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Characterization of Phosphorus in Subtropical Coastal Sand Dune Forest Soils

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Abstract: Continuous research into the availability of phosphorus (P) in forest soil is critical for sustainable management of forest ecosystems. In this study, we used sequential chemical extraction and ^{31}P-nuclear magnetic resonance spectroscopy (^{31}P-NMR) to evaluate the form and distribution of inorganic P ($P_{i)}$ and organic P (P_o) in *Casuarina* forest soils of a subtropical coastal sand dune at Houlong in Taiwan. The soil samples were collected from humic (+2-0 cm) and mineral layers (mineral-I: 0-10, mineral-II: 10-20 cm) at two topographic locations (upland and lowland) by elevation. Sequential chemical extraction revealed that the NaOH-P_o fraction, as moderately recalcitrant P, was the dominant form in humic and mineral-I layers in both upland and lowland soils, whereas the cHCl-P_i fraction was the dominant form in the mineral-II layer. Resistant P content, including NaOH-P_i, HCl-P_i, cHCl-P_i, and cHCl-P_o fractions, was higher in the upland than lowland in the corresponding layers; however, labile P content, $NaHCO_3$-P_o, showed the opposite pattern. Content of resistant P_i (NaOH-P_i, HCl-P_i, and cHCl-P_i) increased significantly with depth, but that of labile P_i (resin-P_i and $NaHCO_3$-P_i) and recalcitrant P_o ($NaHCO_3$-P_o, NaOH-P_o, and cHCl-P_o) decreased significantly with depth at both locations. ^{31}P-NMR spectroscopy revealed inorganic orthophosphate and monoesters-P as the major forms in this area. The proportions of P_i and P_o evaluated by sequential chemical extraction and ^{31}P-NMR spectroscopy were basically consistent. The results indicated that the soils were in weathered conditions. Furthermore, the P distribution and forms significantly differed between the upland and lowland by variation in elevation and eolian aggradation effects in this coastal sand dune landscape.

Keywords: sequential chemical extraction; ^{31}P-nuclear magnetic resonance spectroscopy (^{31}P-NMR); phosphorus; coastal sand dune; *Casuarina* forests

1. Introduction

Phosphorus (P) plays an essential role in nutrient cycles [1], and the availability of P in soil is controlled by biogeochemical processes [2]. Investigating P in soil organic matter is often overlooked in many studies [1]. As compared with nitrogen, which can be replenished through biological N_2 fixation, sources of total P are weathering bedrocks and atmospheric deposits [3], and the loss of P due to leaching or conversion into occluded P can be replenished by a weathering process [4]. Phosphorus dynamics and availability in soil is determined by soil properties, soil types, parent materials, vegetation, environmental conditions and land management practices [5].

Organic P (P_o) is important in biogeochemical cycling [6]. P_o contains a variety of compounds in the soil environment with different stability and biological availability [3,7]. The chemical composition of P and plants/microbes have interactive effects on each other [3]. When inorganic P (P_i) content decreases, P_o can be decomposed by microbial communities to replenish the limited P_i. Determining the relation between P_i and P_o is important to understand P availability and predict soil development [4]. In addition, microbial respiration rate and organic matter decomposition was affected by the amount of P, labile carbon (C) and nitrogen (N) in soil [8]. We need to understand the sources and transformations of P_o because they can provide information for the development of sustainable management practices and predicting nutrient changes (e.g., C: N: P) and biogeochemistry processes [9].

Hedley et al. [10] developed chemical fractionation techniques to quantify different pools of P based on different availability and used the sequential chemical extraction method to estimate subtle changes in P pools in cultivated field soil. The chemical sequential extraction results for P were used to evaluate mineralization, microbial turnover, and plant-utilization mechanisms and to compare with carbon and nitrogen dynamics [11]. However, sequential chemical extraction could not evaluate the structural composition of the different P forms; thus, ^{31}P-NMR spectroscopy was developed to improve on the deficiencies [2,12]. In fact, ^{31}P-NMR spectroscopy is less complicated to use than chemical extraction methods for characterizing organic P [13,14]. Furthermore, it can be difficult to compare research results with the use of different chemical extractants [9]; thus, ^{31}P-NMR has been widely used to quantify soil organic P and compare P forms with standard extractants (e.g., NaOH-ETDA) [15,16].

Sand dune is a typical landscape on the western coast of Taiwan, and *Casuarinas* forests were planted 8 decades ago to stabilize the sand dune and preserve the inland vegetation [17]. This type of forest was grown under a hostile environment, including drought, low fertility, and high soil salinity. As well, this region is in a subtropical area with high temperature and abundant rainfall; the soil is weakly developed from sand and has low water-holding capacity. Because P is generally limited in forest ecosystems [18-20], we need to evaluate the plant-available P and characterize the availability of organic P for these ecosystems [21].

Negassa and Leinweber [22] indicated that only a few studies have characterized P in soils by comparison of ^{31}P-NMR and sequential chemical extraction; thus, more natural ecosystems and anthropogenic field experiments are required for evaluating the basic principles and mechanisms of P transformation [9]. Although several authors have investigated P distribution and forms in sand dune forest soils in temperate areas [3,4], to our knowledge, our research is the first to characterize P in a subtropical sand dune forest.

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Phosphorus distribution and forms are influenced by changes in soil oxidation-reduction along a toposequence [23]. We hypothesized that P distribution and forms are affected by different topographies (upland and lowland) in a sand dune under varied soil conditions and biogeochemical processes. Thus, the main objective of this research was to evaluate the effect of topography on P composition in a sand dune ecosystem by using sequential chemical extraction and ^{31}P-NMR methods.

2. Materials and Methods

2.1. Site description

The study site was at the coastal sand dune forests at Houlong, Miaoli County, northwestern Taiwan (24˚39'N, 121˚54'E). The mean annual temperature is 22.4 ℃. The climate is hot and wet in the summer (mean temperature 28.6 ℃) and mild and dry in the winter (mean temperature 16.3 ℃). The mean annual rainfall is about 1700 mm, with a rainfall season from February to August. The rainfall is relatively low from September to January. *Casuarina spp.* as the dominant vegetation was established more than 8 decades ago to protect the inland plants and stabilize the sand dunes. *Hibiscus tiliaceus* and *Broussonetia papyrifera* are less dominant tree species. *Lantana camara*, as an understory shrub, distributes commonly in the forests. According to the topography of the sand dune forests, the study site was separated into two locations: upland and lowland. Upland areas distribute on the flat ridges of the sand dune and lowland areas face toward the inland side. The average slope between upland and lowland is 5%. The distance and mean elevation between the two locations are about 200 m and 6 m, respectively.

The soils at the two locations are classified as Typic Udipsamment, which contains a very shallow litter layer (1-2 cm) and humic layer (2-3 cm) on weakly developed sandy soil [17,24]. Soil samples were collected from three layers: humic (+2-0 cm), mineral-I (0-10 cm), and mineral-II (10-20 cm) at the upland and lowland. The general soil physical and chemical properties of the study site are summarized in Table 1. The soil texture was sand, and the soil pH values increased with depth at both locations. In addition, the content of organic carbon (C), total nitrogen (N), cation exchange capacity (CEC), and moisture was highest in the humic layer and decreased sharply in the two mineral layers. The content of crystalline iron (Fe_d) and aluminum (Al_d) oxides, amorphous iron (Fe_o) and aluminum (Al_o) oxides was similar in the two mineral layers. Because both locations are close, the climate features and mineral compositions of the parent material were similar. Thus, the typographic variations might be the most important factors causing the different distribution of P forms.

Table 1. General physical and chemical properties of studied coastal forest soils.

Site	Layer	Texture	pH (H_2O)	Organic C (%)	Total N (%)	Moisture (%)	CEC ($\mathrm{cmol}_{(+)}\ \mathrm{kg}^{-1}$)	Fe_d (g/kg)	Fe_o (g/kg)	Al_d (g/kg)	Al_o (g/kg)
Upland	Humic (+2-0 cm)	–	4.2	16.2 A	1.2 A	45.0 A	45.0	–	–	–	–
	Mineral-I (0-10 cm)	Sand	4.9	1.1 b	0.1 b	3.7 a	7.9	5.8	1.6	1.5	0.7
	Mineral-II (10-20 cm)	Sand	6.6	0.1 c	0.1 c	3.4 a	3.8	6	1.7	2.1	0.9
Lowland	Humic (+2-0 cm)	–	4.2	9.6 B	0.7 B	27.6 B	13.1	–	–	–	–
	Mineral-I (0-10 cm)	Loamy sand	4.7	1.4 a	0.1 a	5.0 a	2.8	4.6	1.3	1.9	0.6
	Mineral-II (10-20 cm)	Sand	5.2	0.3 c	0.1 c	4.1 a	2.1	5.3	1.5	1.7	0.8

Soil pH, organic C, total N contents and moisture were adapted from Chen et al. (2005); Values followed by the same letters in each column are not significantly different ($p > 0.05$) by Duncan's multiple range test; Capital letters and lowercase letters represent humic and soil mineral layers, respectively.

2.2. Soil sampling

At the two locations, one composite sample consisting of three subsamples was collected from each of the three layers. Soil samples in the humic layer (+2-0 cm) were collected with 50×50 cm quadrats after undecomposed litter/roots were removed carefully. The samples of the underneath mineral layers (mineral-I [0-10 cm] and mineral-II [10-20 cm]) were collected by using a soil auger with 4.25-cm diameter. Before the soil samples were sieved through a 2-mm sieve, the litter/roots were manually removed from the samples once more. Then, the soil samples were air-dried.

2.3. Sequential chemical extraction of soil P

A method of sequential chemical extraction of P [25] was used to obtain various P_i and P_o forms. The general concept for the P fractionation method is based on the bioavailability and chemical forms of P in specific reagents. The extraction procedure was started with 0.5 g air-dried soil. First, an anion exchange resin was used to extract biologically available P_i [26], then the other P_i was extracted by applying 0.5 M $NaHCO_3$, 0.1 M NaOH, and 1M HCl solutions sequentially. $NaHCO_3$ and NaOH was used to extract P, which was adsorptive to soil colloids [27] and combined strongly with Fe and Al [11]. The 1 M HCl extraction was specific for extracting Ca-associated P [28]. The residue was then extracted with concentrated HCl (cHCl) to evaluate P in stable residual pools [25] and particulate organic debris.

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In each extraction, the soil-extractant solution was centrifuged at 20,000 × *g* for 10 min and filtered through Whatman No. 42 filter paper. Then, P_i in each extraction was determined by the colorimetric method with the malachite green procedure [29]. The extraction was then digested with H_2SO_4 (97%) and H_2O_2 (30%) at 300 °C, and P in digested solution was determined by the colorimetric method. P_o in each extraction was determined by subtracting P_i from total P in the solution. For data presentation, the P fractions extracted in this study were grouped: (1) P_i forms: resin-P_i, $NaHCO_3$-P_i, NaOH-P_i, HCl-P_i, and cHCl-P_i; (2) P_o forms: $NaHCO_3$-P_o, NaOH-P_o, and cHCl-P_o. Total P (TP) of the soil samples was determined by digesting the soil with H_2SO_4 (97%) and H_2O_2 (30%) at 300 °C.

2.4. ^{31}P-NMR measurements

Air-dried soil (5 g) was dispersed in 20 mL of 0.25 M NaOH-0.05 M EDTA (1:1) for 2 h at 76 °C. To reduce paramagnetic interference of iron and other metals, the chelating resin was added in the soil mixture for 6 h at room temperature. Then, the resin was removed by filtering the soil mixture with Whatman 42 filter paper. The remaining soil mixture was centrifuged at 10000 × *g* for 30 min and the extractant was freeze-dried for storage. Then the freeze-dried extractant (0.1 g) was dissolved in 0.5 mL of 0.5 M NaOH and 0.1 mL D_2O. Finally, the solution was transferred to a 5-mm NMR tube for ^{31}P-NMR analysis [30].

The ^{31}P-NMR spectra were attained at 242.86 MHz and 25 °C. on a Bruker-600 NMR spectrometer with a 60° pulse, 3.5-s delay and 0.33-s acquisition time. To overcome the nuclear Overhauser enhancement and obtain a quantitative result, the ^{31}P spectra were proton-decoupled by applying an inverse-gated pulse sequence [12,31]. Depending on the P content in the alkaline extract, 500 to 2500 scans were applied to obtain an acceptable signal-to-noise ratio. Spectra were verified with a line-broadening of 20 Hz. The chemical shift was evaluated relative to an external 85% H_3PO_4/D_2O standard. Quantities of P components (inorganic orthophosphate, phosphonate, orthophosphate monoesters, orthophosphate diesters, pyrophosphate, polyphosphates) were estimated by relative peak areas calculated by electronic integration [18,30,32]. The inorganic orthophosphate and orthophosphate monoester signals were detected on the basis of a boundary determined from the valley between the two signals to the baseline [18].

2.5. Statistical analyses

The three subsamples (replicates) were used for sequential chemical extraction and ^{31}P-NMR measurements. To evaluate the main effect of two variables (location [upland and lowland] and layer [humic, mineral-I, and mineral-II]) and the interaction effect between the two variables (location × layer) for P composition and forms, two-way ANOVA and Tukey's honestly significant difference (HSD) test were applied with use of R software [33]. $P < 0.05$ was considered statistically significant.

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3. Results

3.1. Sequential chemical extraction of soil P

The concentrations and statistical results of sequential chemical P-extraction are summarized in Table 2. As stated by several authors [25,34], resin-P_i and $NaHCO_3$-P are labile forms of P, NaOH-P is moderately recalcitrant, HCl-P and cHCl-P are recalcitrant, and residual-P is highly recalcitrant. Content of the labile P_i forms (resin-P_i and $NaHCO_3$-P_i) decreased significantly with depth at both locations (Table 2). However, content of NaOH-P_i, HCl-P_i, and cHCl-P_i as the recalcitrant P_i forms, increased significantly from the humic to mineral-I and mineral-II layers at the upland and lowland sites. The content decreased with depth for all P_o forms, residual-P, and TP.

When considering the effect of location, besides $NaHCO_3$-P_o, the content of NaOH-P_i, HCl-P_i, cHCl-P_i, sum of P_i, cHCl-P_o, residual-P, sum of P, and TP were significantly higher in the upland than lowland. For the location × layer effect, the content of residual-P, sum of P and TP in the humic layer was significantly greater in the upland than lowland and that of NaOH-P_i and cHCl-P_o in the mineral-I layer was significantly greater in the upland than lowland. The content of NaOH-P_i and HCl-P_i in the mineral-II layer was significantly lower in the lowland than upland.

The summation of P_o ($NaHCO_3$-P_o + NaOH-P_o + cHCl-P_o) was greater than 55% of the total extractable P in the humic layer; thus, P_o was the major source in humic soils (Figure 1 and Figure 2). NaOH-P_o and $NaHCO_3$-P_o were the most abundant P fractions in the humic layer. The summation of P_i (resin-P_i + $NaHCO_3$-P_i + NaOH-P_i + HCl-P_i + cHCl-P_i) was slightly greater than the summation of P_o in mineral-I soils in the upland site. However, in the lowland, the summation of P_i was slightly lower than that of P_o in the mineral-I layer. Nevertheless, NaOH-P_o and $NaHCO_3$-P_o were still the major P fractions in mineral-I soils. The P_i forms were the dominant sources, and HCl-P_i as well as cHCl-P_i were the most abundant P_i fractions in mineral-II soils.

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Table 2. Summary of P concentrations (mean values, unit: mg kg^{-1}) and two-way ANOVA significance tests (p-value) for sequential chemical extraction.

Site	Layer	Inorganic P (P_i)					Sum of P_i	Organic P (P_o)			Sum of P_o	Residual-P	Sum of P	TP
		Resin-P_i	$NaHCO_3$-P_i	NaOH-P_i	HCl-P_i	cHCl-P_i		$NaHCO_3$-P_o	NaOH-P_o	cHCl-P_o				
Upland	Humic (+2-0 cm)	60.9	50.0	18.2	11.5	28.9	169.5	85.5	170.1	55.2	310.8	78.5	558.8	633.8
	Mineral 1 (0-10 cm)	24.5	22.6	27.5	19.7	40.0	134.4	42.7	55.5	34.8	132.9	43.3	310.6	379.1
	Mineral 2 (10-20 cm)	13.0	17.4	48.6	54.3	54.1	187.4	16.1	37.3	12.3	65.7	22.8	275.9	275.0
Lowland	Humic (+2-0 cm)	54.3	42.4	10.2	9.5	24.5	140.8	91.9	149.1	45.6	286.6	55.7	483.1	501.3
	Mineral 1 (0-10 cm)	25.3	21.5	15.1	24.6	33.6	120.1	56.3	66.6	22.2	145.1	43.2	308.4	376.4
	Mineral 2 (10-20 cm)	15.4	19.6	20.4	33.7	44.6	133.7	27.6	41.2	18.2	87.0	22.4	243.1	210.9

Two-way ANOVA significance tests (p-value)

Effects	Resin-P_i	$NaHCO_3$-P_i	NaOH-P_i	HCl-P_i	cHCl-P_i	Sum of P_i	$NaHCO_3$-P_o	NaOH-P_o	cHCl-P_o	Sum of P_o	Residual-P	Sum of P	TP
Locations	0.673	0.407	2.16×10^{-7} *	4.95×10^{-4} *	0.015 *	2.57×10^{-4} *	4.22×10^{-3} *	0.817	0.016*	0.711	7.04×10^{-3} *	1.54×10^{-3} *	1.66×10^{-3} *
Layers	3.82×10^{-8} *	1.83×10^{-6} *	6.03×10^{-7} *	1.67×10^{-10} *	2.08×10^{-5} *	2.07×10^{-3} *	1.62×10^{-9} *	1.45×10^{-7} *	1.72×10^{-8} *	1.9×10^{-10} *	1.46×10^{-8} *	4.3×10^{-11} *	7.16×10^{-9} *
Location x Layer	0.366	0.306	4.34×10^{-4} *	6.82×10^{-6} *	0.687	0.068	0.611	0.302	4.5×10^{-3} *	0.091	2.94×10^{-3} *	0.020	0.024 *

HCl-P_i: inorganic P extracted by 1.0 M HCl; cHCl-P_i: inorganic P extracted by 11.3 M HCl; cHCl-P_o: organic P extracted by 11.3 M HCl; Sum of P_i: Resin-P_i+NaHCO3-P_i+NaOH-P_i+HCl-P_i+cHCl-P_i; Sum of P_o: $NaHCO_3$-P_o+NaOH-P_o+cHCl-P_o; Sum of P: summation of Resin-P_i + $NaHCO_3$-P_i + $NaHCO_3$-P_o + NaOH-P_i + NaOH-P_o + HCl-P_i + cHCl-P_i + cHCl-P_o + residual-P; TP: by separate H_2SO_4/H_2O_2 digest of whole sample; *: significant difference ($p < 0.05$).

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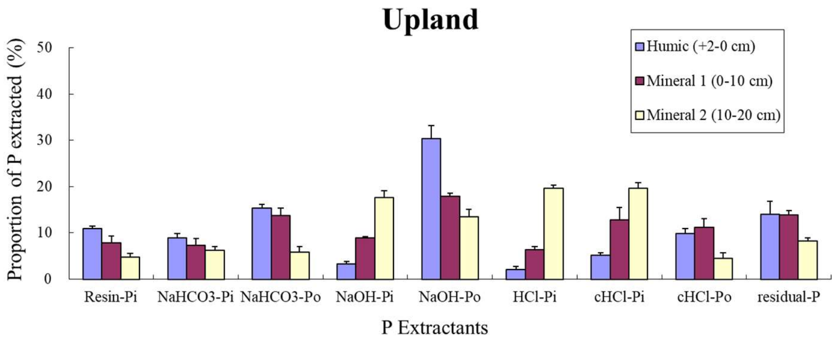

Figure 1. Proportion of P fractions from the studied coastal forest upland soils determined by the sequential-chemical extraction method.

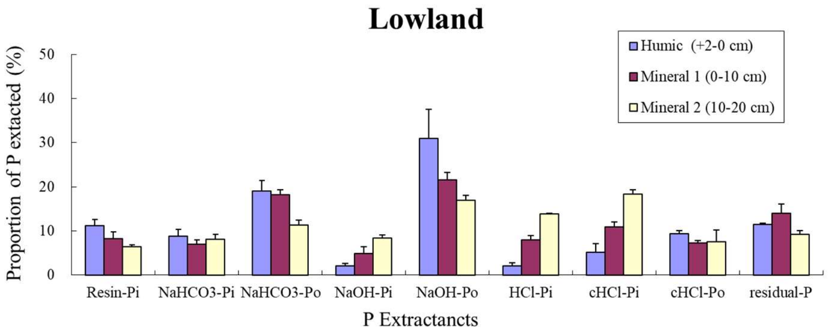

Figure 2. Proportion of P fractions from the studied coastal forest lowland soils determined by the sequential-chemical extraction method.

3.2. ^{31}P-NMR spectra analyses

The ^{31}P-NMR spectra for NaOH-EDTA extracts demonstrated the detection of inorganic orthophosphate, orthophosphate monoesters, orthophosphate diesters, and pyrophosphates in soil samples in this study site (Figure 3 and Figure 4). ^{31}P-NMR measurements revealed no phosphonate in soils. The forms of P were grouped into P_i compounds, including inorganic orthophosphate and pyrophosphates, and organic P compounds, including orthophosphate monoesters and orthophosphate diesters [12].

As compared with pyrophosphate, inorganic orthophosphate was the major form of P_i, and the relative proportions increased significantly with soil depth (Figure 5 and Table 3). In addition, the proportions of inorganic orthophosphate were greater in the upland than lowland.

The proportions were higher for orthophosphate monoesters than diesters in each soil layer. The orthophosphate monoesters were the predominant compounds of P_o. The relative proportions of orthophosphate monoesters and diesters significantly decreased with soil depth at the upland and lowland. Additionally, the proportions of monoester-P and diester-P were lower in the upland than lowland. ^{31}P-NMR results indicated that the proportions of monoester-P and diester-P in the mineral-II layer were significantly lower in the upland than lowland.

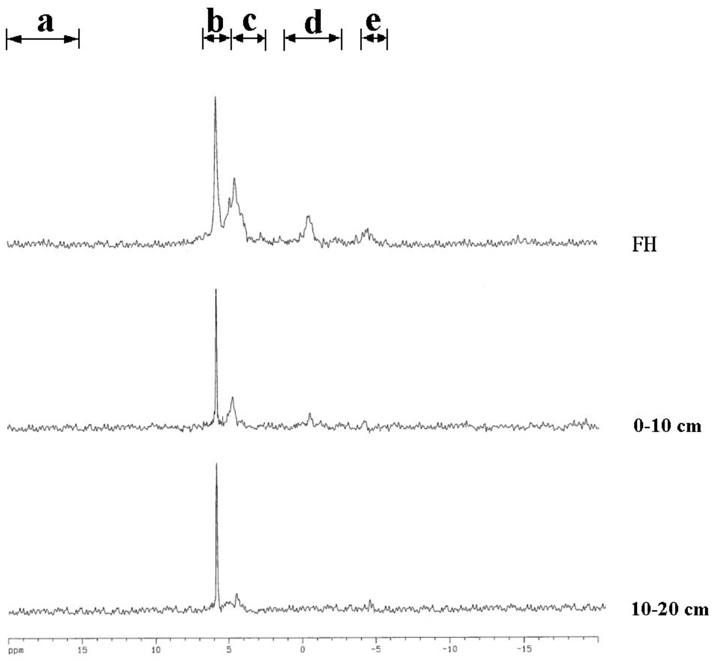

Figure 3. ^{31}P-NMR spectra for NaOH-EDTA extracts from humic and mineral layers of upland soils. a: phosphonate, b: inorganic orthophosphate, c: orthophosphate monoesters, d: orthophosphate diesters, e: pyrophosphate.

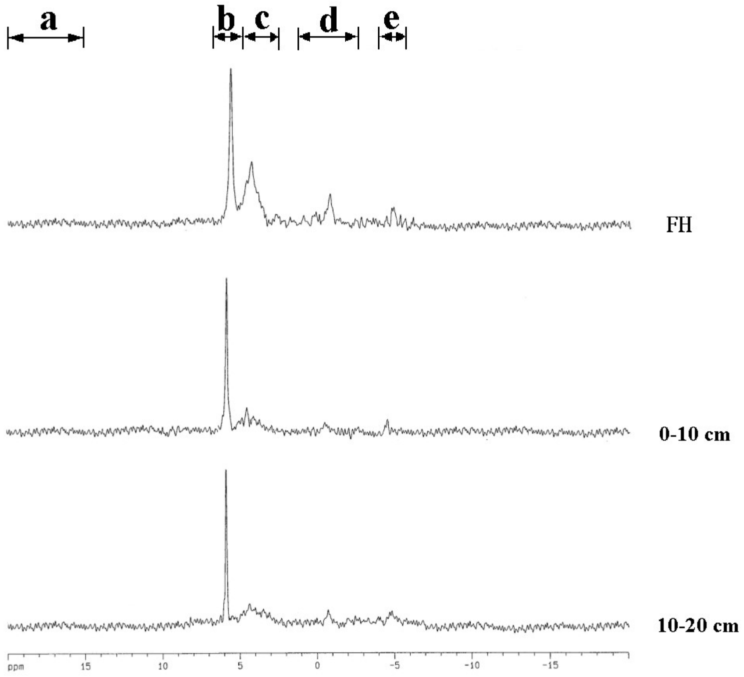

Figure 4. ^{31}P-NMR spectra for NaOH-EDTA extracts from humic and mineral layers of lowland soils. a: phosphonate, b: inorganic orthophosphate, c: orthophosphate monoesters, d: orthophosphate diesters, e: pyrophosphate.

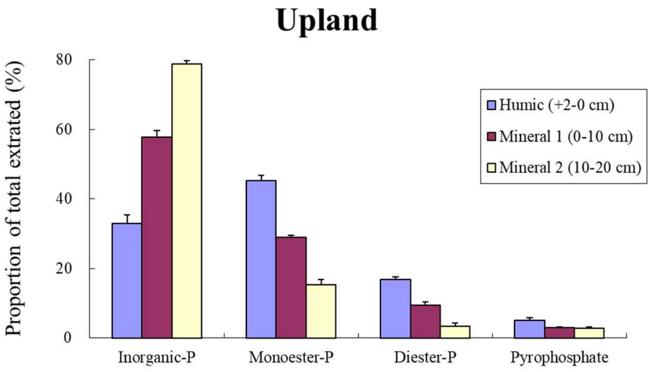

Figure 5. ^{31}P-NMR spectroscopy of proportion of extracted P in various classes from humic and mineral layers of upland soil.

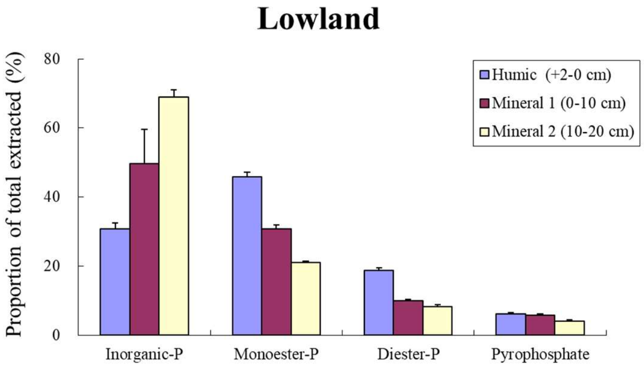

Figure 6. ^{31}P-NMR spectroscopy of proportion of extracted P in various classes from humic and mineral layers of lowland soil.

Table 3. Summary of two-way ANOVA significance tests (*p*-value) for ^{31}P-NMR spectroscopy.

Effects	Inorganic-P	Monoester-P	Diester-P	Pyrophosphate	Sum of P_i	Sum of P_o
Locations	7.00×10^{-3} *	3.85×10^{-4} *	9.94×10^{-6} *	1.63×10^{-6} *	0.031 *	9.44×10^{-7} *
Layers	5.4×10^{-9} *	7.48×10^{-14} *	4.79×10^{-12} *	2.19×10^{-6} *	9.42×10^{-9} *	1.41×10^{-15} *
Location x Layer	0.313	4.74×10^{-3} *	7.67×10^{-4} *	4.94×10^{-3} *	0.355	6.07×10^{-5} *

*: significant difference ($p < 0.05$).

4. Discussion

4.1. Sequential chemical extraction of soil P

From previous research at the same subtropical coastal sand dune forest site [35], the rapid decomposition of soil organic matter in the surface layers and low adsorption capacity of organic C in sandy soil enhanced the eluviation process in the soil profile. Thus, there was less accumulation of organic matter in the deeper depth in the soil profile [35]. In addition, most of the P was taken up by plants and returned as litter with P_o forms in soil [18,36]. As a result, P_o concentrations were highest in the humic layer at this site, with less accumulation of organic matter in mineral layers.

Resin-P was associated with the content of organic C, which indicated that resin-P was mineralized from organic matter [22]. Because organic C content was the highest in the humic layer and decreased in the deeper layers (Table 1), the trend of resin-P content was identical to organic C content. Labile P_i was positively related to microbial uptake in soil [10,37] and was replenished by dissolution or desorption mechanisms of less labile P_i and the mineralization of P_o [1,2]. Consequently, the labile P_i concentrations were also highest in the humic layer, and the labile P_i content was associated with P_o content at both upland and lowland sites.

However, as compared with labile P_i, resistant P_i content had an opposite pattern, which increased with depth. Phosphorus sorption was affected by variations in mineralogy between soil layers, with low sorption in organic matter–enriched top layers and high sorption in sesquioxide-enriched deep layers [1,34]. Because Fe and Al oxides were observed in mineral-I and mineral-II layers, sorption of the recalcitrant P_i to sesquioxides via downward migration may induce accumulation of the recalcitrant P_i in the two mineral layers. Additionally, the recalcitrant P_i may have originated from the parent materials.

Spatial variability of the distribution of P pools and total P were ascribed to parent materials and to redistribution in layers and landscapes [1]. Our previous study [38] confirmed that eolian aggradation was affected differently between the upland and lowland in this study area. Eolian aggradation occurred continually on the original surface soils, thereby leading to multi-stratification of soil profiles in the upland. However, eolian aggradation was much reduced at the inland side of the lowland. Because of more sources of P via eolian aggradation in the upland, TP content was higher in the upland than lowland. The large molecules of P forms (i.e., NaOH-P_i, HCl-P_i, cHCl-P_i, and cHCl-P_o) were less mobile than the small molecules of P forms (e.g., $NaHCO_3$-P_o). As well, residual-P was in large molecular-weight complexes (e.g., lignin and organometallic content) [10,39], which was not decomposed easily. As a result, the content of immobile P forms was higher in the upland than lowland.

Cross and Schlesinger [40] and Solomon and Lehmann [2] found a large proportion of P_o in highly weathered tropical soils. Because P_o was the major form in both the humic layer and mineral-I layer in our lowland site, a weathering process may occur in these sand dune soils. Additionally, the content of P was similar in the humic and mineral-I layers. The plant litter was decomposed via biological cycling, so a great quantity of P_o accumulated in soil surfaces [23]. NaOH-P_o is mineralized slowly [2,41], which explained the large amount of NaOH-P_o in the sand dune soils.

With sufficient moisture under warmer climates, the rate of microbial decomposition is higher and organic matter is decomposed rapidly [42], for less amount of organic matter accumulated in the mineral-II layer in our site. Because the recalcitrant P_i forms were not easily decomposed, most of the

HCl-P_i and cHCl-P content accumulated in the mineral-II soils. Thus, the proportion of P_i increased and that of P_o decreased with depth in the sand dune forests at Houlong.

4.2. ^{31}P-NMR spectra analyses

Decomposition processes are facilitated in a weakly acidic environment [18]. As the pH increased with depth, organic matter was decomposed rapidly in the deeper soil profiles in our site; thus, the proportion of inorganic orthophosphates increased with depth. As compared with orthophosphate diesters, orthophosphate monoesters were the dominant species of P_o pools. These patterns were consistent with temperate coastal sand dune forests in New Zealand [3]. However, as compared with findings from Turner et al. [3] (inorganic-P: 17-39%; monoester-P: 51-76%), the proportion of inorganic-P and orthophosphate monoesters was higher and lower, respectively, in mineral layers at our study site. Because the annual temperature was much higher and the distribution of rainfall was more uneven in our subtropical sand dune than a temperate sand dune [3], P_o was decomposed rapidly by microbial communities and led to the reduced P_o content in our study soils.

Orthophosphate monoesters are more stable than diester forms [1], so monoester-P could accumulate in soil with long residence time [43]. Soil organic matter in sandy soil is usually composed of particulate plant debris [44]; thus, we assumed that diester-P was plant-derived. In addition, diester-P is more labile and degrades more rapidly than monoester-P [2,45,46] and it also mineralizes and hydrolyzes more easily and is absorbed more weakly than monoester-P [2,3,18]. The above statements explain why the proportion was higher for orthophosphate monoesters than diesters in our soils.

Overall, the peak values of inorganic orthophosphate, pyrophosphate, and orthophosphate monoesters and diesters determined by ^{31}P-NMR were similar or identical to previous findings [15,18,47]. The peak signal positioned around 4.5 ppm was myo-inositol hexakiphosphate [9,48,49]; and the peak signal centered at -0.5 ppm was DNA [12,42]. *Myo*-inositol hexakiphosphate and DNA represented the dominant forms of orthophosphate monoesters and diesters, respectively, in this study. Because myo-inositol hexakiphosphate is positively related to plant-available P_i, the biologically available P is considered to regulate the quantity of *myo*-inositol hexakiphosphate under P-limited conditions [47]. These findings support that orthophosphate monoester content had a similar trend as labile P_i content, which decreased with depth in soils at the study site. DNA as the diester form is favoured in acidic soils [9,50,51]; however, pH increased from the top to bottom soil layers. Consequently, the proportion of diesters decreased with depth at both locations.

Most pyrophosphates were from the plant and microbial communities and were the hydrolysis product of polyphosphates [18]. Because pyrophosphates are hydrolyzed to orthophosphate rapidly through enzymatic mechanisms in soil [18], the proportion of pyrophosphates in our soil was low. Phosphonate is produced by bacteria (e.g., *Bacillus cereus*) via enzymatic activity; however, bacteria

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are uncommon in acidic soil [23,34,43]. Thus, phosphonate was not detected in our sand dune forest soils.

Under aerobic conditions, the content of P_o pools in soil from dominant to minor were monoester-P, diester-P, and phosphonate [7,9]. Furthermore, abiotic reactions (e.g., sorption and occlusion) into minerals contributed to the preservation of orthophosphates [52].

4.3. Sequential chemical extraction and ^{31}P-NMR measurement comparisons

The differences in relative proportions of P_o measured by sequential chemical extraction and ^{31}P-NMR spectroscopy were less than 10% in this study (Table 4). Negassa and Leinweber [22] found that orthophosphate diester content was correlated with $NaHCO_3$-P_o content, which is easily mineralized, and orthophosphate monoester content was related to NaOH-P_o content, which is more stable. The proportions were consistent between $NaHCO_3$-P_o and diesters and between NaOH-P_o and monoesters in our study (Figures 2-3 and Figures 5-6). However, the proportion of P_i was much higher by ^{31}P-NMR spectroscopy than sequential chemical extraction. The differences in proportions between the two measurements were relatively higher for P_i than P_o, especially in the mineral-I and mineral–II layers (Table 4).

The variances might be due to occlusion of orthophosphate within large humic molecules [3]. Furthermore, sequential chemical extraction was an indirect chemical method to estimate organic P [18]. As stated by Tiessen and Moir [25], P_o is estimated by the difference between P_t and P_i from each extract, so there are uncertainty factors (e.g., precipitation) when using sequential chemical extraction. The precision of ^{31}P-NMR spectroscopy was also limited by background noise and the overlapping signals, especially for P_i and orthophosphate monoesters [43]. In addition, paramagnetic impurities (e.g., Fe and Mn) would restrict the quality of ^{31}P-NMR because they reduce the signal-to-noise ratio and amplify the intensity of spinning side bands [1]. Thus, these uncertainties caused differences between the two methods.

Vestergren et al. [53] and Huang et al [9] identified that the use of 2-D ^{1}H-^{31}P correlation would effectively enhance the resolution of overlapping signals after eliminating the paramagnetic impurities. This new technique was suggested for use in future applications of ^{31}P-NMR spectroscopy. Overall, the proportions of P_i and P_o evaluated by sequential chemical extraction and ^{31}P-NMR spectroscopy were mostly consistent and the results were similar to those from other studies [34,42,54].

Table 4. Mean proportions (%) with standard deviation of total P extracted for inorganic P (P_i) and organic P (P_o) forms in NaOH-EDTA extracts from soil samples as determined by sequential-chemical extraction and ^{31}P-NMR spectroscopy.

Site	Layer	P_i		P_o	
		Chemical	^{31}P-NMR	Chemical	^{31}P-NMR
Upland	Humic (+2-0 cm)	30.3±1.5	38.2±2.9	55.6±2.2	62.2±1.6
	Mineral 1 (0-10 cm)	43.3±1.0	60.8±2.1	42.8±1.5	38.5±1.4
	Mineral 2 (10-20 cm)	67.9±1.9	81.7±1.0	23.8±1.7	18.8±0.5
Lowland	Humic (+2-0 cm)	29.1±4.3	37.0±2.0	59.3±4.2	64.6±1.2
	Mineral 1 (0-10 cm)	38.9±4.1	55.6±9.5	47.0±2.1	40.7±1.2
	Mineral 2 (10-20 cm)	55.0±1.6	72.9±2.4	35.8±1.4	29.1±0.7

5. Conclusions

The results indicated that soils in our study site, a subtropical coastal sand dune forest site, were in a weathered environment. The P distribution and content significantly differed among the soil layers and between the upland and lowland sites by variation in elevation and eolian aggradation effects. The P_o and labile P_i content was highest in the humic layer and decreased with depth; however, recalcitrant P_i content had the opposite pattern. The content of recalcitrant P forms (e.g., NaOH-P_i, HCl-P_i, and cHCl) was greater in the upland than lowland site. ^{31}P-NMR spectroscopy revealed that inorganic orthophosphate and monoester-P forms were the most abundant in this study. The proportions of P_i and P_o observed by sequential chemical extraction and ^{31}P-NMR spectroscopy were mostly consistent. Because P distribution can be affected by microbial activity, the relation between microbial processes and P content require further investigation.

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