

Alpine climate alters the relationships between leaf and root morphological traits but not chemical traits

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Received: 26 June 2013 / Accepted: 5 March 2014 / Published online: 17 March 2014
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Abstract Leaves and fine roots are among the most important and dynamic components of terrestrial ecosystems. To what extent plants synchronize their resource capture strategies above- and belowground remains uncertain. Existing results of trait relationships between leaf and root showed great inconsistency, which may be partly due to the differences in abiotic environmental conditions such as climate and soil. Moreover, there is currently little evidence on whether and how the stringent environments of high-altitude alpine ecosystems alter the coordination between above- and belowground. Here we measured six sets of analogous traits for both leaves and fine roots of 139 species collected from Tibetan alpine grassland and Mongolian temperate grassland. N, P and N:P ratio of leaves and fine roots were positively correlated, independent of biogeographic regions, phylogenetic affiliation or climate. In contrast, leaves and fine roots seem to regulate morphological traits more independently. The specific leaf area (SLA)–specific root length (SRL) correlation shifted from negative

at sites under low temperature to positive at warmer sites. The cold climate of alpine regions may impose different constraints on shoots and roots, selecting simultaneously for high SLA leaves for rapid C assimilation during the short growing season, but low SRL roots with high physical robustness to withstand soil freezing. In addition, there might be more community heterogeneity in cold soils, resulting in multidirectional strategies of root in resource acquisition. Thus our results demonstrated that alpine climate alters the relationships between leaf and root morphological but not chemical traits.

Keywords Alpine grassland · Climate · Fine root · Leaf · Trait correlations

Introduction

Leaves and fine roots are among the most important and dynamic components of terrestrial ecosystems. The morphological, anatomical, physiological and biochemical characteristics of leaf and fine roots usually capture fundamental trade-offs that determine species' ecological roles. Over the past two decades, a growing body of studies has quantified the variation pattern of key leaf traits along climatic, edaphic and topographic gradients (e.g. Choler 2005; Orwin et al. 2010; Liu et al. 2012). In particular, a number of studies have compiled global data sets to assess broad-scale patterns of leaf traits and identified a 'leaf economics spectrum' (Reich and Oleksyn 2004; Wright et al. 2005; Ordoñez et al. 2009). Fine roots, usually defined as roots with a diameter <2 mm, have the function of water and nutrient uptake. For tree species, roots are often branched and the most distal branch orders primarily serve for absorption (Pregitzer et al. 2002; Comas and Eissenstat

Communicated by Tim Seastedt.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-014-2919-5) contains supplementary material, which is available to authorized users.

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2009). Roots of grassland species, on the other hand, are of the spreading fibrous type and usually do not form a typical hierarchical structure (Sperry 1935).

Plant above- and belowground components are tightly linked. However, the exploration of root traits lags behind that of their aboveground counterparts. A whole-plant resource economics spectrum has not yet been successfully established, despite some existing evidence which indicates that within a site, there can be certain correlations between analogous leaf and root traits. For example, leaf and fine root N (Craine et al. 2001, 2005; Craine and Lee 2003; Tjoelker et al. 2005; Freschet et al. 2010; Liu et al. 2010) and P concentrations (Kerkhoff et al. 2006; Newman and Hart 2006; Holdaway et al. 2011) have consistently been found to be positively correlated. However, for morphological traits, results reported to date are fragmented and inconsistent. For example, specific leaf area (SLA; the light-capturing surface area per unit mass investment) and specific root length (SRL; root length per unit biomass) in most cases were found to be only weakly correlated (Tjoelker et al. 2005; Holdaway et al. 2011; Birouste et al. 2012; Pérez-Ramos et al. 2012). The relationship between leaf and fine root tissue density was positive (Eissenstat et al. 2000; Craine et al. 2001), negative (Ryser 1996), or non-significant (Craine and Lee 2003; Craine et al. 2005; Holdaway et al. 2011; Kembel and Cahill 2011; Birouste et al. 2012). Freschet et al. (2010) examined published studies and found that relationships between pair-wise leaf and root morphological traits such as SLA/SRL, tissue density and thickness varied among different plant types and in different ecosystems. Reviewing previous work, Mommer and Weemstra (2012) suggested that the lack of consistency among existing results may be partly due to the differences in abiotic environmental conditions such as climate and soil nutrient status. Several studies have tested the environmental constraints on the linkage between leaf- and root-trait syndromes. For instance, Liu et al. (2010) demonstrated a relatively stable SRL compared with a notable shift in SLA along a rainfall gradient in Chinese arid and semi-arid ecosystems, reflecting a stronger selection of leaf traits by drought stress and multidirectional strategies of root in water acquisition. Holdaway et al. (2011) found that along a soil chronosequence, the highest SLA occurred in fertile sites while the highest SRL were consistently associated with nutrient-limited sites.

The Tibetan plateau is the highest and largest alpine grassland region in the world. High altitudes, low growing season temperature, great intensity of solar radiation and other factors of the high-altitude environment on the plateau have been strong selective forces on the flora (Zhang et al. 1988). Previous research found that alpine plants have evolved cold-tolerant traits such as slow growth but a long

lifespan (Boratynski et al. 2009), small size, thick leaves (Hultine and Marshall 2000), high water use efficiency (Marshall and Zhang 1994), and sometimes high mineral nutrient concentrations (Körner 1989). Another feature of alpine plants is a large underground root system that provides strong anchorage and facilitates water absorption. However, detailed information on root characteristics of alpine plants and how alpine plants regulate the functioning of above- and belowground components is lacking. Ruess et al. (2003) observed that *Picea mariana*, growing in nutrient-poor high mountains of Alaska, had long leaf longevity but short root longevity. The freeze–thaw dynamics of alpine soils may impose more constraints on rooting zones, therefore roots are more subject to freeze–thaw disturbances than are leaves. Other aspects of the alpine environment may also have a large impact on shoot and root systems, constraining the trait correlations between above- and belowground.

During the past several years we have systematically investigated leaf traits across Chinese grassland biomes (He et al. 2006a, b, 2008). Here we unravel patterns of important fine root traits using a large-scale sampling approach and test whether the coordination between leaf and fine root traits found by previous studies (e.g. Freschet et al. 2010) could be also applied in the Tibetan alpine grassland and Mongolian temperate grassland across Chinese grassland biomes. In particular, we tested whether the very cold climate of the alpine grassland selects for a particular suite of plant traits, and further, what are the patterns of trait correlations between above- and belowground components. For these purposes, we measured six sets of analogous traits for both leaves and fine roots, including C and nutrient concentrations (N, P and N:P ratio), tissue density and the growth-related traits SLA and SRL.

Materials and methods

Site description

Leaf and fine root samples of 139 species belonging to 75 genera and 24 families were collected from temperate grassland on the Inner Mongolian Plateau and alpine grassland on the Tibetan Plateau. Over 81 sites were selected, at latitudes from 30.31 to 50.19°N, longitudes from 90.80 to 120.12°E, and altitudes from 553 to 5,105 m (Table 1). Mean annual temperature and mean annual precipitation range from −5.8 to 2.6 °C and 148 to 604 mm, respectively (Table 1). The vegetation represents natural grassland in these regions, including five main vegetation types: typical steppe, meadow steppe and desert steppe on the Inner Mongolian Plateau, and alpine steppe and alpine meadow on the Tibetan Plateau (Appendix S1) (Zhang et al. 1988).

Table 1 Description of the study regions

Vegetation type	No. of sites	Longitude (°E)	Latitude (°N)	Altitude (m)	MAT (°C)	MAP (mm)
Inner Mongolia						
Typical steppe	22	115.82–119.99	43.60–49.96	553–1,213 (797)	–2.6 to 1.6 (0.0)	232–404 (303)
Meadow steppe	6	117.46–120.12	44.77–50.19	555–1,080 (864)	–2.1 to 0.7 (–1.1)	328–436 (377)
Desert steppe	7	111.83–116.33	41.79–44.13	955–1,421 (1,037)	1.2–4.1 (3.0)	148–299 (214)
Tibet						
Alpine steppe	26	90.80–101.17	30.31–37.28	2,925–4,756 (3,933)	–5.4 to 2.6 (–1.8)	218–600 (388)
Alpine meadow	20	91.45–101.48	30.56–36.78	3,302–5,105 (4,254)	–5.8 to 0.0 (–2.7)	348–604 (497)

Mean altitude, annual temperature (*MAT*) and annual precipitation (*MAP*) of the sampling sites are shown *in parentheses*

Trait measurement

At each site, the dominant species were selected. Five replicate samples per species per site were collected. A 10- to 20-cm-diameter patch was carefully excavated to a depth of 20 cm and the roots and shoots were separated. A representative subset of fully expanded leaf blades was removed and their thickness determined with calipers and leaf area measured using a portable leaf-area meter (AM200; ADC BioScientific, Hertfordshire, UK). Leaf volume was calculated as the product of leaf thickness and leaf area. Roots of each sample were washed free of soil under running water and fine roots, defined as living roots with diameter <2 mm (Cornelissen et al. 2003), were separated from other belowground material. Within the <2-mm diameter class, 78 % of sampled roots belonged to the <1-mm diameter class. Mean diameter of fine root samples among species was 0.20 mm. Diameter distributions and mean values suggested that only the finest root-branch orders were absorptive roots, which are most analogous to leaves. A small subsample of fine roots was removed, placed in a clear plastic tray, spread out in water with minimal overlap and imaged using WinRhizo (Regent Instruments, Quebec) to determine average root diameter, total root length and volume. Roots were scanned at a resolution of 400 dots per inch. Following area, length and volume determinations, the tissues were oven-dried at 60 °C and weighed to calculate SLA (cm² g⁻¹) and SRL (m g⁻¹) as well as tissue density (mass per unit volume, g cm⁻³). Total C and N concentrations were determined on ground material using an elemental analyser (2400 II CHN elemental analyser; Perkin-Elmer, USA). Total P concentrations were measured by a molybdate/stannous chloride method (Kuo 1996).

Climate data

The climate data at each site were calculated based on linear models using latitude, longitude and altitude as predictors from 55-year (1951–2005) averaged annual temperature and precipitation records at 680 evenly distributed climate stations across China (Climate Database, National

Meteorological Bureau of China). Growing season temperature (GST; May–August) and growing season precipitation were also used to more accurately capture the climatic variation important for plant growth.

Statistical analysis

Comparisons of fine root traits were made among different biogeographic regions and vegetation types. The region effect was tested using *t*-tests while one-way ANOVA with a Tukey's post hoc test was used to test the vegetation-type effects. Trait correlations were tested between leaf and fine root traits. Bivariate relationships were examined with a type II regression as there are no independent or dependent variables and measurement error exists for both variables. Both correlation coefficients and standardized major axis (SMA) slopes were calculated using the SMART software package (Falster et al. 2006). Quantile regression was used to estimate variations of fine root traits as a function of GST for 5, 25, 50, 75 and 95 percentile distributions. A principal components analysis (PCA) was performed for all traits pooled to test whether the trait syndrome for fine roots is comparable to that for leaves.

In addition, pairwise correlations between leaf and fine root traits were re-evaluated by calculating phylogenetically independent contrasts (PIC) (Felsenstein 1985) to remove the phylogenetic relatedness among species due to shared evolutionary history. The assignments to plant order and family were based on the Angiosperm Phylogeny Group III classification (Angiosperm Phylogeny Group 2009). The freely available software Phylomatic (<http://www.phylodiversity.net/phyloomatic>) was used to build a supertree for all taxa in this study. Contrasts were calculated with the analysis of traits module in Phylocom version 4.0.1 software (Webb et al. 2008). Fitted lines of PIC regressions were forced to pass through the origin (Garland et al. 1992).

Leaf-fine root trait relationships were also compared between temperate and alpine grasslands. Heterogeneity of regression slopes was tested and common slopes were calculated. Species were further grouped into temperature bands

Table 2 Fine root traits for different biogeographic regions and vegetation types

	C (mg g ⁻¹)	N (mg g ⁻¹)	P (mg g ⁻¹)	N:P	SRL (m g ⁻¹)	Tissue density (g cm ⁻³)
Overall	439.6 (258)	11.38 (259)	0.83 (256)	13.04 (254)	64.67 (252)	0.40 (252)
Region						
Inner Mongolia	437.2 a (105)	12.08 a (105)	0.84 a (105)	13.75 a (105)	68.53 b (105)	0.45 a (105)
Tibet	441.3 a (153)	11.58 a (154)	0.81 a (151)	12.55 a (149)	61.76 a (147)	0.53 b (147)
Vegetation type						
Typical steppe	433.9 (23)	12.21 (23)	0.93 (23)	14.30 (23)	59.01 (23)	0.43 (23)
Meadow steppe	437.9 (6)	12.36 (6)	0.90 (6)	14.12 (6)	63.68 (6)	0.35 (6)
Desert steppe	439.9 (7)	11.40 (7)	0.86 (7)	13.29 (7)	70.76 (7)	0.55 (7)
Alpine steppe	432.6 (20)	11.03 (20)	0.90 (20)	12.60 (20)	41.53 (20)	0.51 (20)
Alpine meadow	447.6 (26)	11.09 (26)	0.91 (26)	12.64 (26)	55.66 (26)	0.41 (26)
	n.s.	*	n.s.	**	***	***

Significant differences between each group at $P < 0.05$ are indicated by *different letters*. Numbers of observations are shown in *parentheses*

SRL Specific root length, n.s. not significant

* $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$

Table 3 Covariations between leaf and fine root traits

Leaf-fine root trait	Species level			Species PIC			Population level		
	<i>r</i>	<i>P</i>	<i>n</i>	<i>r</i>	<i>P</i>	<i>n</i>	<i>r</i>	<i>P</i>	<i>n</i>
Leaf N-fine root N	<i>0.55</i>	<0.001	139	<i>0.32</i>	0.013	60	<i>0.42</i>	<0.001	254
Leaf P-fine root P	<i>0.51</i>	<0.001	136	<i>0.33</i>	0.011	60	<i>0.57</i>	<0.001	256
Leaf N:P-fine root N:P	<i>0.51</i>	<0.001	136	<i>0.36</i>	0.004	60	<i>0.33</i>	<0.001	254
SLA–SRL	0.05	0.541	136	0.09	0.477	60	0.01	0.880	250
Leaf tissue density–fine root tissue density	<i>0.31</i>	0.020	136	0.20	0.121	60	0.09	0.293	250

Significant relationships ($P < 0.05$) are presented in *italic* PIC phylogenetically independent contrasts, SLA specific leaf area

and SMA slopes of these bands were tested for heterogeneity. Afterwards we conducted multiple regressions with fine root traits as the dependent variable and leaf traits, GST and their interactions as the predictors. GST was treated as a four-level factor in the models Fine root trait ~ GST + Leaf trait + GST × Leaf trait to test whether the leaf-fine root trait relationships change across levels of GST. Data of leaf and fine root traits (C, N, P, N:P, SLA, SRL and tissue density) were log transformed for normality. Statistical analyses were conducted with the software R (2011).

Results

Variations of fine root traits

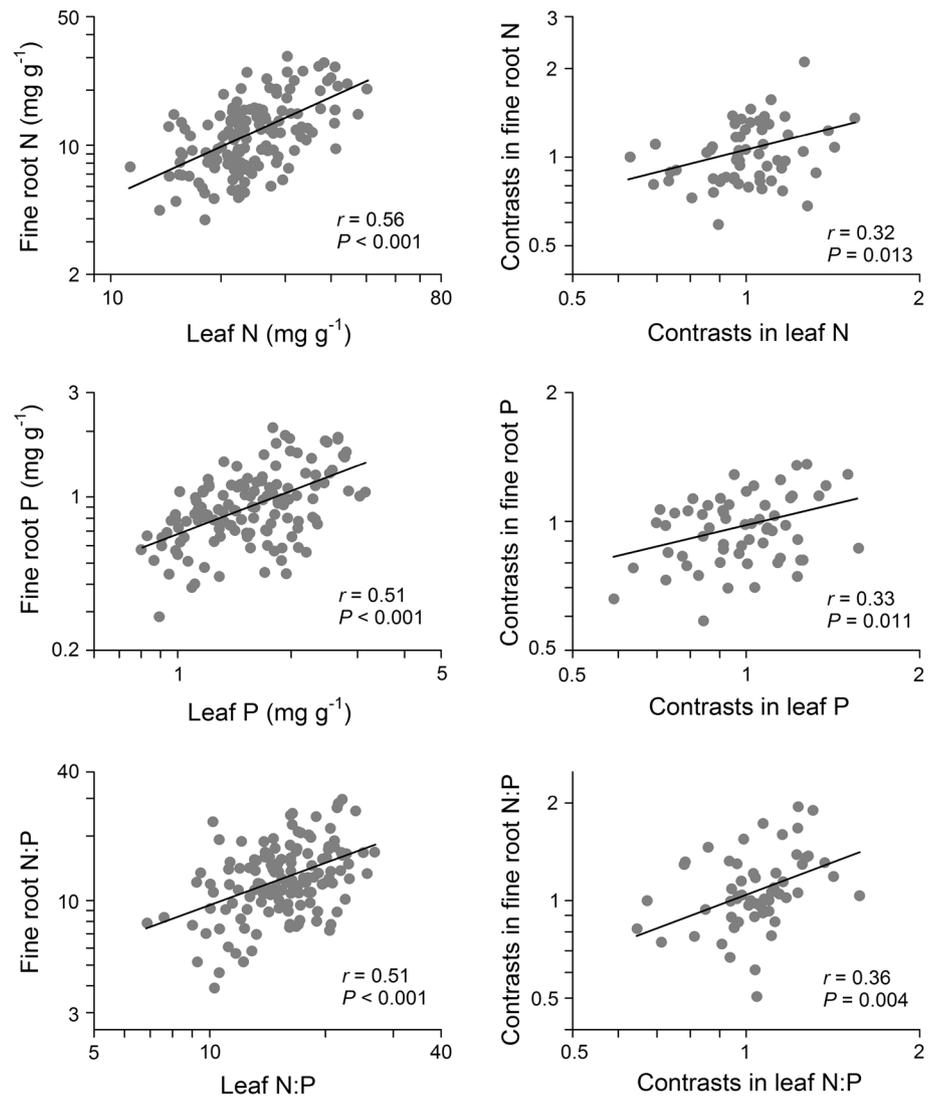
The average values of fine root C, N and P concentrations for all species were 439.6, 11.4 and 0.83 mg g⁻¹ and species varied 1.9-, 7.7-, and 7.8-fold, respectively (Table 2). The C:N:P ratio was 530:14:1, similar to the global terrestrial value of 522:12:1 (Gordon and Jackson 2000). Compared with the global averages (480, 11.1 and 0.92 mg g⁻¹) reported by Gordon and Jackson (2000), fine root P concentration in Chinese grasslands was lower, resulting in a

higher N:P ratio. Mean SRL and fine root tissue density were 64.7 m g⁻¹ and 0.40 g cm⁻³, respectively, and SRL varied considerably among species (Table 2), with the highest being 75 times the lowest. Nutrient concentrations did not differ significantly between Tibet and Inner Mongolia, while on average Tibet had a lower SRL and a higher tissue density (Table 2). Fine roots of plants in meadow steppe were richest in N but lowest in tissue density, while C and P concentrations did not show significant differences among the five vegetation types. There was no significant difference for fine root traits between basal and families which have been more recently derived phylogenetically (Appendix S2).

Trait correlations between leaf and fine root

N, P and N:P ratio of leaves and fine roots were positively correlated (Table 3; Fig. 1). There was no overall relationship between leaves and fine roots in SLA/SRL ($r = 0.05$, $P = 0.54$). Tissue density of above- and belowground components were weakly correlated ($r = 0.31$, $P = 0.02$; Table 3; Fig. 2). When using PIC, pairwise relationships for N and P remained significant as well as their ratio, though the degree of correlation slightly decreased (Table 3; Fig. 1). However, the relationship between leaf and root

Fig. 1 Covariations of N, P and N:P ratio between leaf and fine root. *Left panels* Inter-specific correlation, *right panels* phylogenetically independent contrast (PIC) correlation. Note log scale used on both *x*- and *y*-axis



tissue density became insignificant after accounting for phylogeny (Table 3; Fig. 2).

For the relationships of N, P and N:P ratio between leaf and fine root, SMA tests for common slopes revealed no significant difference when comparing temperate and alpine plants (Table 4). By contrast, SLA–SRL correlations were positive ($r = 0.37$, $P = 0.03$) for temperate species but negative ($r = -0.47$, $P = 0.01$) for alpine species (Fig. 3), with SMA slopes differing significantly between these two groups (Table 4), indicating that environmental gradients of Mongolia and Tibet may drive different relationship patterns between SLA and SRL.

As SRL are composite metrics of diameter and density, SRL and root tissue density are mathematically correlated. Therefore the PCA was conducted in two ways (by including either root diameter or SRL; Appendix S3). Axis 1 was positively related to leaf nutrient concentrations (N and P) and SLA, but negatively related to tissue density and leaf

thickness/root diameter, representing a contrast between ‘productive’ and ‘tough’ leaves. Trade-offs among fine root traits were largely analogous to the economic spectrum seen in leaves; however, SRL was positioned on the ‘costly’ side of the spectrum.

Effect of climate on leaf-fine root trait relationships

Overall climatic factors had negligible impacts on fine root traits. Quantile regression indicated a weak positive relationship between GST and fine root tissue density ($P < 0.05$ for the null hypothesis $b1 = 0$), whereas changes in fine root N, P and SRL were not related to GST (Appendix S4). GST had little effect on SMA slopes describing relationships between leaf and fine root nutrients; however, a temperature-related trend was observed for the SLA–SRL trait pair, from a negative relationship at low temperature to a positive one at high temperature (Table 5). Multiple regressions for

Fig. 2 Relationships between specific leaf area (SLA) and specific root length (SRL), and tissue densities (TD) of leaf and fine root. *Left panels* Inter-specific correlation, *right panels* PIC correlation. Note log scale used on both x- and y-axis

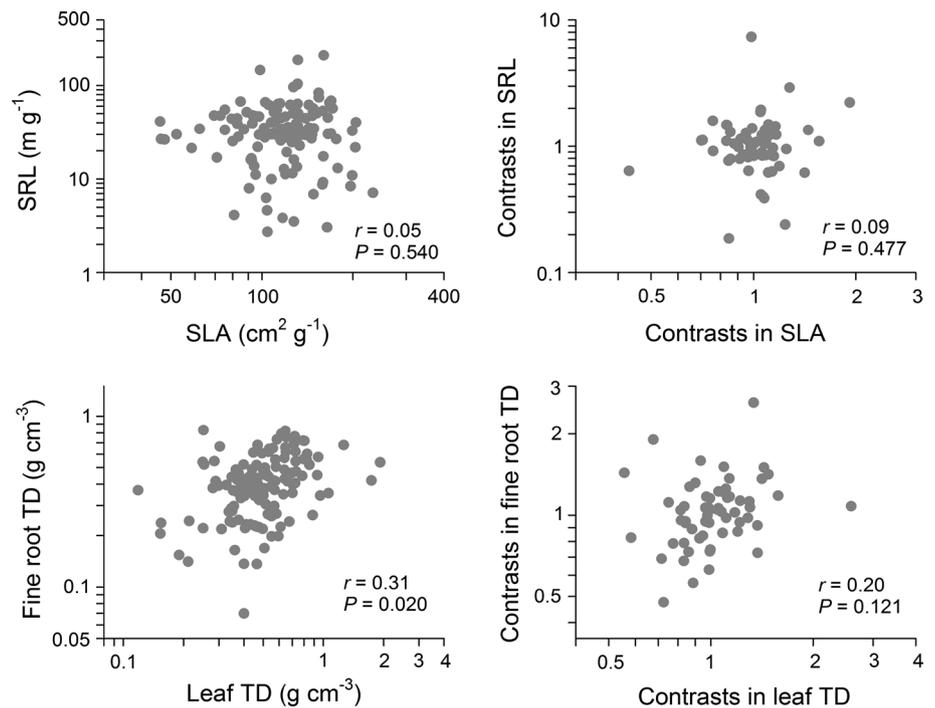


Table 4 Standardized major axis regression (SMA) analyses of pairwise relationships in Inner Mongolian temperature grassland and Tibetan alpine grassland

Bivariate relationship	Group	<i>r</i>	<i>P</i>	Slope	Slope CI	Common slope	<i>P</i>
Leaf vs. fine root N	Temperate	<i>0.46</i>	<0.001	0.963	(0.810, 1.144)	0.998	0.275
	Alpine	<i>0.41</i>	<0.001	1.025	(0.886, 1.202)		
Leaf vs. fine root P	Temperate	<i>0.59</i>	<0.001	0.993	(0.878, 1.127)	1.030	0.654
	Alpine	<i>0.59</i>	<0.001	1.110	(0.934, 1.309)		
Leaf vs. fine root N:P	Temperate	<i>0.30</i>	0.002	0.955	(0.793, 1.150)	0.963	0.921
	Alpine	<i>0.28</i>	0.001	0.969	(0.825, 1.137)		
SLA vs. SRL	Temperate	<i>0.37</i>	0.034	0.470	(0.233, 0.580)	-0.234	0.001
	Alpine	<i>-0.47</i>	0.011	-1.050	(-1.380, -0.806)		
Leaf vs. fine root tissue density	Temperate	<i>0.26</i>	0.006	1.034	(0.823, 1.288)	0.897	0.100
	Alpine	<i>0.21</i>	0.014	0.800	(0.647, 0.980)		

95 % Confidence intervals (CI) of SMA slopes are shown in *parentheses*. Significant relationships ($P < 0.05$) are shown in *italic*

fine root traits predicted by corresponding leaf traits, GST and leaf trait \times GST showed that the interaction terms were significant for SRL ($P = 0.02$) but not for fine root nutrients or tissue density, indicating that SLA–SRL relationships change across levels of GST (Appendix S5).

Discussion

Variations in fine root traits of alpine species

Plants growing on temperate and alpine grasslands had similar N and P concentrations, but differed significantly

in morphological traits such as SRL and tissue density. Nutrient availability typically decreases with decreasing soil temperature because the mineralization of organically bound nutrients is directly related to the temperature-dependent metabolic activity of soil microbes (Chapin et al. 1995; Zak et al. 1999). As a result, a change in root morphology might ensue. For example, in crop monocots the number of lateral roots increased with increasing meristem temperature (Kaspar and Bland 1992). On the alpine grassland at high latitudes, soils can be permanently frozen close to the surface, inhibiting microbial activity and hence nutrient availability to plants. However, alpine plants may not be short in nutrients. For example, N concentration

was found to be higher in leaves of herbaceous plants growing at high elevation under low temperature (Körner 1989). Thus, plants may vary biomass allocation instead to respond to different resource availability. Fine roots produced by alpine grasses per unit leaf area could be four to five times as many as those of forbs at low altitude (Körner 2003). On the Tibetan Plateau, with increasing altitude the proportion of total biomass allocated to belowground biomass increased, shifting C investment towards roots where photosynthate can be used to increase water and mineral uptake (Ma et al. 2010). The high root biomass densities of

alpine grasslands makes them likely to differ substantially from forest or glasshouse-based measurements.

Another challenge for alpine vegetation is to hold the soil firmly with intact root systems. Fine roots made the largest contribution to topsoil aggregate stability in alpine ecosystems (Pohl et al. 2009). Fine roots stabilize the plant by resisting surface flow (De Baets et al. 2007) and strong winds (Billings and Mooney 1968). Therefore fine roots of alpine plants are not only responsible for resource acquisition, but equally important for anchorage and soil colonization. This structural role of fine roots may result in lower SRL values (thicker roots) for plants in the Tibetan grassland. The typical and desert steppes in the Inner Mongolian grassland, on the other hand, are characterized by a continental, semi-arid climate with mean annual precipitation about half of that in the alpine meadow (Table 1). This water stress may select for thinner roots with higher SRL to maximize absorptive surface. For instance, SRL showed an inverse power relationship with soil moisture for fine roots with diameter <0.5 mm of oak trees, and SRL peaked in summer when the soil water content was the lowest (Montagnoli et al. 2012). This high SRL under warm and dry conditions indicates that thin roots may be better equipped to lower soil moisture content at warm sites, whereas low SRL at high elevation might be more responsible for roots to resist mechanical stresses such as soil freezing.

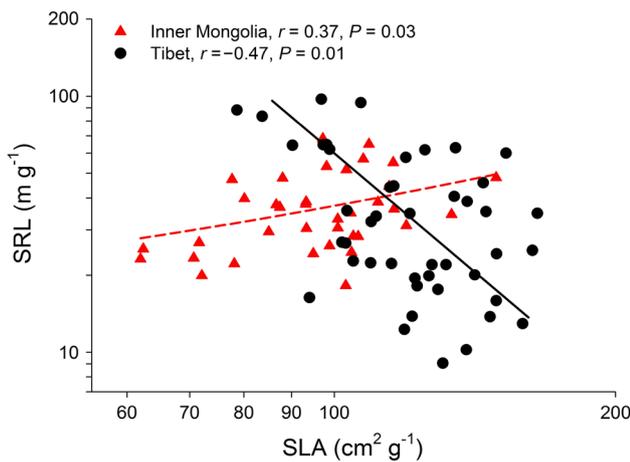


Fig. 3 SLA–SRL relationships in Inner Mongolia and Tibetan Plateau at the site-mean level, by averaging species trait values within each site. Note log scale used on both x- and y-axis. For abbreviations, see Fig. 2

Cold climate alters the correlations between leaf and root morphological traits

Nutrient concentrations in leaves and fine roots were consistently correlated across species and sites, despite

Table 5 Comparisons of leaf-fine root trait relationships at the site-mean level for sites grouped into climate classes

		GST (°C)				
		0–5	5–10	10–15	15–20	
Leaf vs. fine root N	Slope	1.07	1.03	0.82	0.92	ns
	r ²	0.30	0.36	0.28	0.30	
	n	14	30	15	23	
Leaf vs. fine root P	Slope	1.14	1.08	1.05	1.12	ns
	r ²	0.35	0.30	0.32	0.40	
	n	14	30	15	23	
Leaf vs. fine root N:P	Slope	0.90	1.03	0.86	0.67	*
	r ²	0.26	0.46	0.10	0.14	
	n	14	30	15	23	
SLA vs. SRL	Slope	–1.27	–1.01	0.38	0.47	***
	r ²	0.09	0.15	0.05	0.14	
	n	14	30	15	23	
Leaf vs. fine root tissue density	Slope	0.64	0.87	1.14	0.84	*
	r ²	0.05	0.07	0.18	0.05	
	n	14	30	15	23	

Data shown are the SMA slope, coefficient of determination (r²) and sample size. Slopes were tested for heterogeneity

GST Growing season temperature
 * P < 0.1, ** P < 0.05,
 *** P < 0.01

contrasting climatic and topological conditions in temperate Mongolian and alpine Tibetan grasslands. The consistency of leaf-root N and P correlations indicate integrated roles of N and P in plant functioning (Güsewell and Koerselman 2002), with N important for growth and P for the formation of new leaves and roots. Thus the general allocation rule governing the partitioning of nutrients above- and belowground (Kerkhoff et al. 2006) can also be applied to alpine species. In addition, slopes of N–P scaling do not differ between the above- and belowground components (Fig. 4). This highly constrained N:P stoichiometry indicated a ‘stoichiometric homeostasis’ between leaf and fine root, reflecting that underlying physiological processes are coordinated among plant tissues. Here in our study, PIC showed nearly identical patterns of leaf-fine root N, P and N:P correlations, again confirming a universal rule that constrains the allocation of N and P between above- and belowground components, rather than a result of common lineages.

Overall, fine root traits examined in this study showed little response to changes in temperature and precipitation. However, we did observe an effect of temperature on the relationships between corresponding leaf and fine root traits. For chemical traits, leaf-fine root N (and P) fitted within climate bands did not differ in slope, again demonstrating a tight correlation of essential nutrients between above- and belowground organs. Nevertheless, the SLA–SRL correlation shifted from negative at sites under low temperature to positive at warmer sites.

Soil temperature has the potential to influence the morphology and functioning of roots in many ways, including the initiation of new lateral roots, root elongation and root-branching patterns (Kaspar and Bland 1992). At high altitudes, cold soils might inhibit root activity at certain soil depths during most of the year. The large area of alpine grasslands on the Tibetan Plateau is featured by high-altitude and low-latitude permafrost. In these cold regions soil temperatures must play a key role in determining root growth, functioning and mortality. Freezing followed by thawing may lead to certain chemical changes, burst root cells and change in the chemical composition of roots and the surrounding soils. This freezing-thaw cycles of alpine soils may impose more constraints on roots than on leaves. As SLA and SRL showed a negative relationship at the coldest sites (climate band with the lowest GST), it could be possible that the responses of shoots and roots to changes in temperature might be different at high elevation. The extreme conditions could select simultaneously for higher SLA leaves to permit rapid assimilation of C during the short growing season, and lower SRL roots with high physical robustness to withstand soil freezing.

Quantile regression also indicates that SRL strategies are more variable at low temperature while more ‘fixed’ at

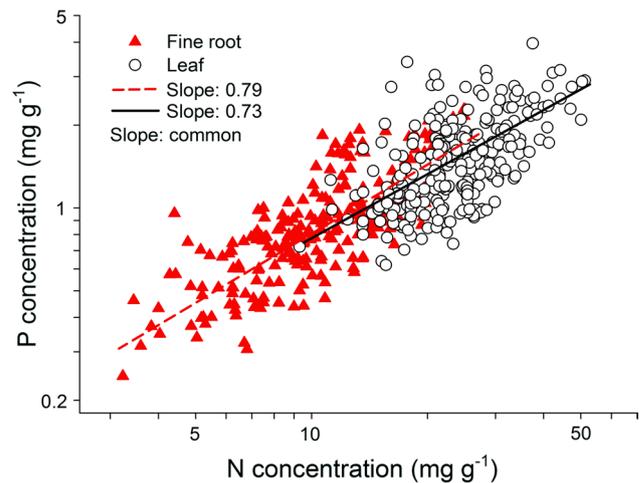


Fig. 4 N and P scaling of leaf and fine root. *Solid line* Regression between leaf N and P, *dashed line* regression between fine root N and P. Note log scale used on both x- and y-axis

high temperature (Appendix S4). Warm sites in this study distribute in Mongolia temperature grassland, where water stress is a limiting factor to growth (for example, typical steppe and desert steppe). Plants tend to produce long thin roots (higher SRL) to overcome water stress (as seen in Table 2). Cold sites of alpine grasslands on the Tibetan Plateau, however, are in fact more diverse in climate. For example, several temperature and moisture regimes can be present on one mountain, depending on the elevation and direction the slopes face. Moreover, different depths and duration of snow cover may lead to heterogeneity of soil temperature and moisture, as well as soil microbes, and further, have complicated effects on the mineralization and immobilization of soil nutrients such as N.

Is the trait syndrome of roots similar to that of leaves?

Trait correlations within roots did not follow the patterns predicted by leaf resource economics theory. For example, we did not find that roots with high SRL also had higher N concentration (Appendix S6), in contrast with the general correlation between SLA and leaf N content. SRL describes the root length as the quantity of invested biomass, and is thus considered to reflect root costs and benefits. Therefore SRL and root nutrients such as N concentration are expected to be related. However, for herbaceous species, recent studies found that SRL and N were not correlated (Birouste et al. 2012), or even varied in opposite directions (Holdaway et al. 2011), in contrast with woody species, the roots of which with high SRL generally had high N concentrations (Pregitzer et al. 1998; Reich et al. 1998; Comas and Eissenstat 2004; Withington et al. 2006). The inconsistency of the SRL–[N] correlation between

herbaceous and woody species might partly stem from the different structure of their root system. A hierarchical root system is a common feature of trees. Metabolic rate and life cycle usually vary systematically with branch orders. It has been found that both tissue nutrients and SRL decline with increasing root orders (Pregitzer et al. 2002; Li et al. 2010). The absorbing region of grasses, on the other hand, could be nearly as great as the extent of the root systems, which is in marked contrast to the hierarchical branching architecture found in tree plants. Until now, the efficiency of the branching-order-based approach in investigating root traits of grassland species has not been supported by the data. The first two to three orders of roots of herbaceous species may have relatively small variation in trait values, and as a consequence, trait relationships are likely to be insignificant. In addition, for many grassland species, roots of small diameter may serve not only for mineral uptake, but also for anchorage and transport. Fine roots sampled in the previous studies could not be exclusively absorptive roots, but absorptive and structural roots are difficult to separate. SRL may thus not have the same meaning as SLA.

The soil environment is far more complex than the air environment, leading to fundamentally different resource economic syndromes in roots compared with leaves (Mommer and Weemstra 2012). For leaves, a high Rubisco concentration (which strongly depends on leaf N content) and high SLA that leads to the capture of more light per unit mass, both enhance photosynthetic performance. Leaf [N], SLA and photosynthetic rate are tightly coupled (Reich et al. 1999). In soil, N and P are stored in both organic and inorganic forms. A large absorptive surface area and efficient resource uptake for nutrients may be achieved by either rapid elongation of fine roots (thus high SRL), or by association with mycorrhizal fungi. Across plant species there can be diverse strategies for resource acquisition. As a result, the strong relationship between leaf N content and SLA may not have equivalent parallels in roots, due to somewhat different meanings of SLA and SRL depending on environmental context. These effects of environments on trait relationships suggest widely varying resource uptake strategies across different habitats rather than consistent selection for coordinated leaf and root traits (Kembel and Cahill 2011). There may be fundamentally different environmental constraints and selective pressures on traits above- and belowground.

Conclusion

Our results demonstrated that the coordination above- and belowground in Chinese grassland species was strong for chemical but not morphological traits. This coupling of chemistry between leaves and fine roots was independent

of regional differences, climate and phylogenetic affiliation, indicating that nutrient concentrations between leaves and fine roots are usually equilibrated within plants. This high coordination in leaf and root tissue chemistry might be extrapolated to other organs, with importance in resource use strategies. Morphological traits between leaves and fine roots, on the other hand, were poorly correlated, probably due to somewhat different meanings of these traits depending on the environmental context. For example, SLA–SRL relationships changed with temperature, suggesting that environmental constraints may be fundamentally different for plant above- and belowground components, instead of consistent selection for coordinated leaf and root traits. Therefore, patterns of large-scale trait relationships across plant organs should be obtained with consideration of the major environmental pressures which are exerted on these organs.

Acknowledgments The authors are grateful to Cunzhu Liang, Yi Wu, Chao Song and Wenhong Ma for assistance with field sampling. We thank Jacob Weiner, Bernhard Schmid and Dan Flynn for help with the manuscript. This study was supported by the National Natural Science Foundation of China (grant no. 31025005) and the National Program on Key Basic Research Project (grant no. 2010CB950602). The authors have declared no conflict of interest.

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