activity and vesicular-arbuscular infection associated with roots of four wheat cultivars. J. Plant Nutr. 13, 585–598.

Satti, P., Mazzarino, M.J., Roselli, L., Crego, P. 2007. Factors affecting soil P dynamics in temperate volcanic soils of southern Argentina. Geoderma, 139, 229–240.

Steward, J., Oades, J. 1972. The determination of organic phosphorus in soils. J. Soil Sci. 23, 38–49.

Taranto, M.T., Adams, M.A., Polglase, P.J. 2000. Sequential fractionation and characterization (^{31}P-NMR) of phosphorus-amended soils in Banksia integrifolia (L.F.) woodland and adjacent pasture. Soil Biol. Biochem. 32, 169–177.

Tate, K.R., Newman, R.H. 1982. Phosphorus fractions of a climosequence of soils in New Zealand tussock grassland. Soil Biol. Biochem. 14, 191–196.

Thomas, S., Johnson, A., Frizano, J., Vann, D., Zarin, D., Joshi, A. 1999. Phosphorus fractions in montane forest soils of the Cordillera de Piuché, Chile: biogeochemical implications. Plant Soil, 211, 139–148.

Tiessen, H., Moir, J.O. 1993. Characterization of available P by sequential extraction. In Carter MR (ed) Soil Sampling and Methods of Analysis. Lewis Publishers, Boca Raton, FL, USA, pp 75–86.

Turner, B.L., Mahieu, N., Condron, L.M. 2003. Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH-EDTA extracts. Soil Sci. Soc. Am. J. 67, 497–510.

Turner, B.L., Richardson, A.E. 2004. Identification of *scyllo*-inositol phosphates in soil by solution phosphorus-31 nuclear magnetic resonance spectroscopy. Soil Sci. Soc. Am. J. 68, 802–808.

Turner, B.L., Cade-Menun, B.J., Condron, L.M., Newman, S. 2005. Extraction of soil organic phosphorus. Talanta, 66, 294–306.

Turner, B.L., Condron, L.M., Richardson, S.J., Peltzer, D.A., Allison, V.J. 2007. Soil organic phosphorus transformations during pedogenesis. Ecosystems, 10, 1166–1181.

Zhu, Y., Wu, F., He, Z., Guo, J., Qu, X., Xie, F., Giesy, J.P., Liao, H., Guo, F. 2013. Characterization of organic phosphorus in lake sediments by sequential fractionation and enzymatic hydrolysis. Environ. Sci. Technol., 16, 7679-7687.

.