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Rhizosphere organic phosphorus fractions of Simon poplar and Mongolian pine plantations in a semiarid sandy land of northeastern China

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Abstract: The aim of this study was to investigate the role of rhizosphere organic phosphorus (P) in soil P supply in semiarid forests and the effects of tree species on rihizosphere organic P. We examined organic P fractions in rhizosphere and bulk soils of mono-specific Simon poplar (*Populus simonii*) and Mongolian pine (*Pinus sylvestris* var. *mongolica*) plantations in a semiarid sandy soil of Horqin Sandy Land in Northeast China. Total organic P (TPo) accounted for 76% of total P across the two stands. The concentration of organic P (Po) fractions decreased in the order of NaOH-Po>Res-Po>HCl-Po>$NaHCO_3$-Po in both plantations. The concentration of $NaHCO_3$-Po was 38% and 43% lower in rhizosphere soil than in bulk soil in Simon poplar and Mongolian pine plantations, respectively. In contrast, total P, TPo and NaOH-Po significantly accumulated in rhizosphere soil in Simon poplar plantations, but no change in Mongolian pine plantations. Soil recalcitrant organic P fractions were positively correlated with soil organic carbon. The results suggest that rhizosphere labile organic P was an important source of plant-available P in this semiarid region, but the dynamic of rhizosphere recalcitrant organic P fractions varied with tree species and was correlated to organic carbon dynamics.

Keywords: rhizosphere effect; organic P fractions; *Populus simonii*; *Pinus sylvestris* var. *mongolica*

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Nitrogen (N) and phosphorus (P) are the most common limiting nutrient elements in terrestrial ecosystems (Vitousek et al., 2010). In forests, more than 50% of the total P in topsoils is in organic form, and soil P supply was mainly sustained by the recycling of organic materials (Attiwill and Adams, 1993). In arid and semiarid regions, geochemical processes were traditionally considered as the main control of soil P cycling (Lajtha and Schlesinger, 1988). However, over recent decades, many studies revealed that biological transformation of soil organic P contributes substantially to plant P nutrition not only in humid regions (Vincent et al., 2010) but also in semiarid regions (Cross and Schlesinger, 2001).

Rhizosphere-mediated processes are important for the acquisition of P, because phosphate ions present at low concentrations in soil solution and with poor diffusivity (Richardson et al., 2009). Rhizosphere inorganic P transformation and the underlying mechanisms are well understood (Hinsinger, 2001). In contrast, there are few studies on rhizosphere organic P transformations, particularly the field studies in forests. Several studies found that mineralization of organic P was accelerated by phosphatase secreted by roots and rhizosphere microorganisms (Häussling and Marschner, 1989; Phillips and Fahey, 2006; Zhao et al., 2010), while the contribution of different forms of organic P in rhizosphere soils remains largely

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unknown. Knowledge on dynamics of organic P fractions with different bioavailability is important for a better understanding of the utilization of organic P by plants.

The diffusion of phosphate ions through the soil to the root surface is particularly sensitive to soil moisture regimes (Smith, 2002). Furthermore, inorganic phosphate is easily adsorbed by soil minerals while organic P compounds are much more mobile (Frossard et al., 1989). Thus, rhizosphere organic P transformation may be especially important for soil P supply to tree growing in semiarid regions. Simon poplar (*Populus simonii*) and Mongolian pine (*Pinus sylvestris* var. *mongolica*) are the most widely planted tree species for soil conservation in the southeastern Horqin Sandy Land in Northeast China. These two species are highly adapted to the poor soil conditions and semiarid climate, though with contrasting growth rate and nutrient demands. In previous studies we investigated the basic chemical and biological properties in rhizosphere and bulk soils of these plantations and found the accumulation of Olsen-P and phosphatase activities in rhizosphere soils (Zhao et al., 2010) but the source of available P remains unknown. Thus, in this study, we further examined sequentially extracted organic P fractions in rhizosphere and bulk soils of these plantations, aiming to reveal the contribution of organic P to soil P supply in the study area, and examine the effects of tree species. We hypothesized that labile organic P is an important source of available P in rhizosphere soils, and organic P fractions differ significantly between rhizosphere and bulk soils.

1 Materials and methods

1.1 Site description and soil sampling

The study was conducted at Daqinggou Ecological Station (42°58'N, 122°21'E; 260 m asl), Institute of Applied Ecology, Chinese Academy of Sciences, in the southeastern Horqin Sandy Land, Northeast China. Detailed descriptions of the study area and the soil properties referred to Zhao et al. (2010). Briefly, the area belongs to a semiarid region under temperate climate with annual mean temperature of 6.4°C and mean annual precipitation of 450 mm. The soil is a sandy soil characterized by coarse texture and loose structure (Typic Ustipsamment). The soil is deficient in soil organic carbon (SOC), N and P, with SOC, total N and total P concentrations of 4.35, 0.30 and 0.15 g/kg in 0–30 cm soil layer, respectively.

In July 2009, 18-year-old pure even-aged Simon poplar and Mongolian pine plantations on a degraded grassland were selected for this study. Within a 1-km radius area, five 20 m×20 m plots were randomly established for soil sampling. The distance between adjacent plots was at least 5 m. The soil samples used in the present study was the same as in the study conducted by Zhao et al. (2010). Briefly, bulk and rhizosphere soils were collected at 10–30 cm soil layer. The soil adhering to live fine roots (<1 mm) after gentle shaking was regarded as rhizosphere soil. The remaining soil after removal of rhizosphere soil was regarded as bulk soil (Phillips and Fahey, 2006). One composite soil sample consisting of six soil cores (6 cm in diameter) was taken randomly within each plot. Soils were air-dried for determination of soil organic P fractions. Some chemical and biological properties of bulk and rhizosphere soils are given in Table 1.

1.2 Soil organic P fractionation

The fractionation scheme of soil organic P followed the sequential extraction procedure developed by Bowman and Cole (1978), and modified by Sharpley and Smith (1985) and Ivanoff et al. (1998). Soil organic P was fractionated into fractions with increasing availability: Bic-Po, HCl-Po, NaOH-Po and Res-Po. Specific fractionation methods are as follows: 1) labile organic P (Bic-Po), extracted by 0.5 M $NaHCO_3$ at pH 8.5; 2) moderately labile organic P (HCl-Po), obtained by extraction with 1.0 M HCl; 3) moderately stable organic P (fulvic-Po), related to fulvic acid, which was extracted with 0.5 M NaOH and then the NaOH extract was acidified with concentrated HCl; 4) highly stable organic P (humic-Po), associated with humic acid, which was estimated by subtracting fulvic-Po from the total P measured in the 0.5 M NaOH extract; 5) non-labile residual organic P (Res-Po) fraction is determined by igniting the soil residue at 550°C for 1 h, followed by dissolution in 1.0 M H_2SO_4. NaOH-Po is the sum of funvic-Po and humic-Po. Total organic P (TPo) was calculated as the sum of all organic P fractions. The extracts in every step were analyzed for

Table 1 Chemical and biological properties of bulk and rhizosphere soils of Simon poplar and Mongolian pine plantations

Tree species		pH	APA (μg NP/(g•h))	SOC (g/kg)	DOC (mg/kg)	MBC	Olsen-P	MBP	MBN	Microbial C/N
Simon poplar	Bulk soil	8.3±0.1	58.2±3.1	3.0±0.2	16.4±1.8	73.6±7.2	1.1±0.1	2.6±0.1	6.7±0.2	10.9±1.2
	Rhizosphere soil	7.9±0.1	79.8±3.2	4.7±0.2	23.7±0.9	90.7±6.4	2.2±0.3	2.8±0.2	8.2±0.6	11.1±1.1
	T-test	*	*	*	**	*	*	ns	*	ns
Mongolian pine	Bulk soil	6.2±0.1	171.4±8.9	3.1±0.1	11.4±1.8	45.6±3.2	1.8±0.1	1.6±0.1	4.9±0.5	9.4±1.0
	Rhizosphere soil	6.2±0.1	217.6±9.5	3.1±0.1	22.5±1.8	48.4±4.2	2.8±0.4	1.8±0.2	8.0±0.9	6.1±0.7
	T-test	ns	**	ns	**	ns	*	ns	**	*

Note: Value is mean±SE (n=5). ns denotes no significant difference; * and ** denote significant differences between bulk and rhizosphere soils at P<0.05 and P<0.01 levels, respectively (by paired samples T-test). APA, acid phosphatase activities; SOC, soil organic carbon DOC; dissolved organic carbon; MBC, microbial biomass C; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorus. Original data are from Zhao et al. (2010).

inorganic phosphate and total P. The inorganic phosphate concentration in all extracts was directly analyzed colorimetrically using the molybdate blue method. Total P in the extract was determined after addition of 1 mL 0.5 M H_2SO_4 and 0.2 g of $K_2S_2O_8$ and autoclave digestion during 1 h at 120–125°C (Rowland and Haygarth, 1997). Organic P in the extract was calculated as the difference between total P and inorganic P.

1.3 Statistical analysis

Differences in soil variables between the rhizosphere and bulk soil samples were compared with paired samples *T*-test for each tree species separately (P<0.05). Independent-sample *T*-test was used to determine the differences in soil organic P fractions between Mongolian pine and Simon poplar plantations. Pearson's correlation analyses were performed between soil organic P fractions and SOC and labile C (original data from Zhao et al. (2010)). All statistical analyses were performed using the SPSS 11.5 software package (SPSS Inc., Chicago, IL. USA).

2 Results

Soil total P ranged from 72 to 107 mg/kg, TPo ranged from 53 to 81 mg/kg and accounted for 76% of total P across the study site. The distribution of organic P among various fractions generally followed the same pattern in Simon poplar and Mongolian pine plantations; the concentration of organic P fractions decreased in the order of NaOH-Po>Res-Po>HCl-Po>Bic-Po. NaOH-Po (fulvic-Po+humic-Po) was the dominant form of organic P, which accounted for 62% and 72% of TPo in Simon poplar and Mongolian pine plantations, respectively. Bic-Po was less than 3.0 mg/kg across the study site, accounting for only 2.8% and 4.6% of TPo in Simon poplar and Mongolian pine plantations, respectively. The concentration of fulvic-Po was similar to that of humic-Po in Simon poplar plantations, but was about 2 times of humic-Po in Mongolian pine plantations (Table 2).

Bic-Po was significantly lower in rhizosphere soil than in bulk soil in both Simon poplar and Mongolian

Table 2 Concentrations of organic P fractions and TPo in bulk and rhizosphere soils of Simon poplar and Mongolian pine plantations

Tree species		Bic-Po	HCl-Po	Fulvic-Po	Humic-Po	Res-Po	TPo	Total P
		(mg/kg)						
Simon poplar	Bulk soil	2.6±0.4	3.9±0.8	25.0±1.5	24.9±1.6	13.3±0.9	69.2±3.4	87.0±3.1
	Rhizosphere soil	1.6±0.5	4.8±0.7	28.9±1.7	30.7±1.9	15.4±0.6	81.6±2.4	107.2±4.7
	T-test	*	ns	*	*	ns	*	*
Mongolian pine	Bulk soil	2.8±0.4	3.3±0.8	22.0±1.1	10.9±0.8	14.9±0.7	54.7±2.5	72.4±3.9
	Rhizosphere soil	1.6±0.6	3.4±0.4	21.9±0.9	11.5±0.9	13.9±0.5	52.3±1.6	74.2±2.8
	T-test	*	ns	ns	ns	ns	ns	ns

Note: value is mean±SE (n=5). NS denotes no significant difference; * denotes significant difference at P<0.05 level (by paired samples T-test); TPo, Total organic P; Po, organic P.

pine plantations. In Mongolian pine plantations there was no significant difference between rhizosphere and bulk soils for TPo and all other organic P fractions. In contrast, in Simon poplar plantations fulvic-Po, humic-Po and TPo were significantly higher in rhizosphere soil than in bulk soil but there was no difference for HCl-Po and Res-Po in rhizosphere and bulk soils (Table 2).

Effects of tree species on soil organic P fractions were similar in bulk and rhizosphere soils. Concentrations of total P, TPo, fulvic-Po, humic-Po in bulk and rhizosphere soils were both significantly higher in Simon poplar plantations than those in Mongolian pine plantations while the concentrations of Bic-Po, HCl-Po and Res-Po in both soils had no significant difference between the two plantations (Table 3).

Pearson's correlation analysis was conducted between SOC, microbial indices and different forms of organic P fractions. SOC, dissolved organic carbon, microbial biomass carbon, microbial biomass P and acid phosphatase activities were more or less positively correlated with TPo and organic P fractions which was relatively stable. There were no significant correlations between SOC, dissolved organic carbon, microbial biomass carbon and Olsen-P, Bic-Po (Table 4).

3 Discussion

In the present study, the proportion of soil organic P in total P is 76%, which is not only greatly higher than that in arid and semiarid desert shrubs (<13%) (Lajtha and Schlesinger, 1988; Cross and Schlesinger, 2001), but also greatly higher than that in woodlands across a semiarid chronosequence (0.8%–8.0%) (Selmants and Hart, 2010). The distribution of P in different forms is closely related to the soil age, parent material and environmental conditions. According to the conceptual model of Walker and Syers (1976), soils derived from silica-rich rocks at the middle stage of development

Table 3 Summary results of independent-sample *T*-test for the differences in organic P fractions between Simon poplar and Mongolian pine plantations

Soil	Bic-Po	HCl-Po	Fulvic-Po	Humic-Po	Res-Po	TPo	TP
Bulk soil	ns	ns	*	**	ns	**	**
Rhizosphere soil	ns	ns	*	**	ns	**	**

Note: * denotes significant at $P<0.05$ level (2-tailed); ** denotes significant at $P<0.01$ level (2-tailed); ns indicates that the difference is not statistically significant.

Table 4 Pearson's correlation coefficients between SOC and different forms of organic P fractions across Simon poplar and Mongolian pine plantations (n=20)

	SOC	DOC	MBC	MBP	APA	Olsen-P	Bic-Po	HCl-Po	Fulvic-Po	Humic-Po	Res-Po	TPo
SOC	1.00											
DOC	0.56*	1.00										
MBC	0.48*	0.29	1.00									
MBP	0.47*	0.61**	0.69**	1.00								
APA	–0.25	–0.05	–0.65**	–0.64**	1.00							
Olsen-P	–0.05	0.38	0.14	0.01	0.36	1.00						
Bic-Po	–0.20	–0.44	0.11	0.01	0.22	0.03	1.00					
HCl-Po	0.65**	0.39	0.48	0.51*	–0.38	–0.09	–0.28	1.00				
Fulvic-Po	0.66**	0.53*	0.46	0.63**	–0.46	–0.08	–0.07	0.46	1.00			
Humic-Po	0.68**	0.40	0.67**	0.76**	–0.79**	–0.39	–0.21	0.66**	0.63**	1.00		
Res-Po	0.65**	0.18	0.03	–0.01	0.15	–0.27	0.00	0.46	0.43	0.28	1.00	
TPo	0.78**	0.48*	0.66**	0.74**	–0.69**	–0.33	–0.11	0.69**	0.81**	0.95**	0.46*	1.00

Note: * denotes significant at $P<0.05$ level (2-tailed); ** denotes significant at $P<0.01$ level (2-tailed). Original data of SOC, DOC, MBC and MBP are from Zhao et al. (2010).

usually had more organic P. Soils in the present study are just in that case, which belong to Typic Ustipsamment derived from alluvial sand deposit with low cation exchange capacity and P sorption capacity.

Only a very small fraction (<5%) of organic P was in labile form, and more than 60% of organic P was associated with fulvic acid and humic acid in the present study (Table 2). Studies done by other researchers (Redel et al., 2008; Dieter et al., 2010; Slazak et al., 2010) also showed that NaOH-Po had the largest fraction of organic P in forest soil. Fulvic-Po and humic-Po are mainly composed of large molecular inositol phosphate combined with other organic compounds (Makarov and Leoshkina, 2009). In soil P cycling, "immediately available" pool of P is constantly replenished through the mobilization of "less available" P pool. The large pool of NaOH-Po is an important source of bioavailable P during long-term tree growth (Cross and Schlesinger, 1995).

In both plantations, the great depletion of labile Bic-Po in rhizosphere soil was accompanied by the significant accumulation of Olsen-P (Tables 1 and 2). This reflects that labile organic P is an important source of plant available P. Accelerated mineralization of labile organic P by greatly increased phosphatase activities in rhizosphere soil is responsible for the depletion of labile organic P (Table 1). Similar results were found in Chinese fir plantation (Chen, 2003), mature Norway spruce forest in humid regions (Häussling and Marschner, 1989) and temperate silvopastoral system (Scott and Condron, 2003). Conversely, depletion of labile inorganic P and accumulation of labile organic P in rhizosphere soils have been reported for crops and tree seedlings (Chen et al., 2002; Hassan et al., 2012). Thus, rhizosphere P dynamics is highly variable.

Rhizosphere effects on TPo and more stable organic P fractions differed obviously with tree species (Table 2). Accumulations of total P and TPo in rhizosphere soil compared with bulk soil were observed only in the Simon poplar plantations (Table 2). The effects can be ascribed to the differences of the SOC accumulation and microbial activities in rhizosphere between the tree species. The importance of rapid microbial P immobilization and turnover in P dynamics has been stressed by many researchers (Bünemann et al., 2012). In the present study, SOC, soil MBC and P were positively correlated with TPo and recalcitrant organic P fractions (Table 4). Moreover, SOC and soil microbial biomass carbon was significantly accumulated in rhizosphere soil in Simon poplar plantations, but not in Mongolian pine plantations (Table 1). Accumulation of organic P was also found in other forest stands (Clegg and Gobran, 1997; Chen, 2003; Toberman et al., 2011). The results, therefore, suggest that the accumulation of organic carbon in rhizosphere controls the organic P dynamics on a long-term basis while rhizosphere microbial processes control short-term P availability in the present study.

The correlation between SOC and labile P was always in argument. There was no significant correlation between SOC and labile organic P fractions in the present study (Table 4). However, converse results were observed in topsoils of tropical and temperate forests (Johnson et al., 2003; Dieter et al., 2010). The disagreement between the present study and others may be due to the very low content and high stability of soil organic matter in our study. Dissolved organic carbon and microbial biomass carbon accounted for less than 2.5% of total SOC. Fulvic-Po and humic-Po predominated in organic P. Brams (1973) found a positive correlation between soil organic matter and acid extractable P in superficial soil horizons but not in subsoils with low organic matter content.

4 Conclusions

This study shows a strong predominance of soil organic P, NaOH-Po in particular, in plantations in a semiarid sandy soil. Significant depletion of labile organic P was concurrent with the accumulation of Olsen-P in rhizosphere soil, confirming the essential role of biological organic P transformation in soil P supply in this semiarid region. The dynamic of recalcitrant organic P in rhizosphere soil was highly related to the dynamic of soil organic matter. Tree species differed greatly in rhizosphere effects on recalcitrant organic P fractions, which may be ascribed to the differences of the accumulation of SOC and microbial communities between the tree species.

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