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Ecophysiological response in leaves of *Caragana microphylla* to different soil phosphorus levels

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Abstract

Phosphorus (P) is one of the limiting mineral nutrient elements in the typical steppe of Inner Mongolia, China. In order to find out the adaptive strategy of *Caragana microphylla* to low soil P status, we grew plants in P-deficient soil in April 2009 and gave a gradient of P addition ranging from 0 to 60 mg(P) kg^{-1}(soil) from May 2010. Leaf traits were measured in September 2010. Both leaf growth and light-saturated photosynthetic rate (P_{max}) were similar among different groups. Leaf nitrogen (N):P ratio indicated that the growth of *C. microphylla* was not P-limited in most of the Inner Mongolia typical steppe, which had an average soil available P content equal to 3.61 mg kg^{-1}. The optimal P addition was 20 mg(P) kg^{-1}(soil) for two-year-old plants of *C. microphylla.* Leaf mass area (LMA) and leaf dry matter content (LDMC) were enhanced with low P, and significantly negatively correlated with photosynthetic N-use efficiency (PNUE). Photosynthetic P-use efficiency (PPUE) increased with decreasing soil P and increasing leaf inorganic P (Pi): organic P (Po) ratio, and showed no significant negative correlation with LMA or LDMC. P_{max} of *C. microphylla* did not decline so sharply as it was anticipated. The reason for this phenomenon might be due to the increased PPUE through regulating the leaf total P allocation. *C. microphylla* had high P-use efficiency *via* both high PPUE and long P-retention time at low-P supply. The adaptation of *C. microphylla* to low-P supply provided a new explanation for the increased distribution of the species in the degraded natural grassland in Inner Mongolia, China.

Additional key words: adaptive strategy; leaf functional traits; leaf phosphorus allocation; soil phosphorus level.

Introduction

The typical steppe of Inner Mongolia is one of the important parts of the Eurasia grassland ecosystems (Christensen *et al*. 2004). The soils of the steppe have often a P-deficiency problem, especially in the degraded areas (Wu *et al*. 2006). Over the past several decades, grassland degradation has been aggravated, associated with the increased distribution of *C. microphylla*, which is a widely spread, N_2-fixing shrub species in the Inner Mongolia steppe, changing grassland into shrubland finally. The expansion of *C. microphylla* in the degraded grassland was thought to be caused by the resistance of the species to grazing, drought, and low-N status (Xiong *et al.* 2003). In the previous field study, we found that light-saturated photosynthetic rate of *C. microphylla,* calculated per unit of leaf area (P_{area}), was above 10 µmol(CO_2) m^{-2} s^{-1} even at the lowest P status (Zhao *et al*. 2011). The study of the adaptive strategy of *C. microphylla* to low-P status could help us to understand better the increasing distribution of its populations in the degraded grassland.

P is an essential nutrient element for plant growth and development, and it plays an important role in the photo-

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Abbreviations: CLA – leaf area per compound leaf; DM – dry mass; FM – fresh mass; g_s – stomatal conductance; ILN – increase of compound leaf number; LDMC – leaf dry matter content; LMA – leaf mass area; LPt – leaf total phosphorus; LPt_{area} – leaf total phosphorus content per unit of leaf area; LPt_{mass} – leaf total phosphorus content per unit of leaf dry mass; N_{mass} – leaf total nitrogen content per unit of leaf dry mass; P_{area} – light-saturated photosynthetic rate per unit of leaf area; P_{mass} – light-saturated photosynthetic rate per unit of leaf dry mass; P_{max} – light-saturated photosynthetic rate; Pi – inorganic phosphorus; Pi_{area} – leaf inorganic phosphorus content per unit of leaf area; Pi_{mass} – leaf inorganic phosphorus content per unit of leaf dry mass; PNUE – photosynthetic nitrogen-use efficiency; Po – organic phosphorus; Po_{area} – leaf organic phosphorus content per unit of leaf area; Po_{mass} – leaf organic phosphorus content per unit of leaf dry mass; PPUE – photosynthetic phosphorus-use efficiency; RR – response ratio.

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synthetic carbon assimilation. P-impoverished soils limit the growth and primary productivity in terrestrial plant. Ecologists have paid much attention to leaf traits for the analysis of plant adaptions to soil nutrient status (Read *et al*. 2005). They found that a number of leaf traits reflected the adaptive strategy of plants to low soil P supply. McIntyre (2008) found that an increase of LMA and LDMC was associated with decreased soil P availability in an Australian eucalyptus–grass ecosystem. Plants invest more in a leaf toughness to achieve a prolonged leaf life span at low-P habitat to have a long P residence time. It caused then a decrease of PNUE, because plants cannot maximize both PNUE and leaf toughness at the same time (Hikosaka 2004). However, unlike PNUE, PPUE could increase with the leaf toughness at the same time at low-P environment (Hidaka and Kitayama 2011). Photosynthesis requires inorganic phosphorus (Pi) as a substrate and it is correlated much closer with Pi than organic phosphorus (Po) (Thomas *et al*. 2006, Hidaka and Kitayama 2011). Plants might regulate the leaf total P (LPt) allocation to increase PPUE at low-P condition, *i.e.*, plants allocate a greater P fraction to Pi than to Po (Hidaka and Kitayama 2009). The increasing PPUE with decreasing soil P supply was thought to be one of the plant adaptive strategies to low-P supply (Denton *et al.* 2007, Hidaka and Kitayama 2009).

We aimed to find out the responsive characteristics of *C. microphylla* to different soil P status, especially in P-deficient conditions. We hypothesized that *C. microphylla* could regulate the leaf Pi:Po ratio, and then raise PPUE to maintain P_{max} at low soil P supply, and increase LMA and LDMC at the same time. This study could provide the insights into the reasons, why the expanded distribution of *C. microphylla* occurred in the degraded grassland in Inner Mongolia steppe of China.

Materials and methods

Plant material and treatments: Seeds of *C. microphylla* were collected from the natural grassland in Inner Mongolia, germinated in April 2009, and then transferred into pots (20 × 20 cm) containing 3 kg of sterile sandy loam. The soil had low level of nutrients: total N was 104 mg kg^{-1}, total P was 0.04%, and Olsen P was 1.5 mg kg^{-1}. After one year, seedlings, similar in the growth at the beginning of treatment, were maintained for 21 weeks (since April 30, 2010) before their leaf traits were measured.

The experiment was carried out in the glasshouse in the College of Life Science of Nankai University, Tianjin (39.10°N, 117.16°E; 5 m a.s.l; the mean annual temperature of 12.9°C; the mean growing-season temperature of 21.8°C). During the experimental period, the midday mean photosynthetically active radiation varied between 1,300 μmol m^{-2} s^{-1} and 1,500 μmol m^{-2} s^{-1}. A randomized block design with 9 levels of P supply and 15 replicates was used. Standard Hoagland's solution was modified in order to vary P concentration, whilst keeping an optimal availability of the other nutrients. Different soil P levels were obtained by addition of Hoagland's solution with different P content [KH_2PO_4 with the rate of 0, 1.5, 3.0, 4.5, 6.0, 8.5, 20, 30, and 60 mg(P) l^{-1}]. 750-ml equivalents were added monthly from May to August, 2010. The total P contents were 0, 1.5, 3.0, 4.5, 6.0, 8.5, 20, 30, and 60 mg(P) kg^{-1}(soil). 500 ml of water was added to each pot every other day to ensure that plants were not water-stressed. Some seedlings were infested by the spider mite during the experiment, thus, they were excluded from measurements. Nine to twelve replicates per treatment were finally used for data analysis.

Measurements of leaf traits: Upper fully-expanded leaves were collected in September 2010 for the measurements (Cornelissen *et al*. 2003).

P_{area}, light-saturated photosynthetic rate per unit of leaf dry mass (P_{mass}), stomatal conductance (g_s), PNUE, and PPUE were measured between 09:00–11:30 h on sunny day; three upper fully-expanded leaves per seedling were used to measure the leaf gas exchange (Cornelissen *et al*. 2003). P_{area} and g_s were measured by using a portable open gas-exchange system (*LI-6400*, *LiCor*, Lincoln, NE, USA). The photosynthetic photon flux density, relative humidity, and leaf temperature in the leaf chamber were set at 1,500 μmol m^{-2} s^{-1}, 60 ± 5%, and 25 ± 0.5°C. The ambient CO_2 concentration was 390 ± 10 μmol mol^{-1}. The three values per seedling were averaged as the trait value of the individual. P_{mass} was calculated as P_{area}/LMA, PNUE was calculated as the ratio of P_{mass} to leaf total N content per unit of leaf dry mass (N_{mass}) (Cheng *et al*. 2012), and PPUE was calculated as the ratio of P_{mass} to LPt content per unit of leaf dry mass (LPt_{mass}) (Hidaka and Kitayama 2009).

Leaf area per compound leaf (CLA) and the increase of compound leaf number (ILN) were measured after photosynthetic measurements; the same leaves were scanned to make computer images. CLA was measured with the *Adobe Photoshop 7.0.1 program* (*Adobe Systems Inc*., San Jose, CA, USA) (Cornelissen *et al*. 2003). The 3 values were averaged as the CLA of the individual. ILN was calculated as the difference between the compound leaf number on July 10th and the compound leaf number on May 10th.

LMA and LDMC: The same leaves, used to measure P_{area} and CLA, were oven-dried at 60°C for 72 h, and their dry mass (DM) was determined. LMA was calculated as DM/CLA (Cheng *et al*. 2012). Other 10 upper fully-expanded leaves per seedling were placed in water in the darkness at 5°C for 12 h after the stem or petiole was removed. This procedure ensured full leaf saturation. Leaves were then dried with a tissue paper to remove surface water and immediately weighed to determine their saturated fresh mass (FM). The samples were then oven-dried at 60°C for 72 h, and their DM was determined. LDMC was calculated as DM/saturated FM (Garnier *et al*. 2001).

$\mathrm{N}_{\mathrm{mass}}$, LPt, Pi, and Po content per unit of leaf dry mass ($\mathrm{LPt}_{\mathrm{mass}}$, $\mathrm{Pi}_{\mathrm{mass}}$ and $\mathrm{Po}_{\mathrm{mass}}$) and per unit of leaf area ($\mathrm{LPt}_{\mathrm{area}}$, $\mathrm{Pi}_{\mathrm{area}}$ and $\mathrm{Po}_{\mathrm{area}}$) were determined from 12 upper, fully-expanded, compound leaves per seedling. All petioles and rachis were cut off before leaf nutrient content analysis (Cornelissen *et al*. 2003). Ten leaves were used to measure $\mathrm{N}_{\mathrm{mass}}$ and $\mathrm{LPt}_{\mathrm{mass}}$. They were oven-dried at 100°C for 10 min in order to fix chemical components of leaves and to maintain constant chemistry of leaves, ground to pass through a 0.15-mm mesh sieve, and oven-dried at 60°C for at least 72 h. The other two leaves were immersed in liquid N, freeze-dried and ground for $\mathrm{Pi}_{\mathrm{mass}}$ measurement. $\mathrm{N}_{\mathrm{mass}}$ was determined following the standard Kjeldahl method (Nelson and Sommers 1972) and $\mathrm{LPt}_{\mathrm{mass}}$ following the molybdate colorimetric method after ascorbic acid reduction (Murphy and Riley 1962). $\mathrm{Pi}_{\mathrm{mass}}$ was measured according to the ascorbic acid-molybdate method after 1% HClO_4 extraction (Thomas *et al*. 2006). $\mathrm{LPt}_{\mathrm{area}}$ and $\mathrm{Pi}_{\mathrm{area}}$ were calculated as $\mathrm{LPt}_{\mathrm{mass}} \times \mathrm{LMA}$ and $\mathrm{Pi}_{\mathrm{mass}} \times \mathrm{LMA}$, respectively. $\mathrm{Po}_{\mathrm{mass}}$ and $\mathrm{Po}_{\mathrm{area}}$ were calculated as $\mathrm{LPt}_{\mathrm{mass}} - \mathrm{Pi}_{\mathrm{mass}}$ and $\mathrm{LPt}_{\mathrm{area}} - \mathrm{Pi}_{\mathrm{area}}$, respectively.

Data analysis: All statistical analyses were conducted using *SPSS 13.0* (*SPSS 13.0*, Chicago, IL, USA). One-way *ANOVA* was used to determine the effects of soil P treatments on leaf traits. Response ratio (RR) was calculated to indicate the ecophysiological response to different P levels referring by Violle *et al.* (2006), as $\ln \mathrm{RR}_{i,j} = \ln(\mathrm{T}_{i,j}/\mathrm{T}_{i,0})$, where $\mathrm{T}_{i,j}$ and $\mathrm{T}_{i,0}$ are mean value of trait i at the P addition of j and 0 mg(P) kg^{-1}(soil), respectively. The $\mathrm{LPt}_{\mathrm{mass}}$ were divided arbitrarily into 3 groups. We tested the responses of plant traits to soil P supply and the relationships between P_{mass} and $\mathrm{N}_{\mathrm{mass}}$ in different $\mathrm{LPt}_{\mathrm{mass}}$ groups using the best-fitting linear and nonlinear regression curve estimations. *Spearman*'s rank coefficients were used to test the relationships among leaf functional traits because some traits were not found to be normally distributed using *Shapiro-Wilk*'s test. In all cases, $p<0.05$ was taken as significant.

Results

***C. microphylla* response to soil P supply**: *C. microphylla* showed no evident P-deficiency symptom at any level of the P supply, *i.e.*, either darkish green leaves or reddish stems. Each plant produced at least 20 leaves since the treatment started.

Both the growth traits and the P_{max} were rather similar among different levels of soil P supply (Table 1). Only CLA varied significantly among the 9 levels, and there was no significant correlation between the response ratio of CLA and soil P supply (Table 1, Fig. 1*A*). The response

Table 1. One-way *ANOVA* test and the maximum and minimum values of plant traits in different soil P supply. Leaf area per compound leaf (CLA), increase of compound leaf number (ILN), light-saturated photosynthetic rate per unit of leaf dry mass and area (P_{mass} and P_{area}), stomatal conductance (g_{s}), photosynthetic nitrogen-use efficiency (PNUE), photosynthetic phosphorus-use efficiency (PPUE), leaf total phosphorus content per unit of leaf dry mass and area ($\mathrm{LPt}_{\mathrm{mass}}$ and $\mathrm{LPt}_{\mathrm{area}}$), leaf inorganic phosphorus content per unit of leaf dry mass and area ($\mathrm{Pi}_{\mathrm{mass}}$ and $\mathrm{Pi}_{\mathrm{area}}$), leaf organic phosphorus content per unit of leaf dry mass and area ($\mathrm{Po}_{\mathrm{mass}}$ and $\mathrm{Po}_{\mathrm{area}}$), leaf mass area (LMA), and leaf dry matter content (LDMC).

Parameter	F	*p*	Maximum value	Minimum value
CLA [cm^2]	3.301	0.003	5.65	3.70
ILN	1.731	0.103	86	52
P_{mass} [nmol(CO_2) $\mathrm{g}^{-1}\ \mathrm{s}^{-1}$]	1.599	0.137	296.34	245.24
P_{area} [μmol(CO_2) $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$]	1.437	0.193	20.70	18.64
g_{s} [mol($\mathrm{H_2O}$) $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$]	2.179	0.037	0.37	0.24
PNUE [μmol(CO_2) mol^{-1}(N) s^{-1}]	5.342	<0.001	166.96	113.91
PPUE [μmol(CO_2) mol^{-1}(P) s^{-1}]	13.07	<0.001	5344	2469
$\mathrm{LPt}_{\mathrm{mass}}$ [g kg^{-1}]	36.55	<0.001	3.32	1.46
$\mathrm{LPt}_{\mathrm{area}}$ [g m^{-2}]	20.59	<0.001	0.25	0.11
$\mathrm{Pi}_{\mathrm{mass}}$ [g kg^{-1}]	23.19	<0.001	1.29	0.67
$\mathrm{Pi}_{\mathrm{area}}$ [g m^{-2}]	13.082	<0.001	0.10	0.05
$\mathrm{Po}_{\mathrm{mass}}$ [g kg^{-1}]	15.1	<0.001	2.13	0.79
$\mathrm{Po}_{\mathrm{area}}$ [g m^{-2}]	11.55	<0.001	0.15	0.06
Pi:Po	–2.132	0.041	0.91	0.55
N:P	46.863	<0.001	21.06	7.05
LMA [g m^{-2}]	–0.757	0.641	76.70	70.24
LDMC [g kg^{-1}]	–2.843	0.008	312.48	273.63

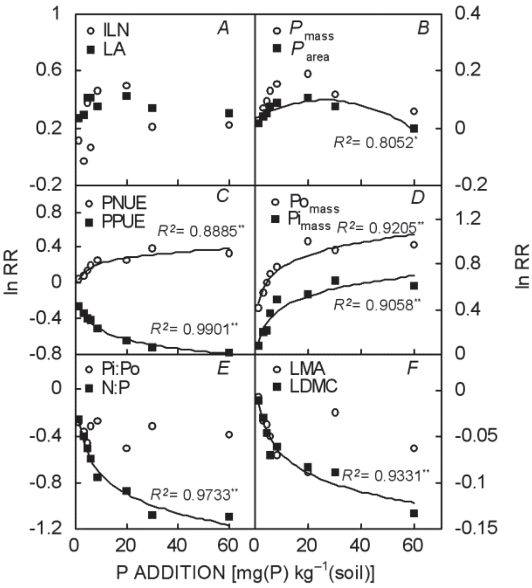

Fig. 1. Ln response ratio (RR) of traits among treatments for *C. microphylla*. (*A*) ○, leaf area per compound leaf (CLA), ■, increase of compound leaf number (ILN); (*B*) ○, light-saturated photosynthetic rate per unit of leaf dry mass (P_{mass}), ■, light-saturated photosynthetic rate per unit of leaf area (P_{area}); (*C*) ○, photosynthetic nitrogen-use efficiency (PNUE), ■, photosynthetic phosphorus-use efficiency (PPUE); (*D*) ○, leaf organic phosphorus content per unit of leaf dry mass (Po_{mass}), ■, leaf inorganic phosphorus content per unit of leaf dry mass (Pi_{mass}); (*E*) ○, Pi:Po, ■, N:P; (*F*) ○, leaf mass area (LMA), ■, leaf dry matter content (LDMC). Coefficient of determination (R^2) was shown at $p<0.05$. ** – highly significant ($p<0.01$) and * – significant ($0.01<p<0.05$), ($n = 8$).

Table 2. *Spearman*'s rank correlation coefficients (ρ_s) between leaf phosphorus contents and other plant traits. Leaf inorganic phosphorus content per unit of leaf dry mass and area (Pi_{mass} and Pi_{area}), leaf organic phosphorus content per unit of leaf dry mass and area (Po_{mass} and Po_{area}), leaf area per compound leaf (CLA), increase of compound leaf numbers (ILN), light-saturated photosynthetic rate per unit of leaf dry mass and area (P_{mass} and P_{area}), stomatal conductance (g_s), photosynthetic nitrogen-use efficiency (PNUE), photosynthetic phosphorus-use efficiency (PPUE), leaf mass area (LMA), and leaf dry matter content (LDMC). ** – highly significant ($p<0.01$) and * – significant ($0.01<p<0.05$), ($n = 93$).

Parameter	Pi_{mass}	Pi_{area}	Po_{mass}	Po_{area}	Pi:Po
CLA	0.188	0.135	0.262^{*}	0.257^{*}	–0.187
ILN	0.136	0.138	0.164	0.150	–0.076
P_{mass}	0.292^{**}	-	0.238^{*}	-	0.003
P_{area}	-	0.224^{*}	-	0.081	0.103
g_s	–0.015	–0.081	0.083	0.057	–0.074
PNUE	0.575^{**}	0.363^{**}	0.515^{**}	0.332^{**}	–0.089
PPUE	-0.633^{**}	-0.700^{**}	-0.815^{**}	-0.911^{**}	0.469^{**}
LMA	–0.117	0.250^{*}	-0.248^{*}	0.080	0.138
LDMC	-0.364^{**}	-0.354^{**}	-0.377^{**}	-0.384^{**}	0.137

ratio of P_{area} decreased, when the P addition was greater than 20 mg(P) kg^{-1}(soil), and P_{mass} had the same trend though the relationship was not significant (Fig. 1*B*). g_s varied significantly among different soil P levels (Table 1), mainly due to the low values in 60 mg(P) kg^{-1}(soil) addition level. The variation of g_s changed into not significant, when comparing the first 8 soil P levels. PNUE and PPUE showed opposite correlations with soil P supply; PNUE decreased, while PPUE increased (Table 1, Fig. 1*C*). Leaf nutrient contents showed significant differences among the soil P supply levels (Table 1). The response ratio of Pi_{mass} was smaller than that of Po_{mass}, and both of them decreased with the decreasing soil P supply (Fig. 1*D*). P_{max} of *C. microphylla* varied less than leaf Po and leaf Pi (Fig. 1*B*,*D*). Though the response ratio of Pi:Po showed no significant relationship with soil P supply, there was an increasing trend with the decreasing P addition below 6 mg(P) kg^{-1}(soil). A linear negative correlation existed between Pi:Po ratio and P addition at the individual plant level below 20 mg(P) kg^{-1}(soil) ($R^2 = 0.066$, $p=0.030$, $n = 72$). Leaf N:P ratio increased with decreasing P addition, which was 21.06 at 0 mg(P) kg^{-1}(soil) addition and 16.16 at 1.5 mg(P) kg^{-1}(soil) P addition. At other levels, leaf N:P ratio were all lower than 16 (Table 1, Fig. 1*E*). For the leaf material resistant traits, LDMC varied significantly among 9 levels and increased with the decreasing soil P supply, while there was no significant difference in LMA among 9 levels, though there was a linear decrease with decreasing P addition at the individual plant level below 20 mg(P) kg^{-1}(soil) ($R^2 = 0.068$, $p=0.028$, $n = 72$) (Table 1, Fig. 1*F*).

Relationships between leaf traits: CLA showed the significant, positive correlation with leaf Po, while the ILN showed no significant correlation with either leaf Pi or Po. There was a significant, negative relationship between LMA and leaf Po_{mass}. LDMC increased with both decreasing leaf Po and Pi. P_{max} decreased with decreasing leaf Pi both on the area and mass basis, while the positive correlation between P_{max} and Po was only significant on the mass basis. g_s showed no significant relationship with either leaf Pi or Po. PNUE showed a significant, positive correlation with both Po and Pi, while PPUE showed a significant, negative correlation with them. PPUE increased significantly with increasing leaf Pi:Po ratio (Table 2). PNUE showed negative correlations with both LMA and LDMC, while PPUE was positively correlated with LDMC (Fig. 2). P_{area} showed no significant relationship with either LMA or LDMC. The significant, positive correlation between N_{mass} and P_{mass} only existed within high LPt group, and the slope showed a steeper trend with higher LPt content (Fig. 3).

Discussion

The seedlings showed no evident, P-deficiency symptoms even at the lowest soil P supply level, and both the leaf growth and $P_{\max}$ showed no significant difference among the different soil P supply levels. Leaf N:P ratio was a useful indicator for nutrient limitation. Koerselman and Meuleman (1996) suggested that N:P<14 indicated N limitation, while N:P>16 indicated P limitation, and Güsewell (2004) suggested corresponding values of N:P<10 and N:P>20. The leaf N:P ratios were all lower than 16 at P addition above 3 mg(P) kg^{-1}(soil) in the present study, indicating that *C. microphylla* was not P-limited in the most typical steppe in Inner Mongolia, which had an average soil available P content equal to 3.61 mg kg^{-1} (Geng *et al.* 2011). This coincided with the conclusion that N_2-fixing plants had an advantage in P-limited environments (Houlton *et al.* 2008). Additionally, *C. microphylla* showed the reduced $P_{\max}$, when P addition was greater than 20 mg(P) kg^{-1}(soil), and the growth traits had a similar trend. The P addition level above 20 mg(P) kg^{-1}(soil) exceeded the value, which was needed and caused the reduced $P_{\max}$ for *C. microphylla*. For species, which were adaptable to low-nutrient environments, a moderate P supply was able to satisfy the growth of plants and to cause P toxicity (Shane *et al.* 2004, Lambers *et al.* 2008). For example, in Australia, where the problem of low soil P is widespread, Pang *et al.* (2010) found that the novel perennial pasture legumes, such as *Lotononis bainesii* Baker, which adapted to low-P environment, showed reduced biomass at 24 mg(P) kg^{-1}(soil) supply, while the species, which did not adapt to low-P habitat, such as *Medicago sativa* L., showed no significant reduction of biomass at even 384 mg(P) kg^{-1}(soil) supply. In the present study, the relatively low optimal soil P supply for *C. microphylla* indicated that it adapted well to low P soil status.

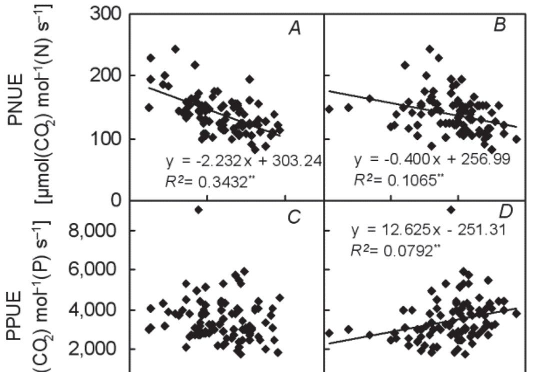

Fig. 2. Relationships between leaf mass area (LMA) and leaf dry matter content (LDMC), and photosynthetic nutrient-use efficiency (PNUE) and photosynthetic phosphorus-use efficiency (PPUE). Regression relationship, coefficient of determination (R^2) was shown at p<0.05. ** – highly significant (p<0.01), (n = 93).

Increasing a leaf toughness to achieve a prolonged leaf lifespan is a common strategy to adapt to low-P environment, because longer resident time for P was a crucial impact on long-term P-use efficiency by increasing the photosynthetic return of invested P (Denton *et al.* 2007, Hidaka and Kitayama 2011). We found that the leaf traits correlated to leaf lifespan, such as LMA and LDMC, which were enhanced by the low soil P supply. The negative correlations between LMA/LDMC and PNUE indicated a negative correlation between the leaf toughness and PNUE. The possible explanation was that high PNUE requires a leaf to invest more nitrogen into the photosynthetic machinery, while increasing leaf toughness needs more N invested into cell walls (Onoda *et al.* 2004). The significant correlation between the photosynthetic rate and the leaf N content was found in disparate species within or among communities (Reich *et al.* 2009, Kobayashi *et al.* 2010, Cheng *et al.* 2012). However, we found that P_{mass} of *C. microphylla* was not affected by leaf N_{mass} content at low LPt_{mass} in the present study, which might explain the relatively high $P_{\max}$ at low-P supply. There was a trend that the slope of P_{mass}:N_{mass} were smaller with low LPt contents, which was in agreement with the result of Reich *et al.* (2009). The smaller slope indicated that plants in low LPt content group would have higher $P_{\max}$ at a given low N value compared to those in high LPt content group. Domingues *et al.* (2010) suggested that the limitation of photosynthesis would be caused by the least abundant nutrient, and it was then less sensitive to the

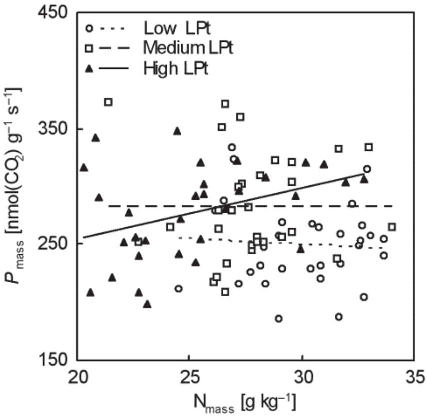

Fig. 3. Scatter plot of the relationship of light-saturated photosynthetic rate per unit of leaf dry mass (P_{mass}) *vs.* leaf total nitrogen content per unit of leaf dry mass (N_{mass}) by three P groups. The groups were divided arbitrarily into the lowest, middle, and the highest thirds according to leaf total phosphorus content per unit leaf dry mass (LPt_{mass}) level. Only the relationship at high LPt group was significant (p=0.037). The slope, intercept, and R^2 of the relationship were 4.343, 168.29, and 0.141 (n = 31), respectively.

allocation of the nonlimiting nutrient to nonphotosynthetic pools. Thus the allocation of the LPt would be more important for the response to low-P supply for *C. microphylla*. PPUE showed an opposite correlation trend to PNUE with decreasing soil P supply, and we found that Pi showed stronger correlations with the light-saturated photosynthetic rate than Po, supporting the result of Thomas *et al*. (2006), who found Pi was a better predictor for photosynthesis of *Eucalyptus grandis*. Thomas *et al*. (2006) suggested that low-P supply and, correspondingly, low leaf Pi, might limit the photosynthetic rate by limiting the capacity to regenerate ATP and RuBP, or Rubisco activity and/or content. Hidaka and Kitayama (2009) indicated that P_{mass} of plants could be maintained at low-P supply by increasing the allocation of LPt to Pi fraction and that there would be a higher PPUE. We found that the *C. microphylla* showed a smaller variation in P_{max} leaf Po and leaf Pi, and there was an increasing allocation to Pi at low P supply. We also found a significantly positive correlation between PPUE and leaf Pi:Po ratio, consistent with the idea of Hidaka and Kitayama (2009). We suggested that raising PPUE was an effective adaptive strategy and an important functional trait in *C. microphylla* in P-poor soils. Moreover, unlike the relationship between PNUE and LMA/LDMC, *C. microphylla* could maximize PPUE and the leaf toughness at the same time. As shown in the work of Hidaka and Kitayama (2011), there was no trade-off in the P allocation between metabolic and structural P, unlike the situation of the N allocation.

In conclusion, we found that the two-year-old plants of *C. microphylla* maintained photosynthesis at low-P supply by increasing PPUE through regulating the LPt allocation, which could simultaneously increase leaf toughness in the present study. *C. microphylla* had high P-use efficiency *via* both high PPUE and long P-retention time at low-P supply. The performance of young plants might determine plant distribution (Fang *et al*. 2011). The adaptation of *C. microphylla* to low-P status provided an explanation of the expanding distribution of *C. microphylla* in the degraded grassland of Inner Mongolia, which often had low-P soil.

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