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Soil Phosphorus Composition and Phosphatase Activities along Altitudes of Alpine Tundra in Changbai Mountains, China

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Abstract: Alpine tundra ecosystems have specific vegetation and environmental conditions that may affect soil phosphorus (P) composition and phosphatase activities. However, these effects are poorly understood. This study used NaOH-EDTA extraction and solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy to determine soil P composition and phosphatase activities, including acid phosphomonoesterase (AcP), phosphodiesterase (PD) and inorganic pyrophosphatase (IPP), in the alpine tundra of the Changbai Mountains at seven different altitudinal gradients (i.e., 2000 m, 2100 m, 2200 m, 2300 m, 2400 m, 2500 m, and 2600 m). The results show that total P (TP), organic P (OP), OP/TP, NaOH-EDTA extracted P and AcP, PD, and IPP activities over the altitude range of 2500–2600 m are significantly lower than those below 2400 m. The dominant extracted form of P is OP (73%–83%) with a large proportion of monoesters (65%–72%), whereas inorganic P is present in lower proportions (17%–27%). The activity of AcP is significantly positively correlated with the contents of soil OP, total carbon (TC), total nitrogen (TN), and TP (P < 0.05), indicating that the AcP is a more sensitive index for responding P nutrient storage than PD and IPP. Soil properties, P composition, and phosphatase activities decrease with increased altitude and soil pH. Our results indicate that the distribution of soil P composition and phosphatase activities along altitude and AcP may play an important role in P hydrolysis as well as have the potential to be an indicator of soil quality.

Keywords: alpine tundra ecosystem; altitude; phosphatase activity; phosphorus (P) composition; solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy

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1 Introduction

The Changbai Mountains is the highest mountain in the northeastern China and is the head of three large rivers (the Songhua River, Yalu River, and Tumen River) (He *et al*., 2002). A typical alpine tundra belt formed above 2000 m after the glacier receded from the mid-latitudes of the northern hemisphere to the Arctic during the Quaternary period (Dai *et al*., 2002). The alpine tundra on the Changbai Mountains also represents the best-reserved tundra ecosystems and the highest biodiversity in the northeast Eurasia. The soil contains a large amount of carbon, nitrogen, phosphorus, and sulfur due to the harsh climate (Wu *et al*., 2006). Although the tundra ecosystem contains a large amount of nutrients, the majority of those constitute recalcitrant pools of nutrients in the form of organic matter. For example, 65% of the nitrogen (N) pool in the ecosystem is recalcitrant soil organic matter, and 56%–76% of the NaOH-EDTA extracted soil phosphorus (P) was organic P (OP) (Turner *et al*., 2004) in the Swedish arctic tundra. Thus, vegetative productivity is often limited by nutrient

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availabilities in the tundra ecosystem. However, the proportion of soil organic nutrients in the Changbai Mountains, particularly P and its bioavailability, are still unknown.

In tundra soil, the cold climate restricts P release from weathering and mineralization by affecting plants and microorganisms (Stark, 2007). For example, the input of plant residues and excretion by plant roots can influence soil P storage and release (Wu *et al.*, 2013). In addition, microorganisms in tundra soil can affect soil P availability by their ability to immobilize soil P and to hydrolyze soil OP (Jonasson *et al.*, 1999; Reiner *et al.*, 2012). The P has been proven to be a primary limiting nutrient at high altitudes, with the degree of limitation varying markedly across the heterogeneous landscape (Turner *et al.*, 2004). Soil P is dominated by organic forms in the alpine tundra (Turner *et al.*, 2004). The OP acquired by plants and microorganisms must primarily be mineralized by phosphatases (Schmidt *et al.*, 1999). It is well documented that phosphatases derived from plant roots and soil microorganisms can stimulate the hydrolysis of OP in the soil (Spier and Ross, 1978; Tarafdar and Claassen, 1988; Tadano *et al.*, 1993).

Soil P has various compositions, which play differing roles in the supply of plant nutrients. A ^{31}P nuclear magnetic resonance (NMR) spectroscopy technique that can successfully qualitatively identify the P composition in soil and a NaOH-EDTA extraction method that can quantitatively determine the P composition in the soil were recently developed (Turner *et al.*, 2003). The relationship between soil P composition and soil phosphatases could be important to understanding soil P storage and supply for organisms in agro-ecosystems (Wei *et al.*, 2014a; 2014b; Zhang *et al.*, 2014). However, few studies have assessed these relationships in an alpine tundra ecosystem.

Therefore, the aims of this study were to 1) examine the proportion and composition of the soil OP and 2) explore the relationships between the soil P composition and the phosphatases involved in soil P turnover under seven different altitudinal gradients in the alpine tundra on the northern slope of the Changbai Mountains in Northeast China. Determining the soil P composition and phosphatase activities at different altitudes could provide information for the effective and sustainable management of alpine tundra systems.

2 Materials and Methods

2.1 Study site

The study site was located at the altitudes from 2000 m to 2600 m on the northern slope of the Changbai Mountains Nature Reserve (41°35′–42°25′N, 127°40′–128°16′E) in Northeast China. The alpine tundra area was 15 195 ha. The annual precipitation and the mean annual temperature were 900–1340 mm and –7.4℃, respectively, where the sub-alpine climate was affected by monsoon (Martin, 2003). The topographical, geomorphic, and hydrological conditions had obvious variation, which resulted in the following five soil types: peat alpine tundra soil, meadow alpine tundra soil, grey alpine tundra soil, lithic alpine tundra soil and cold desert alpine tundra soil. The plant community at the site was typical alpine tundra vegetation dominated by moss, lichen, shrubs and herbaceous plants (Wu *et al.*, 2006).

2.2 Soil sampling

Soil sample collection was conducted in early August (in the growing season) of 2011. Five representative plots (20 m × 20 m) with similar aboveground, geomorphic and hydrological conditions were selected at seven altitudes at an interval of 100 m over the range from 2000 m to 2600 m. Five soil samples (0–10 cm) (> 1 kg) were collected within each plot and then passed through a 2 mm sieve after removing stones and coarse roots. A portion of the sieved soils (≥ 200 g) was kept at 4℃ for the analysis of soil phosphatase activities within one month, and the rest of the soil was air-dried and stored at room temperature for the chemical analysis.

2.3 Determination of soil properties

The total P (TP) was determined by the combustion method and the molybdenum blue colorimetric method at 880 nm (Walker and Adams, 1958; Murphy and Riley, 1962). Inorganic P was extracted with 0.5 M H_2SO_4 (1 ∶ 25 soil-to-solution ratio for 16 h) and measured by the method of Kuo (1996). The OP was calculated by subtracting the inorganic P from the TP. Soil P composition was determined by NaOH-EDTA (0.25 mol/L NaOH, 0.05 mol/L Na_2EDTA) extraction and solution ^{31}P NMR spectroscopy according to Zhang *et al.* (2012) and Wei *et al.* (2014a; 2014b). The P composition for each sample was analyzed by NMR Utility Transform Software (NUTS) for Windows (Acorn NMR, Livermore, CA), and the spectra of the samples were plotted with 0.2-Hz line broadening (Zhang *et al*., 2012; Wei *et al*., 2014a; 2014b).

Total carbon (TC) and total nitrogen (TN) of soils were determined via the dry combustion method using an automatic element analyzer (Analyzer vario MICRO cube, Elementar, Germany) (Zhang *et al*., 2012). Soil pH was determined at a 1 ∶ 5 soil/deionized water ratio using a glass electrode (Shen *et al*., 2013).

The activities of acid phosphomonoesterase (AcP), phosphodiesterase (PD) and inorganic pyrophosphatase (IPP) were analyzed using fresh soil as described by Tabatabai (1994). The AcP activity was determined by measuring the release of *p*-nitrophenol by incubating 1 g of soil at 37℃ for 1 h with 0.2 mL of toluene, 4 mL of universal buffer (pH 6.5), and 1 mL of 50 mmol *p*-nitrophenyl phosphate. The enzymatic activity was expressed as mg p-nitrophenol/(kg soil·h). The activity of PD was determined by a similar procedure but with *bis*-*p*-nitrophenyl phosphate as the substrate, and the buffer was adjusted to pH 8.0. The activity of IPP was determined by measuring the concentration of PO_4^{3-}-P, which was released by incubating 1 g of soil with 3 mL of 50 mmol sodium pyrophosphate at 37℃ for 5 h. The enzymatic activity was expressed as mg PO_4^{3-}-P/(kg soil·h).

2.4 Data analysis

The soil data were calculated based on the oven-dried (105℃) weight. The significance of differences in soil chemical properties and phosphatase activities was analyzed by one-way ANOVA with the Duncan test at the P = 0.05 level. The correlation of the soil parameters was based on Pearson′s correlation coefficients. All of the statistical analyses were conducted using SPSS 16.0 software for Windows.

To determine the impacts of soil property and altitude on soil P composition and phosphatase activities, principal component analysis (PCA) was conducted using Canoco Software 4.5 (Microcomputer Power, USA).

3 Results

3.1 Soil chemical properties in alpine tundra

Soil pH ranged from 4.84 to 5.60 in the seven altitudes in the alpine tundra of the Changbai Mountains (Table 1). Soil moisture content varied from 43.7% to 93.9%, with the highest at 2400 m and the lowest at 2600 m, while the others were in little difference. Generally, the contents of TC, TN, and TP at 2500 m and 2600 m were significantly lower than those below 2400 m (Table 1). Soil TP ranged from 222 mg/kg to 644 mg/kg, and OP ranged from 155 mg/kg to 559 mg/kg (70%–87% of the soil TP). The proportion of soil OP was significantly lower at 2500 m and 2600 m at 73% and 70% of soil TP, respectively.

3.2 Soil phosphorus (P) composition in alpine tundra

Extraction of the soil with NaOH-EDTA recovered between 45% and 80% of soil TP from the seven different altitudinal gradients (Table 2, Fig. 1), which indicated that the highest value was at 2000 m and lowest value was at 2600 m. Soil P composition of the soil surface (0–10 cm) was dominated by OP, which accounted for 73%–83% (65%–72% of monoesters and 5%–12% of diesters) of the NaOH-EDTA TP (Table 2). The monoester to diester ratios ranged from 6.1 (2300 m) to 13.3

Table 1 Soil chemical properties in Changbai Mountains alpine tundra along seven altitudinal gradients

Altitude (m)	pH (H_2O)	Soil moisture (%)	TC (g/kg soil)	TN (g/kg soil)	TP (g/kg soil)	OP (mg/kg soil)	OP/TP (%)
2000	5.10	88.4	104±16.45a	5.8±1.10ab	499±32.46ab	432±41.29ab	86±2.90a
2100	5.05	86.6	109±14.38a	5.3±0.67b	644±331.87a	559±335.60a	87±6.31a
2200	5.26	91.9	115±9.36a	6.6±0.53a	574±53.74ab	496±45.66ab	86±0.95a
2300	5.01	78.8	105±17.02a	5.8±0.85ab	424±52.85abc	352±46.97abc	83±2.86a
2400	4.84	93.9	105±15.32a	6.0±0.81ab	598±159.16ab	512±168.25ab	86±7.91a
2500	5.20	83.7	81±21.12b	4.2±0.95c	388±89.41bc	284±77.17bc	73±5.08b
2600	5.60	43.7	46±6.03c	2.2±0.57d	222±46.84c	155±50.21c	70±7.55b

Notes: TC, total carbon; TN, total nitrogen; TP, total phosphorus; OP, organic phosphorus; OP/TP, organic phosphorus/total phosphorus. Values of TC, TN, TP and OP are means ± standard deviation (S.D., n = 5), and different lowercase letters within a column indicate significant differences (P < 0.05) in the values at altitudes from 2000 m to 2600 m

Table 2 Concentrations (mg P/kg soil) of phosphorus (P) composition determined in NaOH-EDTA extracts of soil from alpine tundra of Changbai Mountains via solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy

Altitude (m)	NaOH-EDTA Total P[a]	Inorganic P		Organic P		Organic P/NaOH-EDTA Total P (%)	Monoester/ diester
		Orthophosphate[b]	Pyrophosphate[b]	Monoesters[b]	Diesters[b]		
2000	399(80)	59(15)	18(5)	286(72)	36(9)	81	8.0
2100	291(45)	48(16)	16(5)	200(69)	27(9)	78	7.4
2200	393(68)	55(14)	15(4)	282(72)	41(10)	82	6.9
2300	291(69)	39(13)	12(4)	206(71)	34(12)	83	6.1
2400	380(64)	51(13)	20(5)	265(70)	43(11)	81	6.2
2500	193(50)	35(18)	15(7)	133(69)	10(5)	74	13.3
2600	121(55)	23(19)	9(7)	79(65)	10(8)	73	7.9

Notes: P, phosphorus. a, values in parenthesis are the proportion (%) of total soil P; b, values in parenthesis are the proportion (%) of total NaOH-EDTA extracted P

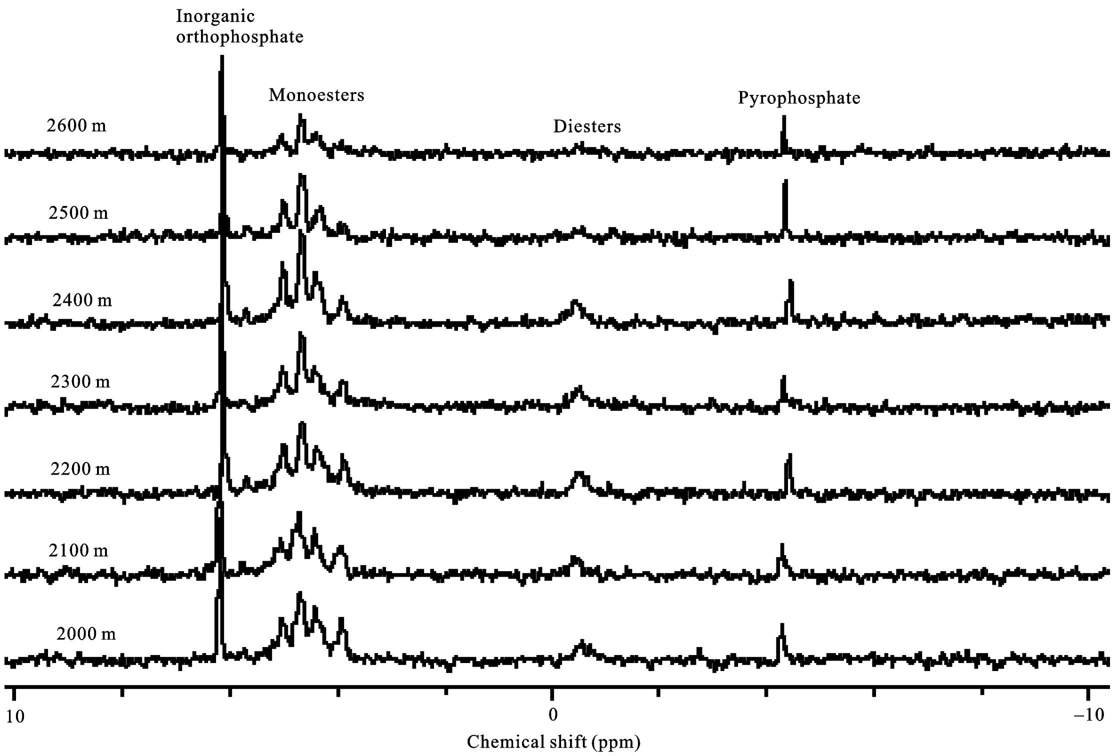

Fig. 1 Solution ^{31}P nuclear magnetic resonance (NMR) spectra of NaOH-EDTA extracts of alpine tundra soil along seven different altitudinal gradients (2000–2600 m) on Changbai Mountains in Northeast China

(2500 m). Inorganic P composition accounted for 17%–26% (13%–19% of orthophosphate and 4%–7% of pyrophosphate) of the NaOH-EDTA TP (Table 2). The TP, inorganic P (except pyrophosphate), OP (monoesters and diesters), and the ratio of OP to TP extracted by NaOH-EDTA at 2500–2600 m were significantly lower than those below 2400 m.

3.3 Soil phosphatase activities in alpine tundra

The AcP activities from 2100 m to 2400 m were not significantly different from each other but were significantly higher than those at 2500 m and 2600 m (Fig. 2). The activities of PD and IPP were the highest at 2100 m, but no significant differences were found among the activities at the other altitudes except for a decrease at 2500 m and 2600 m.

3.4 Relationships among soil property, phosphorus composition, phosphatase activity and altitude

Soil TC, TN, TP, and OP were strongly and positively correlated with soil moisture, AcP, orthophosphate, and monoesters (Table 3). The TC and TN were significantly and positively correlated with TP and OP. Phosphatase activity (AcP and IPP) had significantly positive correlations with TP and OP. However, the soil properties, P composition, and phosphatases activity were negatively correlated with altitude and soil pH. The ratio of monoesters to diesters exhibited a positive correlation with altitude.

The PCA based on the soil properties and altitude parameters explained 92.4% of the total data variation (Fig. 3). The first ordination axis explained 85.2% of the total data variance, while the second explained 7.2%. Generally, the cosines of the angles between the vectors of soil properties, P composition, and phosphatases reflected their relationships. The PCA revealed that soil properties, P composition, and phosphatases were negatively correlated with altitude and soil pH. The values of these parameters at altitudes of 2500 m and 2600 m scattered to the right of axis 2, which indicated a large difference compared to those from 2000 m to 2400 m, which scattered on the left of axis 2.

4 Discussion

4.1 Soil phosphorus in alpine tundra

In our study, OP accounted for over 70% of the soil TP, and the highest proportion was 87%, which might be a result of the low temperature and monsoon climate. In the alpine tundra, the low temperature and frozen soil could inhibit microbial activity and therefore P mineralization, especially during winter, which might be the main cause of OP accumulation in alpine soil (Bowman *et al*., 2003; Litaor *et al*., 2005). Weintraub (2011) and Sundqvist *et al*. (2014) also found that net P mineralization and available P content were typically low in alpine and arctic tundra, which resulted in P limiting plant growth. Therefore, the low mineralization rate associated with the low temperature is probably responsible for the accumulation of OP in this area. For example, over 45% of total P was OP, which accumulated in alpine inceptisols at the altitudes from 2000 m to 2400 m (Cassagne *et al*., 2000). Moreover, the lower OP content at the altitudes of 2500 m and 2600 m might be attributed to the lower plant coverage and the reduced plant diversity in higher altitude tundra, which decreased the input of nutrients, including P, to the soil (Litaor *et al*., 2005).

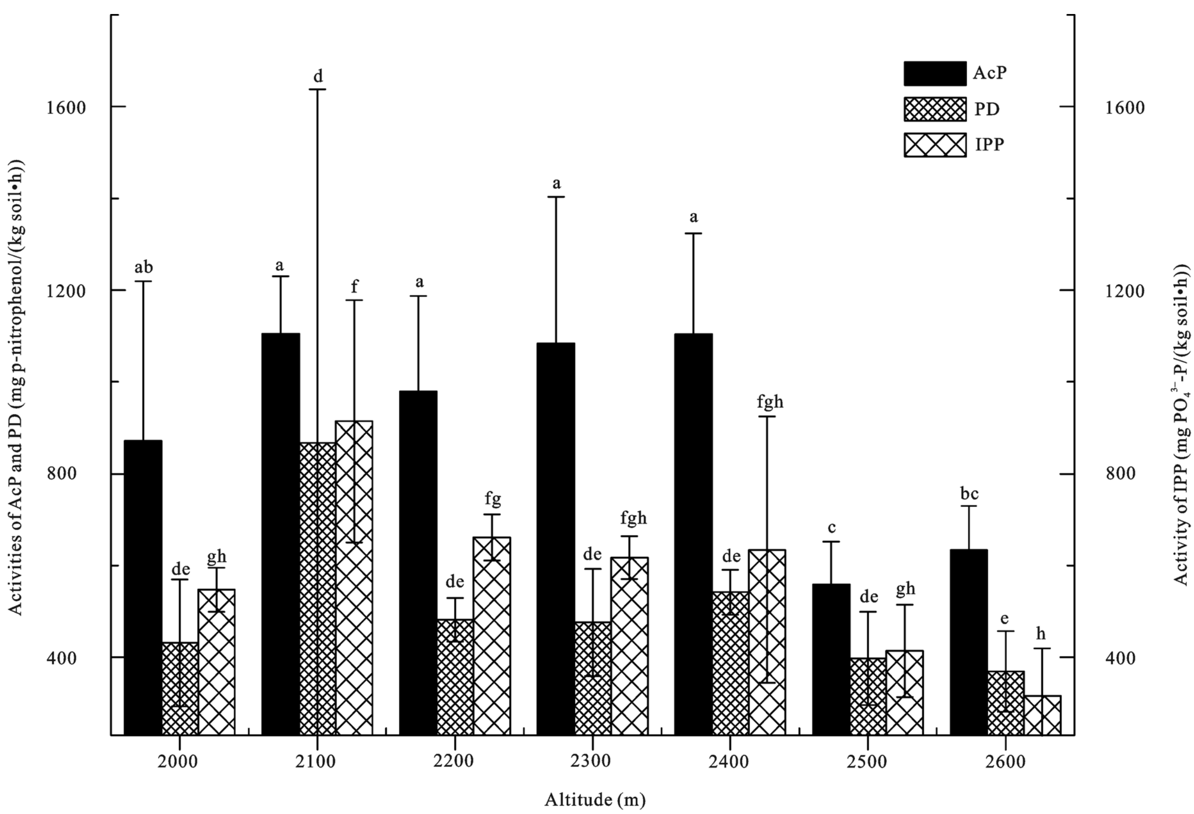

Fig. 2 Soil phosphatase activities (AcP, PD and IPP) at different altitudes in range from 2000 m to 2600 m, expressed as seven different altitude gradients of alpine tundra ecosystem of Changbai Mountains. AcP, acid phosphomonoesterase activity; PD, phosphodiesterase activity; IPP, inorganic pyrophosphatase activity. Different lowercase letters above the bar indicate significant differences (P < 0.05) at different altitudes

Table 3 Correlation coefficients among altitudes, soil chemical properties, soil phosphatase activities, and phosphorus composition in alpine tundra ecosystem on Changbai Mountains

	A	TN	TP	TC	pH	OP	AcP	PD	IPP	P1	P2	P3	P4	P2/P3	SM
A	1														
TN	–0.702	1													
TP	–0.697	0.829*	1												
TC	–0.769*	0.975**	0.897**	1											
pH	0.444	–0.754	–0.743	–0.776*	1										
OP	–0.733	0.839*	0.996**	0.902**	–0.730	1									
AcP	–0.569	0.756*	0.772*	0.787*	–0.720	0.806*	1								
PD	–0.489	0.328	0.724	0.508	–0.450	0.721	0.649	1							
IPP	–0.693	0.677	0.889**	0.805*	–0.623	0.897**	0.840*	0.910**	1						
P1	–0.843*	0.875**	0.843*	0.866*	–0.622	0.861*	0.603	0.337	0.616	1					
P2	–0.757*	0.928**	0.785*	0.875**	–0.655	0.812*	0.681	0.219	0.556	0.964**	1				
P3	–0.616	0.899**	0.740	0.831*	–0.674	0.778*	0.832*	0.250	0.580	0.835*	0.940**	1			
P4	–0.507	0.667	0.789*	0.671	–0.776*	0.771*	0.437	0.333	0.471	0.813*	0.749	0.623	1		
P2/P3	0.338	–0.422	–0.355	–0.380	0.277	–0.425	–0.803*	–0.304	–0.461	–0.338	–0.481	0.714	–0.061	1	
SM	–0.619	0.897**	0.869*	0.910**	–0.811*	0.846*	0.554	0.377	0.633	0.854*	0.824*	0.702	0.856*	–0.064	1

Notes: A, altitudes (m); TC, total carbon; TN, total nitrogen; TP, total phosphorus; OP, organic phosphorus; AcP, acid phosphomonoesterase activity; PD, phosphodiesterase activity; IPP, inorganic pyrophosphatase activity; P1, inorganic orthophosphate; P2, orthophosphate monoesters; P3, orthophosphate diesters; P4, pyrophosphate; P2/P3, monoester to diester ratio; SM, soil moisture. * and **, correlations are significant at the 0.05 and 0.01 level, respectively

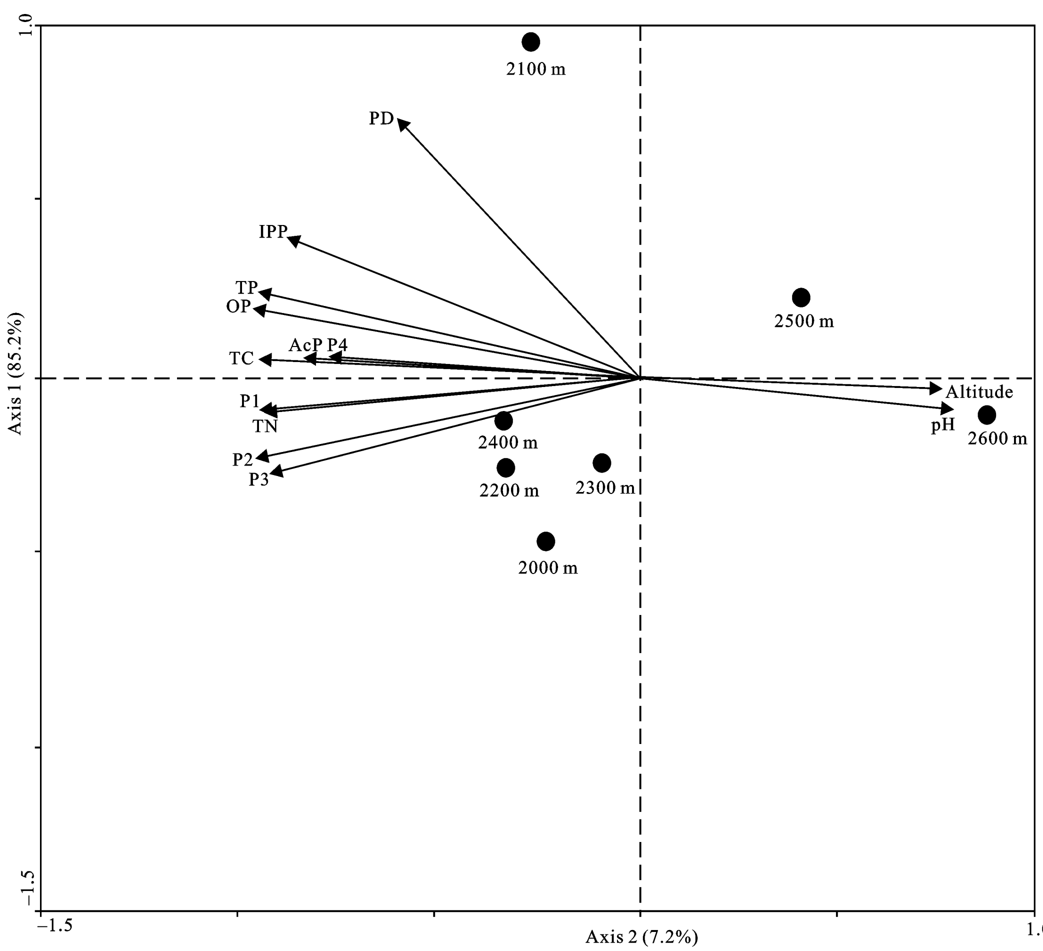

Fig. 3 Soil phosphorus composition, phosphatase activities, and environmental variables (soil properties and altitudes) biplot of principal component analysis (PCA). TC, total carbon; TN, total nitrogen; TP, total phosphorus; OP, organic phosphorus; AcP, acid phosphomonoesterase activity; PD, phosphodiesterase activity; IPP, inorganic pyrophosphatase activity; P1, inorganic orthophosphate; P2, orthophosphate monoesters; P3, orthophosphate diesters; P4, pyrophosphate; ●, altitude

In alpine tundra soil, OP composition was the main constituent (73%–83%) of the NaOH-EDTA extracts, and the proportion of OP composition did not differ significantly from 2000 m to 2400 m but was lower at 2500 m and 2600 m. Similar results were also reported by Makarov *et al*. (1997) and Turner *et al*. (2004) in alpine soils in the northern Caucasus and alpine tundra in the Fennoscandian mountains, respectively. The accumulation of OP might result from the low mineralization of soil P in the alpine tundra. The inorganic P composition was reduced because of the slow decomposition of OP in these cold and acidic soils (Turner *et al*., 2004).

The NaOH-EDTA extractable P consisted of monoesters, diesters, orthophosphate, and pyrophosphate. The concentrations of the various types of P were not significantly different from 2000 m to 2400 m but exhibited a decrease at 2500 m and 2600 m. This result indicated that high altitudes did not contribute to P accumulation, which could be due to the low plant-residue inputs (Turner *et al*., 2004). The OP was strongly and positively correlated with the overall P composition, indicating that OP might have an important influence on soil P composition in this area. The extracted P was dominated by monoesters (Table 2) because diesters are more labile due to their rapid hydrolysis in acidic soils (Condron *et al*., 1990) into monoesters (Zhang *et al*., 2012). In addition, the monoester/diester ratios exhibited a positive correlation with altitude (Table 3), suggesting that the ratios increased with altitude and therefore that diesters are more labile at higher altitudes. Moreover, in the tundra soil studied here, the monoester contents appeared to be relatively higher than that reported by Turner *et al*. (2004) at the altitudes below 1000 m, which suggested that monoester contents might be higher in high-altitude tundra (above 2000 m) than that below 1000 m. This difference could be attributed to lower phosphatase activity in the high-altitude area due to wet and cold conditions (Nannipieri *et al*., 2011), which might reduce the hydrolysis of monoesters and enable more monoesters to accumulate in the soil.

4.2 Soil phosphatase activities in alpine tundra

The higher soil phosphatase activities at low altitudes (from 2000 m to 2400 m) could be attributed to the following two factors: 1) there is little vegetation, which results in a low amount of enzyme activity excreted by plant roots at high altitudes (Stone *et al*., 2013), and 2) the lack of microorganisms, the major phosphatase sources, decreased the phosphatase levels (Ma *et al*., 2011). The AcP activity was significantly and positively correlated with soil OP, TC, and TP, which demonstrated that the AcP is a sensitive index for nutrients storage and could be used as an indicator of soil quality (Yu, 2001; Samuel *et al*., 2010) in the area. In addition, the IPP activity was significantly and positively correlated with TP and OP, suggesting that IPP might play an important role in the hydrolysis and mobilization of soil-bound P in this particular soil.

4.3 Factors impacting soil phosphorus distribution in alpine tundra

The results of this study indicated that the TC and TN were significantly positively correlated with TP and OP contents, which suggested that the elemental nutrients C, N, and P were mutually promoted in alpine tundra ecosystems. Similar results were also reported by Litaor *et al*. (2005), who found that atmospheric deposition of N might cause a change from N limitation to P limitation in the alpine environment. In addition, the microbial community structure often shifted in response to a shift from C limitation to P limitation (Stone *et al*., 2013). The reason for this result could be the effect of climate, plants and microorganisms in transforming the soil nutrient elements (C, N, and P) (Cathy *et al*., 2003). Soil moisture had a significantly positive correlation with TC, TN, TP, OP, and P composition, except for diesters, and a significantly negative correlation with pH, which indicated that the soil moisture could be an important determinant of future plant productivity in this alpine tundra ecosystem. Furthermore, P composition patterns were broadly similar at different altitudes, which might indicate identical soil properties throughout the alpine tundra, which is in agreement with the results of Turner *et al*. (2011). For example, orthophosphate monoester was found at higher proportion in neutral soils, whereas, orthophosphate diesters were more common in very acidic soils. Monoesters and phosphonates might persist in acidic soils because organisms capable of degrading them cannot survive at low pH (Tate and Newman, 1982). In this alpine tundra, the soil pH values were less than 6.0 (Table 1), which might contribute to the accumulation of monoesters and orthophosphate (Turner *et al*., 2004). However, in this study, phosphonates were not detected, which could be attributed to rapid degradation of phosphonates because the soil pH in these areas was not suitable for their accumulation. For example, there was a little amount of phosphonate detected at pH under 4.41 in the tundra ecotone of Fennoscandian soils (Turner *et al*., 2004). However, pH in the alpine tundra soils of the Changbai Mountains was higher than 4.80, which might not be a suitable condition for the existence of phosphonates.

5 Conclusions

The Changbai Mountains alpine tundra ecosystems have specific vegetation and environmental conditions that may affect soil P composition and phosphatase activities. This study used NaOH-EDTA extraction and solution ^{31}P NMR spectroscopy to determine soil P composition and phosphatase activities. The soil P composition and phosphatase activities decreased with increased altitude and soil pH. The OP (73%–83%) was the dominant P type in the NaOH-EDTA extracts, and monoesters (65%–72%) were the main OP constituents. The AcP exhibited a significantly positive correlation with the soil TC, TN, TP, and OP, indicating that AcP might play an important role in OP hydrolysis and could be used as an indicator of soil quality in the alpine tundra. Future studies should be focused on OP mineralization and sustainable utilization of alpine tundra soil in the Changbai Mountains.

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