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Article

Characterization of phosphorus in a toposequence of subtropical perhumid forest soils facing a subalpine lake

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Abstract: The productivity of forests is often considered to be limited by the availability of phosphorus (P). Knowledge of the role of organic and inorganic P in humid subtropical forest soils is lacking. In this study, we used chemical fractionation and ^{31}P nuclear magnetic resonance (NMR) spectroscopy to characterize the form of P and its distribution in undisturbed perhumid *Chamaecyparis* forest soils. The toposequence of transects was investigated for the humic layer from summit to footslope and lakeshore. The clay layer combined with a placic-like horizon in the subsoil may affect the distribution of soil P because both total P and organic P (P_o) contents in all studied soils decreased with soil depth. In addition, P_o content was negatively correlated with soil crystalline Fe oxide content, whereas inorganic P (P_i) content was positively correlated with soil crystalline Fe oxide content and slightly increased with soil depth. Thus, P_i may be mostly adsorbed by soil crystalline Fe oxides in the soils. Among all extractable P fractions, the NaOH-P_o fraction appeared to be the major component, followed by $NaHCO_3$-P_o; the resin-P and HCl-P_i fractions were lowest. In addition, we found no typical trend for P_i and P_o contents in soils with topographical change among the three sites. From the ^{31}P-NMR spectra, the dominant P_o form in soils from all study sites was monoesters with similar spectra. The ^{31}P-NMR findings were basically consistent with those from chemical extraction. Soil formation processes may be the critical factor affecting the distribution of soil P. High precipitation and year-round high humidity may be important in the differentiation of the P species in this landscape.

Keywords: *Chamaecyparis* forest; humic substances, ^{31}P nuclear magnetic resonance spectroscopy (^{31}P NMR); P species; topography

1. Introduction

In terrestrial environments, mountainous forest is one of the canonical ecosystems that contain abundant biodiversity and is a vulnerable ecosystem [1]. Thus, understanding the soil nutrient distributions in a forest ecosystem is vital to maintain ecosystem functions and productivity [2]. In such ecosystems, phosphorus (P) can be a limiting element because unlike nitrogen (N), which is mainly deposited from the atmosphere [3], P is mostly acquired from weathering soil parent material and is continuously lost due to soil erosion [4-6]. The bioavailability of P in soils further relies on the chemical/physical conditions that fractionate the total P into different species [7,8]. Understanding P availability and its transformation among each fraction will help assess the P supply capacity of the soil over the long term and to adapt management practices [9].

Some isolated P fractionation pools have key functions in the P cycle and plant nutrition [9-13]. The organic and inorganic forms of P are usually separated and quantified by their plant availability. The mineralization of organic P is generally responsible for most of the P supply to plants [14,15], especially in mountain forest ecosystems [2,9,16]. Actually, the sequential fraction can provide a general indicator of how biological and geochemical forms of P change during soil weathering in mountain forest ecosystems. In general, organic P was found the dominant fraction of total P (TP) in these forest ecosystems, and available P content was determined by the mineralization processes of organic P [9,17]. Productivity, including plant growth and biomass production of trees in afforested mountain areas, is largely influenced by mineralization and microbial processes of organic P as well as soil organic matter.

However, identifying different fractions of P and evaluating their bioavailability are difficult because of spatial inconsistency and the complexity of geochemical properties. Several wet-chemical methods for determining P fractionations have been established and used in various ecosystems [18-21]. Also, ^{31}P-nuclear magnetic resonance (NMR) spectroscopy has been used for determining the composition of soil P [22-24]. De Feudis *et al.* [25] determined the P availability in subalpine forest soils in Italy and found organic P, bioavailable P contents and alkaline mono-phosphatase activity were all increased with altitude. Similarly, Doolette *et al.* [26] analyzed P composition in five alpine and subalpine forest soils and found that 54% to 66% of extractable P was contributed by organic P such as phosphomonoesters and inositol phosphonates, and the organic P composition was affected by temperature and soil moisture.

To our knowledge, studies of P fractionation in forests were mostly performed in temperate ecosystems [8,27,28], with relatively fewer studies from subtropical and tropical alpine forests. Understanding the biogeochemistry of subalpine/alpine forest soils in tropical/subtropical areas may help explain the transition phenomenon between temperate and subtropical/tropical ecosystems and could well link pedogenic processes or nutrient cycles along a climosequence. In this study, to evaluate the pedogenetic effects on the forms of P, we determined the composition of soil P with both chemical extraction and ^{31}P-NMR methods along a toposequence in a pristine subtropical subalpine

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forest. Because the changes in soil oxidation–reduction status affect the formation of iron (Fe) and consequently the P status in such humid forest soils, we hypothesized that the content of labile P associated with Fe oxides increases with changing topographic sequence from the summit to lakeshore because of leaching and soil erosion, whereas recalcitrant organic P, which is more complex formed, will remain in the summit.

2. Materials and Methods

2.1. Study sites

This study was conducted in the Yuanyang Lake forest ecosystem (24˚35′N, 121˚24′E) in northeastern Taiwan. The study sites covered an elevation of 1,700 to 2,000 m a.s.l., with an average annual temperature of 12.5˚C and a mean annual precipitation of more than 4,000 mm. The ecosystem consists of a primary forest dominated by Taiwan false cypress (*Chamaecyparis obtusa* var. *formosana*) and an evergreen broadleaf shrub (*Rhododendron formosanum*). The bedrock of the study sites is composed of interbedded Tertiary shale and sandstone [29]. This locality can be described as a temperate, very wet and mountainous ecosystem. It has been established as a Nature Reserve and selected as one of the long-term ecological research sites in Taiwan. The forest soils are divided into three main groups, which are closely related to the topography. The soil at the summit, with a slope of about 15˚, is classified as Typic Hapludult [30], which is relatively well drained and develops clear eluvial and illuvial boundaries. The footslope, with a slope of about 28˚, is dominated by Typic Dystrochrept, where poor drainage caused by the clay and silty clay mineral horizon beneath the organic layer limits the downward movement of soluble compounds and thus hampers the soil profile development. Lithic Medihemist stretches from the lakeshore to the toeslope, about 1.5 m above the lake and with a slope of about 10˚, which is inundated by occasional storms. Details of the environment of this ecosystem are described elsewhere [31].

2.2. Soil sampling

A pedon sample was collected from each site to the bedrock. However, the pedon sample in the lakeshore was limited to the O horizon because no mineral layers were developed on the bedrock. Each horizon in the pedon was collected separately to determine the basic soil physiochemical properties.

To further determine the P fractionation along the topography, soil samples were collected from three selected sites along a topographic sequence in the forest that covered the summit, footslope and lakeshore. At each sampling site, three composite samples, each containing five subsamples, were collected with a soil auger with 8 cm in diameter and 10 cm in depth (O_e and O_a horizons).

Visible coarse organic materials, such as roots and litter were manually removed before sieving. The remaining soil samples were air dried and sieved through a 2-mm sieve for chemical analysis.

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2.3. General soil chemical properties

Soil pH was measured at a soil:water ratio of 1:1. Total organic C (TOC) and total N (TN) contents in the soil were determined with an NCS Elemental Analyzer (Model NA1500 Fisons, Italy). Cation-exchange capacity was determined by the NH_4/Na exchange method [32]. Crystalline Fe (Fe_d) and Al (Al_d) oxide contents were determined by the dithionite-citrate-bicarbonate extraction method [33]. Amorphous Fe (Fe_o) and Al (Al_o) oxide contents were measured by an ammonium oxalate extraction method [34].

2.4. Sequential fractionation of P

Sequential fractionation was performed as described in [35]. The sequential fractionation procedure removes progressively less available P with each subsequent soil extraction [36]. The fractionation started with 0.5 g dried sieve soil. An anion exchange resin was used first to extract plant-available inorganic P (P_i) [37]. Then, the other P_i content was determined directly in 0.5 M $NaHCO_3$, 0.1 M NaOH, 1M HCl and concentrated HCl extractions. The extracted solutions were then digested with H_2SO_4 (97%) and H_2O_2 (30%) at 300 °C to determine the total dissolved P (P_d) content of each fraction. The organic P (P_o) content was calculated by subtracting P_i content from P_d content in each fraction ($P_o = P_d - P_i$). The remaining soil was digested with H_2SO_4 (97%) and H_2O_2 (30%) at 300 °C to determine the residual P content. All extracts and digestions obtained were measured colourimetrically by the malachite green procedure [38].

Summed P_i content was calculated as the sum of all analyzed P_i fractions including resin-P_i, $NaHCO_3$-P_i, NaOH-P_i, HCl-P_i and cHCl-P_i. Summed P_o content was calculated as the sum of all analyzed P_o fractions including $NaHCO_3$-P_o, NaOH-P_o and cHCl-P_o. Summed P content was calculated as the sum of P_i, P_o and residual P content. Total P content of the soil samples was determined by digestion with H_2SO_4 (97%) and H_2O_2 (30%) at 300 °C.

2.5. ^{31}P-NMR measurements

Air-dried soil (5 g) was dispersed in 20 mL of 0.25 M NaOH-0.05 M EDTA for 2 h, and the suspension was centrifuged at 12,100 × *g* for 30 min. The extractant was then reacted with chelating resin for 6 h at room temperature to reduce the paramagnetic interference of iron and other metals in the NMR spectra. After being stirred, the resin was separated by filtration through Whatman 42 filter paper. The extract was freeze-dried for storage. A freeze-dried sample (0.1 g) of NaOH-EDTA extractant was dissolved in 0.5 mL of 0.5 M NaOH, then 0.1 mL D_2O was added and the solution was transferred to a 5-mm NMR tube for ^{31}P-NMR analysis [39]. The ^{31}P-NMR spectra were obtained at 242.86 MHz and 25 ˚C on a Bruker-600 NMR spectrometer with 60˚ pulse, 3.5-s delay and 0.33-s acquisition time. The ^{31}P-NMR spectra were proton-decoupled by using an inverse-gated pulse sequence to overcome the nuclear Overhauser enhancement and for quantification [40,41]. Depending on the P content in the alkaline extract, 500-2,500 scans were used for an acceptable signal-to-noise ratio. Spectra were recorded with a line-broadening of 20 Hz. The chemical shift was measured relative to an external 85% H_3PO_4/D_2O standard. The assignment of signals was based on Newman and Tate [40], Dai *et al.* [42], Condron *et al.* [22], and Robinson *et al.* [39]. Contents of the various P components (phosphonate, inorganic orthophosphate, orthophosphate monoesters, orthophosphate diesters, pyrophosphate, polyphosphates) were determined according to relative resonance areas obtained by electronic integration. Inorganic orthophosphates and orthophosphate monoesters signals were separated by using a boundary determined from the valley between the two signals to the baseline [42].

2.6. Statistical analyses

All extraction experiments were carried out in triplicate. Simple linear regression was used to compare the relation between soil P and soil Fe_d, Fe_o Al_d and Al_o concentrations. Differences in the P factions among the three sites were analyzed using one-way analysis of varience (One-Way ANOVA) and Tukey’s honestly significant difference (HSD) test. JMP 11.0 (SAS Inc., Cary, NC, USA) was used for these statistical analyses. $P < 0.05$ was considered as statistically significant.

3. Results

The basic chemical properties of the studied soils are in Table 1. The soils were strongly acidic; pH values ranged from 3.3 to 4.5 in the three sampling sites. Both TOC and TN contents were high in the O horizon and decreased from the surface to the low horizons. Cation-exchange capacity basically coincided with TOC content, decreasing from the surface to the low horizons. Total P content also decreased from the surface to the low horizons, but the difference was much less than for TOC and TN contents.

Table 1. General chemical properties of soils studied

Site	Horizon	Depth (cm)	pH)	CEC ($cmol_{(+)}$ kg^{-1})	Base saturation (%)	TOC (g kg^{-1})	Total N (g kg^{-1})	Total P (mg kg^{-1})	P_i (mg kg^{-1})	P_o (mg kg^{-1})	Fe_o (g kg^{-1})	Fe_d (g kg^{-1})	Al_o (g kg^{-1})	Al_d (g kg^{-1})
Summit	Oi	10─7	3.7	73.1	1.2	352.4	8.88	945.8	125.5	571.6	0.5	4.1	1.0	1.8
	Oe	7─2	3.5	124.9	3.3	492.5	17.49	1040.2	147.2	621.4	0.4	2.6	1.3	2.2
	Oa	2─0	3.5	146.9	2.3	492.4	22.1	606.7	178.6	535.8	1.2	4.3	1.5	2.5
	A	0─1	3.5	60.1	1.8	207.3	11.9	667.8	171.7	305.2	1.5	4.1	1.0	1.6
	E	1─8	3.8	19.5	4.6	38.0	1.9	308.1	89.3	226.1	1.0	1.9	0.8	1.0
	Bt1	8─20	4.1	19.6	4.1	10.0	0.95	194.2	116.0	131.9	17.2	38.5	2.3	7.7
	Bt2	20─30	4.2	12.4	6.5	11.9	1.07	269.0	162.2	132.8	25.8	41.3	1.8	7.8
	BC	30─45	4.3	12.7	3.9	9.0	1.06	245.8	129.3	102.6	23.9	34.9	1.6	7.5
Footslope	Oi	11─8	3.8	98.9	10.6	541.7	12.8	905.0	122.3	714.5	0.4	2.2	0.8	1.5
	Oe	8─4	3.5	120.3	5.3	511.8	19.1	701.6	120.8	653.2	0.6	3.0	0.6	1.2
	Oa	4─0	3.3	102.9	5.0	476.7	22.9	402.5	193.6	545.5	0.4	2.2	0.8	1.9
	A	0─5	3.5	65.3	2.6	237.6	12.9	305.9	213.4	318.9	14.9	18.3	2.5	4.0

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	E	5–10	4	11.5	7.8	7.0	0.7	171.7	139.7	78.7	1.3	5.2	0.4	0.8
	Bw1	10–23	4.1	16.6	2.4	10.0	1.1	283.8	239.7	82.0	18.1	35.9	1.6	4.5
	Bw2	23–42	4.5	17.6	4.5	19.9	1.5	928.3	258.9	125.1	18.3	47.0	2.2	7.9
Lakeshore	Oi	33–22	3.5	36.3	5.7	363.1	12.7	934.1	103.0	561.8	0.7	1.9	0.5	0.9
	Oe	22–9	3.4	18.7	8	186.7	8.7	563.4	107.9	431.7	1.1	3.7	0.6	0.8
	Oa	9–0	3.5	17.7	7.3	176.9	7.4	945.8	124.6	417.3	0.4	1.4	0.3	1.3

CEC: cation-exchange capacity; TOC: total organic C; Fe_o, Al_o: iron and aluminum extracted by the ammonium oxalate method; Fe_d, Al_d: iron and aluminum extracted by the citrate-bicarbonate-dithionite method.

The Fe_o, Fe_d, Al_o and Al_d contents peaked in the Bt_2 horizon at the summit site and in the Bw_2 horizon at the footslope site. P_i content was associated with amorphous (Fe_o and Al_o) and crystalline (Fe_d and Al_d) Al and Fe oxide contents and migrated vertically through the horizons with illuviation. Moreover, P_i and Fe_d contents were positively correlated and P_o and Fe_d as well as P_o and Fe_o contents were negatively correlated in the soil samples (Fig. 1a). However, the relation between P_i and Fe_o contents was not statistically significant (Fig. 1b). In addition, only P_i and Al_o contents were positively correlated and P_o and Al_d contents were negatively correlated but not P_i and Al_d nor P_o and Al_o contents (Fig. 2a; Fig. 2b).

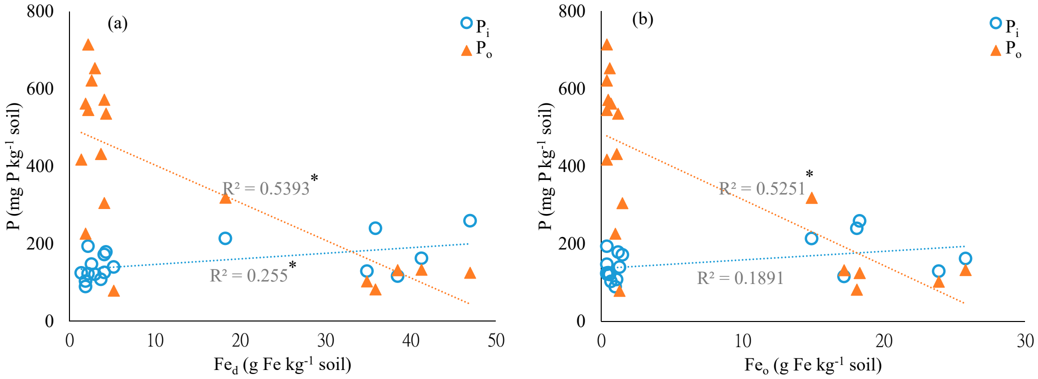

Figure 1. Correlations of contents of P_i and P_o with Fe_d (a) and Fe_o (b) in the pedon samples collected from the three sampling sites. * Statistically significant at $P<0.05$.

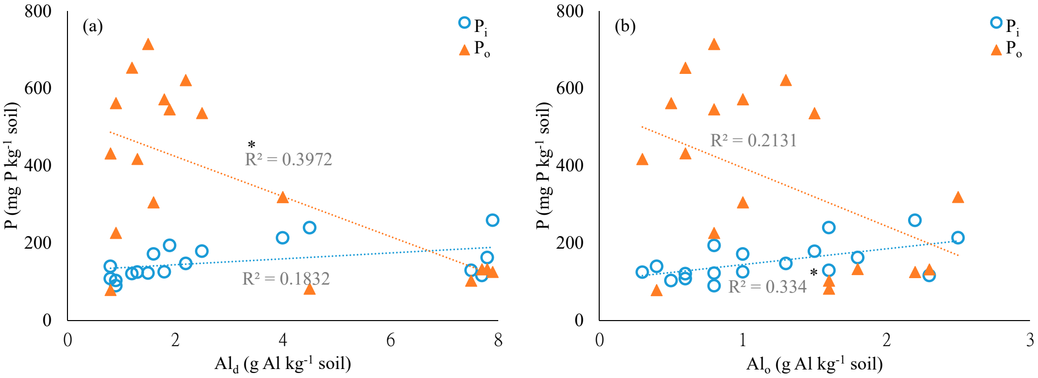

Figure 2. Correlations of contents of P_i and P_o with Al_d (a) and Al_o (b) in the pedon samples collected from the three sampling sites. * Statistically significant at $P<0.05$.

Contents of total P and summed P and P_i in the O/A horizons were greater at the footslope than the lakeshore, whereas the values in the summit site were in between those at the footslope and lakeshore. In addition, summed P_o and residual P contents were similar among the three sites (Table 2). HCl-P_i and cHCl-P_i contents were similar among the three sites, whereas cHCl-P_o content in the footslope soil was similar to that at the lakeshore but higher than that at the summit. NaOH-P_i content was higher at the footslope than the summit and lakeshore. In addition, NaOH-P_o content was higher at the summit than the footslope and lakeshore. $NaHCO_3$ extracted P_i content was higher at the summit than the footslope and lakeshore, whereas $NaHCO_3$-P_o content was the highest at the footslope and was similar at the summit and lakeshore. Resin-P_i content was higher at the lakeshore than the footslope, and resin-P_i content at the summit was in between that at the other two sites.

Table 2. The fractionation of P (mg kg^{-1}) in humic soil samples (O/A horizon) in different topographic sites (sequential extraction)

Site	Inorganic P in extracts					Summed inorganic P (P_i)	Organic P in extracts			Summed organic P (P_o)	Residual-P	Summed P	Total P
	Resin-P_i	$NaHCO_3$-P_i	NaOH-P_i	HCl-P_i^1	cHCl-P_i		$NaHCO_3$-P_o	NaOH-P_o	cHCl-P_o				
Summit	4.8^{ab}	61.2^{a}	46.4^{b}	2.3	9.4	124.1^{ab}	124.4^{b}	415.6^{a}	20.6^{b}	560.6^{ab}	38.0	722.7^{ab}	757.0^{ab}
Footslope	2.4^{b}	35.3^{b}	83.3^{a}	4.4	4.7	130.1^{a}	272.7^{a}	335.3^{ab}	31.6^{a}	639.6^{a}	29.4	799.0^{a}	828.3^{a}
Lakeshore	7.3^{a}	25.4^{b}	36.4^{b}	5.9	9.9	84.8^{b}	177.5^{b}	306.5^{b}	27.6^{ab}	511.5^{b}	34.9	631.2^{b}	668.2^{b}

HCl-P_i: inorganic P extracted by 1.0 M HCl; cHCl-P_i: inorganic P extracted by concentrated HCl; cHCl-P_o: organic P extracted by concentrated HCl; Summed inorganic P: Resin-P_i + $NaHCO_3$-P_i + NaOH-P_i + HCl-P_i + cHCl-P_i; Summed organic P: $NaHCO_3$-P_o+NaOH-P_o + cHCl-P_o; Summed P: sum of P_i + P_o + residual P. Means followed by the same letters in the same column are not significantly different ($p > 0.05$) by Tukey's honestly significant difference test.

Organic P was the dominant P fraction in the mountain forest soils of the three sites (Table 3). Moreover, NaOH-P_o represented the major P fraction and contributed to more than 40% of the summed P content in the three sampling sites (Fig. 3). $NaHCO_3$-P_o was the second most abundant P fraction among the three sites and contributed more than 20% of the summed P content. Summed P_i content (resin-P_i + $NaHCO_3$-P_i + NaOH-P_i + HCl-P_i + cHCl-P_i) in surface soils of all study sites contained less than 18% of summed P, and NaOH-P_i was the major P_i fraction in total P_i.

Table 3. Relative proportions of total P extracted for inorganic (P_i) and organic (P_o) forms in NaOH-EDTA extracts from humic soil samples (O/A horizon) determined by chemical extraction and ^{31}P-NMR spectroscopy.

	P_i		P_o	
Soil	Chemical§	NMR	Chemical§	NMR
	----------------------	%	-------------------------	
Summit	17.2±2.8	17.5±1.2	77.5±2.9	82.5±3.7
Footslope	16.3±2.1	17.3±1.4	80.0±2.4	82.7±2.7
Lakeshore	16.9±1.4	11.6±1.7	81.1±1.5	88.4±5.7

§ P_i: sum of resin-P_i + $NaHCO_3$-P_i + NaOH-P_i + HCl-P_i + cHCl-P_i. P_o: sum of $NaHCO_3$-P_o + NaOH-P_o + cHCl-P_o.

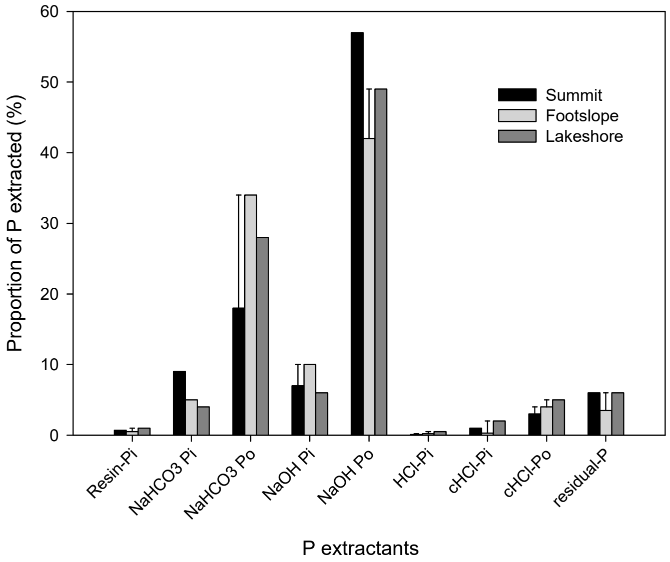

Figure 3. Proportion of P fractions in humic samples at different sites determined by chemical extraction.

Spectra obtained from ^{31}P-NMR analysis of NaOH-EDTA extracts revealed inorganic orthophosphate, orthophosphate monoesters, orthophosphate diesters, pyrophosphates, and phosphonates in the soil extracts (Fig. 4).

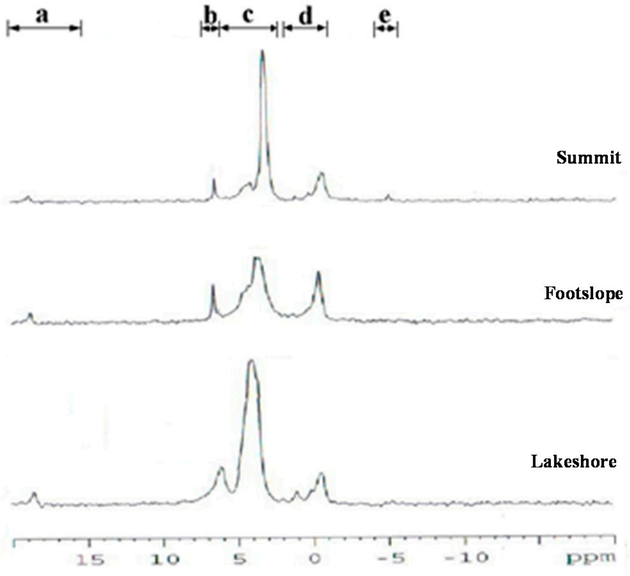

Figure 4. ^{31}P-NMR spectra for NaOH-EDTA extracts from soils at different sites. a: phosphonate, b: inorganic orthophosphate, c: orthophosphate monoesters, d: orthophosphate diesters, e: pyrophosphate

Organic P compounds identified in the NaOH-EDTA extracts included orthophosphate monoesters, orthophosphate diesters, and phosphonates. Orthophosphate monoesters were the

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predominant species of extracted organic P in soil from all sites and contributed to more than 60% of the total P fractions (Fig. 3; Fig. 5). The proportion of orthophosphate diesters was much lower than that of orthophosphate monoesters and only contributed 15% to 20% of the total P pools. Content of phosphonates (18.7 ppm) ranged from only 2.2% to 4.0% of extracted P from the three sites, and the highest content was found at lakeshore, with waterlogged conditions.

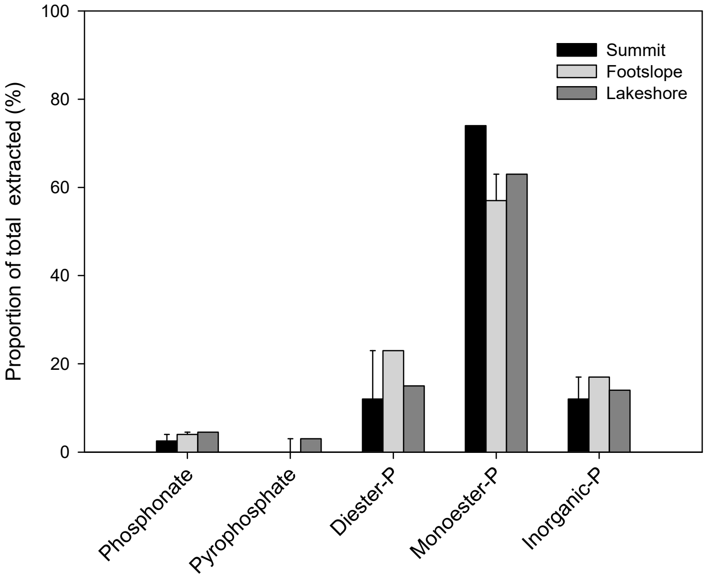

Figure 5. Proportion of extracted P in various classes from humic samples at different sites determined by ^{31}P-NMR spectroscopy.

Inorganic P compounds identified in the NaOH-EDTA extracts included orthophosphate and pyrophosphates. Inorganic orthophosphate signals at 6.1-6.3 ppm ranged from 11.6% to 17.3% of the spectral area for all study sites (Table 3). The highest inorganic P content was found at the footslope. In addition, small additional pyrophosphate resonance (-4.3 ppm) was observed only at the summit site.

4. Discussion

4.1. Soil physiochemical properties and chemical extractable P

The soil in this study site contains high moisture because of the year-round high precipitation [43]. High soil moisture in mountainous forests retards decomposition of soil organic matter, and high precipitation increases the loss of cations, thereby resulting in decreased soil pH and Eh [36,44,45]. This well explains our observations of low soil pH in the studied sites. Moreover, our previous study at the same sites revealed a clay and silt-clay layer under the organic layer [43]. This clay layer may retard the percolation [46] and therefore result in reduced soil TOC, TN and TP contents with increasing soil depth.

The high Fe_o, Fe_d, Al_o and Al_d contents in the B horizons implied that iron moved downwards and accumulated in the subsoil. Because high soil moisture and rich organic matter decreased soil

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redox potential in the surface layer, reduced Fe and Al ions moving from surface to the bottom layer were re-oxidized in the B horizons [47,48]. This formation of accumulated Fe, which may due to the redoximorphic process [49,50], created a placic-like horizon and resulted in slow permeability of P in such perhumid forest soil [47].

The positive correlations between P_i and Fe_d but not P_i and Fe_o contents implied that most inorganic P may be adsorbed by crystalline Fe oxides in soils, whereas the negative correlation between P_o and Fe_d as well as P_o and Fe_o contents implied that organic P was in a complex formation in the soils. In addition, the vertical increase in P_i content, with an opposite trend to P_o content, in each soil profile was significantly related to the content of amorphous and crystalline Fe oxides but not crystalline Al oxides. This observation implied that Fe oxides rather than Al oxides may chemically bind with P_i, and the accumulation of P_i in the subsoils could relate to the downward percolation and reoxidation of Fe. Sollins *et al.* [51] found that soil with high Fe hydrous oxides content tends to irreversibly fix polyvalent oxyanions such as phosphate because of chemosorption and occlusion. A similar trend was found in our previous study of subalpine forest soils [52], in which significant sorption of P_i to sesquioxides was via downward migration.

In addition, acidic soil conditions (pH<4) typically facilitate Fe oxides reduction (i.e., Fe^{2+}), which may help the mobility of Fe in soil [53]. The acidic soils in this study site may further affect the mobility of P_i, thereby resulting in low P_i content in the three study sites.

The depth of O horizons increased from the summit to the lakeshore, which suggests a process of erosion–deposition. In addition, because the mineral clay layer and placic-like horizon reduced vertical percolation, most of the soil organic matter in the water flow is transported laterally [31,43]. This can help the downhill movement of soil nutrients in the O horizon [43,47] and may explain the increased soil TOC and TN contents from the summit to the footslope.

4.2. Chemical extraction of soil P

Because of the low overall P_i concentrations in the three study sites, the different chemical extractable P_i contents increased downhill but not significantly. In addition, the low labile P fractions, $NaHCO_3$-P_i and NaOH-P_i, in the lakeshore may also be due to the vigorous fluctuation of the water level of the lake after showers or storms, which could remove the suspended particles or detritus of litter with the flooding and reduce the accumulation of P in the soil near the lakeshore [28]. This was indirectly supported by the elevated TP concentration in the epilimnion of lake after medium rainfall events (256-620mm) [54].

P_o appeared to be the predominant fraction in the perhumid forest and was mostly non-acid extractable. Because the soil is acidic in the study sites, most P_o fractions may not be labile and remained at higher values at the summit than at the lakeshore. The increase in total P_o content in the surface horizon has been attributed to the input and accumulation of organic matter [55] and factors affected by topography can influence the availability of soil P [56].

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Extracted soil P compounds showed that the contents of highly labile ($NaHCO_3$-P_o), long-term P transformation (NaOH-P_o) and stable residual pool (cHCl-P_o) fractions changed between different sites, which showed that slope position affects the various P pools [57]. As shown in Figure 3, the sum of the highly labile P_o fraction ($NaHCO_3$-P_o) and long-term P transformation (NaOH-P_o) contributed more than 75% of the total extractable P in soils at all study sites, so organic P was the major P source in these soils. The P_o accumulation in soil surfaces resulted from the biological cycling of P through the plant litter to the soil surface.

The cHCl-P_i extract has recalcitrant P forms associated with mainly Fe oxides and/or P derived from non-alkaline extractable debris, whereas cHCl-P_o may include both stable, little and/or bioavailable (non-alkaline extractable) P forms. However, the proportions of cHCl-extractable P_i and P_o were only about 4.5% to 7.5% of sequentially extracted total P in soils of all study sites. The residual P is associated with highly organic materials such as lignin and organometallic complexes [58], but we have no information on the composition of organic matter in this fraction.

4.3. Spectra of ^{31}P-NMR analyses

Orthophosphate monoesters are the most common forms of organic P_o in soils [59-61]. Monoester P includes high proportions of inositol phosphate, sugar phosphate and choline phosphate primarily derived from plant, animal and microbial residues [62].

Depending on the soil types, inositol phosphates are reported to be the predominant organic P forms in Podosols [26], whereas α- and β-glycerophosphate are the predominant organic P forms in Vertosols [63]. In addition, high inositol phosphate contents were reported from several studies with cold and wet climates [64-66].

Inositol phosphates are typically considered of limited bioavailability because of the complex structure with soil minerals, clays, and humic compounds [60,67]. Although ^{31}P-NMR analysis in our study had limited resolution to identify the inositol phosphates content, the low temperature and high precipitation of the study site may likely result in high inositol phosphates content in the soil.

Orthophosphate diesters at about 0 ppm [40] can be further classified into nucleic acids (-1–0 ppm) and phospholipids (0–2 ppm) [60]. Orthophosphate diesters, including nucleic and phospholipids, frequently accumulate in cool and moist acidic forest soils with low microbial activities than in agricultural soils [68-70]. In acidic or wet soils, diester P proportion is between 10% and 36% of extracted P [70-72]. Our findings are consistent with previous studies because the diester P proportion was between 15% and 20% of extracted P in the study sites.

A higher proportion of diester P providing a labile source for available P [70] was found at the footslope site, with poor drainage, than at the other sites. The lower orthophosphate diesters than monoesters content in the three study sites may contribute to the complexity of the chemical compounds. Orthophosphate diesters are more rapidly mineralizable than monoesters because they are generally less adsorbed to soil colloids than monoesters and can be easily hydrolyzed [22,59,74].

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The content of phosphonates in soil are due to bacteria such as *Bacillus cereus*, which has a phosphonatase enzyme that produces phosphonates, but the bacteria are less prevalent in acidic soil [42,72,75]. This observation may explain the low phosphonates concentrations observed in the study sites.

A small amount of pyrophosphate resonance (-4.3 ppm) was observed in spectra of soils at the three sites. Pyrophosphate is believed to be involved in biological P cycling in the soils and may be present in relatively well-drained soil that provides a proper environment for microbial activity and fungus [76]. In addition, pyrophosphate is contributed by fungal P compounds [77]. Because the surface soil of the three sites contained high soil organic matter and high soil moisture, it may provide a less favorable environment for microbial and fungal activities, resulting in low pyrophosphate concentrations.

Organic P represented between 82-88% and 77-81% of total P extracted by NaOH-EDTA and by chemical extraction from all sites studied, respectively. This result is similar to Cade-Menun and Preston [78], who found 77% to 83% of P_o in a low-pH forest perhaps because of the low decomposition of P_o compounds in acidic forest soils that reduced the P_i concentrations [42].

The signal intensity of ^{31}P-NMR spectra caused by paramagnetic Al, Fe and Mn in soils may reduce the spectra quality [45,79,80]. Moreover, chemical hydrolysis of P_o to P_i may occur during alkaline extractions [78,81]. However, in general, the results from NMR analysis were consistent with those of chemical fractionation in this study and other alpine and subalpine forest soils [26,47,52,66].

5. Conclusions

This study demonstrated that soil chemical extractable P_i and P_o can be vertically affected by the formation of Fe oxides in soils. Because of a clay layer combined with a placic-like horizon in the subsoil in our test site, both total P and P_o contents were decreased with increasing soil depth, with P_i content slightly increased in different soil horizons. The low permeable soil layer also favored downhill run-off, however, because P_i contents were relatively low as compared with P_o contents, the contents did not significantly differ among the three study sites. Because most of the P was in organic forms, a negligible amount of P_i may be released to the lake along the slope. Therefore, although topography and soil formation processes affect the distribution of soil P, high precipitation and year-round high humidity might be important for differentiation of the P species in this landscape. Moreover, the similarity of the ^{31}P-NMR spectra among the three sampling sites supports the alleviated differentiation of the P species in this landscape.

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