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RESEARCH ARTICLE

Qiong ZHAO, Xingyu LIU, Dehui ZENG, Jinhuan LIU, Yalin HU

Relationships between foliar phosphorus fractions of *Pinus sylvestris* var. *mongolica* and soil available phosphorus

Abstract In order to find out the best foliar diagnostic index of phosphorus (P) nutrition in Mongolian pine (*Pinus sylvestris* var. *mongolica*) in the southeastern Keerqin Sandy Lands, the concentrations of total nitrogen (N), inorganic P, organic P and total P in needles of different ages and soil available P were examined. The results show that in the study area, soil available P was rather low (0.12–0.63 mg/kg) and was significantly correlated with inorganic P (cPi) and total P (cPt) concentrations in current year needles of Mongolian pine. The significant correlation between soil available P and needle cPt derived from the significant correlation between cPi and cPt. Compared with cPt, cPi did reflect the level of soil P supply more accurately and more directly.

Keywords foliar P fractions, soil P supply, *Pinus sylvestris* var. *mongolica*

1 Introduction

During the past 50 years, Mongolian pine (*Pinus sylvestris* var. *mongolica*) plantations were widely established in the southeastern Keerqin Sandy Lands, China, with the purpose of combating soil desertification and improving local environments; it has proven to be highly effective. However, since the late 1980s, the growth of large areas of Mongolian pine has declined as a result of a combination of insufficient supply of water, nutrients and other factors (Zhu et al., 2005). The soils in the southeastern Keerqin Sandy Lands are mainly sandy soils with little available nitrogen (N) and phosphorus (P) (Jiao, 1989; Jiang et al., 2002). Previous studies revealed that the deficiency of available P in soils was one of the main factors affecting the growth of Mongolian pine in the southeastern Keerqin Sandy Lands and P fertilization clearly improves tree growth (Cao et al., 2002; Liu et al., 2002). Diagnosing nutrition of trees quickly and accurately is indispensable for the management of Mongolian pine plantations.

Foliar nutrient concentration has been widely used as an index of diagnosis of tree nutrition, since foliar nutrient concentration is highly correlated with tree growth and soil nutrient availability (Lee et al., 1991; Elias et al., 2002; Hobbie and Gough, 2002). However, this is not universal and many studies found that total foliar N and P concentrations remained constant with tree growth (Vitousek et al., 1995; Aerts and Chapin, 2000; Thomas et al., 2006). For P nutrition, many studies in recent decades suggested that the level of inorganic phosphate concentration in plant tissues is more sensitive to changes in P nutrition than the levels of organic phosphate and total P concentration (Thomas et al., 2006). However, the use of inorganic foliar P as an index of tree P nutrition diagnosis is still in its infancy. Besides, the sensitivity of foliar nutrient concentration to changes in soil nutrient availability is affected by the age of needles. Fully expanded current-year leaves in evergreen trees and youngest fully expanded leaves in broadleaved trees were recognized as the most sensitive parts of a tree to changes in soil nutrient availability (Palmer et al., 2005; Thomas et al., 2006).

In order to find out the best index of foliar diagnosis of P nutrition in Mongolian pine and to make clear whether or not Mongolian pine plantations in southeastern Keerqin Sandy Lands are limited by P availability, we examined the relationships between P fractions in needles of different ages and soil P availability.

2 Materials and methods

2.1 Site description

The study was conducted at the Daqinggou National Nature Reserve in Inner Mongolia, China (42°45'–42°48'N, 122°13'–122°15'E), geographically located in the southeastern

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Qiong ZHAO, Xingyu LIU, Dehui ZENG (✉), Jinhuan LIU, Yalin HU
Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China
E-mail: zengdh@iae.ac.cn

Keerqin Sandy Lands, at an elevation of 240 m. The study area belongs to a semi-arid region in the middle of a temperate zone, has a continental monsoon climate, characterized by cold and dry winters but warm and moist summers. The mean annual rainfall was 450 mm (ranging from 262 to 740 mm) between 1955 and 2000, with more than 60% of the rain falling during June–August. The annual potential evaporation is 1300–1800 mm. The annual mean temperature is 6.4°C, with the lowest monthly mean temperature occurring in January (-12.5°C) and the highest monthly mean temperature occuring in July (23.8°C). The soil is an aeolian sandy soil (Typic Ustipsamment, sand: 90.9%, silt: 5.0%, clay: 4.1%) developed from sandy parent material through the action of wind and deficient in N and P. The soil pH is around 6.7.

2.2 Sample collection

Seven 20 m×20 m plots were established in Mongolian pine plantations of various densities (1600 to 5000 tree/hm^2) and ages (15 to 30 years old) for soil and leaf sampling. All plots were selected on flat topography within a 1 km^2 area in the Daqinggou National Nature Reserve to ensure similar soil types and climatic conditions. In August 2006, when the current-year needles of the Mongolian pine had been fully expanded, soil and leaf samples were collected simultaneously. In each plot, five composite soil samples, each made up of 10 soil cores (6 cm internal diameter), were collected from 0–20 cm mineral soil horizon. Each soil sample was sieved to pass a 2 mm mesh to remove roots and plant debris and then was stored at 4°C until the measurement of available P.

Twelve sample trees were selected for leaf sampling in each plot. Young branches in all directions of the top 1/3 of the tree crown of three trees were collected as one sample. Branches were brought to the laboratory as soon as possible. In the laboratory, needles of different ages (current-year, one-year-old and two-year old) were picked off from the branches separately. To avoid the hydrolysis of org-anic leaf phosphate, freshly picked needles were dried to constant weight at 140°C (about 5 min) (Zohlen and Tyler, 2004).

2.3 Sample analyses

Soil available P (Olsen-P) was determined after the soil was extracted by 0.5 mol/L $NaHCO_3$ ($pH = 8.5$) for 30 min (Lu, 1999). Dried leaf samples were ground to pass through a 0.25 mm mesh sieve for the measurements of the concentration of total N, total P, inorganic P and organic P. Total leaf N and P concentrations were determined after the samples were treated by H_2SO_4-H_2O_2 digestion at 320°C. Inorganic leaf P was determined according to the method described by Zohlen and Tyler (2004). Briefly, dried leaf samples were extracted by distilled water for 1 h and inorganic phosphate in the extract was the inorganic P; organic leaf P was determined as the difference between total P and inorganic P. All phosphate and N concentrations in the extracts were analyzed colorimetrically on a continuous-flow autoanalyzer (AutoAnalyzer III, Bran + Luebbe GmbH, Germany).

2.4 Statistical analysis

One-way analysis of variance (ANOVA) was used to test for the differences ($p < 0.05$) in nutrient concentrations between needles of different ages. Pearson correlations, partial correlations and regression analyses were conducted to examine the relationships between soil available P and foliar nutrient indices. All statistical analyses were performed using SPSS 11.5.

3 Results and discussion

3.1 Foliar nutrient concentrations

Foliar organic P (Po) concentration did not show significant differences among Mongolian pine needles of different ages, while total foliar P (Pt) and inorganic P (Pi) concentrations decreased significantly with needle age. Total foliar N concentration was significantly higher in current-year and one-year-old needles than in two-year-old needles (Table 1). P in Mongolian pine needles was found

Table 1 Nutrient concentrations in different aged leaves of Mongolian pine ($n = 28$) (unit: $g\cdot kg^{-1}$)

nutrient indices	mean	minimum	maximum	standard deviation
cPi	0.50a	0.31	0.74	0.09
1Pi	0.33b	0.22	0.53	0.08
2Pi	0.26c	0.18	0.44	0.05
cPo	1.19a	0.95	1.84	0.19
1Po	1.15a	0.76	1.50	0.20
2Po	1.06a	0.69	1.61	0.22
cPt	1.70a	1.32	2.31	0.23
1Pt	1.47b	1.03	1.78	0.20
2Pt	1.32c	0.91	1.79	0.22
cN	13.58a	11.94	15.24	0.78
1N	13.43a	11.78	14.60	0.79
2N	12.20b	10.06	14.16	0.94
cN/cPt	8.16b	5.90	11.80	1.60
1N/1Pt	9.29a	6.95	12.97	1.39
2N/2Pt	9.43a	6.52	12.38	1.45

Note: Pi, Po, Pt and N represent leaf inorganic P, organic P, total P and total N concentration, respectively; c, 1 and 2 before Pi, Po, Pt and N represented current-year, one-year-old and two-year-old leaves, respectively (The same comment applies to Table 2). Mean values within columns for each leaf index with different letters were significantly different at 0.05 level among different aged needles.

mainly in organic form, Pi accounted for 30%, 24% and 20% of Pt in current-year, one-year-old and two-year-old needles respectively.

Although inorganic phosphate is usually a small fraction of total P in plant tissues, it is important to leaf metabolism for many reasons, particularly to processes associated with photosynthesis (Pan et al., 1997). What is more, the change in inorganic phosphate content is about an order of magnitude greater than that in organic phosphate over the full range of P nutrition (Bieleski, 1973). As the main fraction of total foliar P, foliar Po is the essential constituent of cell nuclei and cell membranes. Therefore, Po concentration is relatively stable in mature leaves and is hydrolyzed to Pi just before senescence (Chapin and Kedrowski, 1983; Schachtman et al., 1998; Thomas et al., 2006).

Pt is significantly correlated with Po despite needle age, which is probably caused by the fact that Po is the main component of Pt. The correlation between Pi and Pt was only significant in current-year leaves, which suggests that P transformation is much more active in current-year needles than in one- and two-year-old needles. There were no significant correlations between Pi and Po in any of the leaves (Table 2).

3.2 Relationships between foliar P fractions and available soil P

Many studies have shown that Pt concentrations in plant tissues do not respond to changes in available soil P and plant growth as a result of the “dilution effect” during growth, while Pi concentration in plant tissues provided the best index of P status of a plant (Bieleski, 1973; Mclachlan, 1984; Thomas et al., 2006). Since it is affected by plant species and soil P availability, the ratio of foliar Pi to Pt varies greatly. Under the condition of P deficiency, most of the P is used for tissue growth; Pi concentration is very low. Chapin and Kedrowski (1983) found that foliar Pi/Pt was only 12%–25% in unfertilized taiga trees, but this proportion increased to 50%–95% by fertilization. Polgase et al (1992) reported that fertilizer application increased the fraction of Pi in *Pinus taeda* needle litter from 40% to 75%. However, to date, the most widely used foliar diagnostic index of tree P nutrition is total P. The different responses of foliar P fractions to changes in soil P availability and “dilution effect” during tree growth has seldom been taken into account.

Soil available P (Olsen-P) concentration under the Mongolian pine plantations was very low, with a mean value of 0.44 mg/kg (0.12–0.63 mg/kg). Pearson correlation analyses showed that soil Olsen-P concentration was highly and positively correlated with foliar Pi and Pt concentrations in current-year needles, but was not significantly correlated with foliar Po concentration in current-year needles and nutrient concentrations in one- and two-year-old needles (Table 3).

Due to the significant correlation between foliar Pi and Pt in current-year needles (cPi and cPt) (Table 2), the high Pearson correlations between soil Olsen-P and foliar cPi and cPt could not reflect their direct relationships. The direct relationship between two variables can only be examined by partial correlation analysis when the associated variables are controlled. Different from the results of Pearson correlations analyses, partial correlations analyses showed that soil Olsen-P concentration was significantly correlated with foliar cPi concentration, but no significant correlation was found with foliar cPt concentration (Table 4). These results indicate that the significant Pearson correlation between soil Olsen-P and foliar cPt resulted from the high correlation between foliar cPi and cPt. As well, the results suggest the highest sensitivity of cPi to the changes in soil P availability and a significant effect of the soil P supply on the growth of Mongolian pine. Regression analyses with soil Olsen-P

Table 2 Pearson correlation coefficients between Pt, Pi and Po in different aged leaves of Mongolian pine ($n = 28$)

	cPi	cPo		1Pi	1Po		2Pi	2Po
cPt	0.620**	0.921**	1Pt	0.233	0.929**	2Pt	0.133	0.968**
cPi	1	0.265	1Pi	1	−0.144	2Pi	1	−0.119

Note: ** means $p < 0.01$.

Table 3 Pearson correlation coefficients between soil available P (Olsen-P) and foliar nutrient concentrations of Mongolian pine ($n = 7$)

	cPi	1Pi	2Pi	cPt	1Pt	2Pt	cPo	1Po	2Po	cN	1N	2N
Olsen-P	0.763**	0.422	−0.117	0.611*	0.521	0.412	0.475	0.406	0.448	−0.117	0.419	0.465
	(0.002)	(0.151)	(0.802)	(0.026)	(0.068)	(0.358)	(0.101)	(0.168)	(0.313)	(0.493)	(0.154)	(0.112)

Note: values in the table were Pearson correlation coefficients with significance levels in brackets; **: $p < 0.01$; *: $p < 0.05$.

Table 4 Partial correlation coefficients between soil available P (Olsen-P) and foliar inorganic leaf P (cPi) and total P (cPt) in current year

	cPi (cPt as control variable)	cPt (cPi as control variable)
Olsen-P	0.581*(0.047)	−0.085(0.793)

concentration as independent variable (x) and cPi concentration as dependent variable (y) showed that $y = 0.39x + 0.28$ ($R^2 = 0.582$, $p = 0.002$).

3.3 Nutrient limitations in Mongolian pine plantations

There are several definitions of "nutrient limitation" in ecological research. The most widely accepted definition is the one provided by Vitousek and Howarth (1991): "Nutrient limitation to a particular process is defined as being demonstrable when a substantial addition of a particular nutrient increases the rate or changes the endpoint of that process". Thus, according to the results of our present study and previous fertilization experiments, N and P were both limiting nutrients to Mongolian pine growth in the study area (Cao et al., 2002; Deng et al., 2006), but whether N or P is the principal limiting nutrient still needs further investigation. In the last 20 years, N/P stoichiometry has been widely used to assess the nutrient limitation to plant growth (Zeng and Chen, 2005). In the present study, total foliar N concentrations ranged from 11.9 to 15.2 g/kg, total P concentration from 1.3 to 2.3 g/kg and foliar N/P ratio from 5.9 to 10.7. These values differed greatly from the critical values of total foliar N and P concentrations and N/P ratios for N and P limitation diagnostics in other studies (Braakhekke and Hooftman, 1999; Zhang et al., 2004). Besides, the N application experiment showed that the addition of N greatly increases the growth of Mongolian pine seedlings, but had no significant effect on total foliar N and total P concentrations and the N/P ratio (Deng et al., 2006). Therefore, total foliar N and P concentrations and the N/P ratio as indices of tree nutrition diagnostics should be used with great caution.

4 Conclusions

Soil available P was deficient (less than 0.63 mg/kg) under Mongolian pine plantations in the southeastern Keerqin Sandy Lands. Foliar inorganic P concentration was less than 30% of foliar total P concentration and varied significantly among needles of different ages. Both inorganic P and total P concentrations in current-year needles of Mongolian pine were significantly correlated with soil available P concentration. However, as an index of P nutrition diagnosis of Mongolian pine, inorganic P concentration in current-year needles was more accurate and direct than total P concentration. Therefore, instead of taking total P concentration and N/P ratio as diagnostic foliar indices, the different responses of foliar P fractions to the changes in soil P availability should be taken into account in plant P nutrition diagnostics.

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