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Variation of first-order root traits across climatic gradients and evolutionary trends in geological time

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ABSTRACT

Aim Plant roots are crucial for water and nutrient absorption, but large-scale patterns and underlying mechanisms of root trait variation and evolution are poorly understood. Here we quantify the degree of variation in functional traits for the first-order roots across large geographical scales and examine the potential mechanisms underlying these patterns.

Location China.

Methods We collected first-order roots (stream-based ordering system) and leaf samples of 65 tree species in six forests from subtropical to temperate zones and determined the key morphological, architectural and chemical traits.

Results We found wider variation in root morphology in the subtropical zone, where species with very thick and very thin first-order roots coexisted, in contrast with narrower variation among species in the temperate zone. Inconsistent with the predictions of trait economics spectrum, root nitrogen concentration was uncorrelated with root morphology. Furthermore, average root diameter at the plant family level decreased markedly with their divergence time in both piecewise and phylogenetic independent contrast regression analyses.

Main conclusions Higher variation in root morphology in the subtropical zone appears to result from the coexistence of tree species with thick and thin roots, probably because of a more favourable water supply. Patterns of root evolution towards thinner roots were driven by both phylogeny and possible adaptation of newly diverged species to drier habitats from mid to late Cretaceous. Our findings reveal contrasting selection pressures at the root and leaf level in different climatic zones during plant evolution, and suggest that a single vector of variation linking morphology to resource acquisition or life span ('trait economics') remains elusive in roots.

Keywords

Branch order, China, divergence time, economics spectrum, evolution, phylogeny, root functional traits, specific root length.

INTRODUCTION

Variations in plant functional traits and their underlying mechanisms have fascinated scientists for decades, and enormous interspecific trait variation in plant leaves has been observed. Variation in traits like leaf thickness, leaf mass–area ratio, nitrogen concentration ([N]), carbon assimilation rate and longevity has been shown to represent adaptations to the range of environmental conditions that occur in habitats as divergent as

arctic tundra, deserts and tropical rain forests (Wright *et al.*, 2001; Reich & Oleksyn, 2004). Similar divergent variation has been assumed to exist in roots, as roots also need to adapt to a wide range of habitats. Indeed, recent studies have begun to show marked variation in roots for traits such as root branching architecture, morphology and chemistry (Comas & Eissenstat, 2009; Holdaway *et al.*, 2011; Ostonen *et al.*, 2011). However, systematic analyses of root trait variation over a large geographical range have not been reported and are sorely needed for a

better understanding of whole plant functioning (Westoby & Wright, 2006).

Plant traits not only vary along environmental gradients but also show convergence on universal tradeoff surfaces. At the global scale, a 'leaf economics spectrum' has been identified in which species with high photosynthetic rates often have thin leaves, high leaf [N] and short life span ('fast' species), whereas species with low photosynthetic rates often have thick leaves, low leaf [N] and long life span ('slow' species) (Niinemets, 1999; Reich *et al.*, 1999; Wright *et al.*, 2004). A similar 'economics spectrum' has been proposed for root traits such that 'fast' species should have thin roots, high tissue [N] and short life span whereas 'slow' species should have thick roots, low tissue [N] and long life span, yet this 'root economics spectrum' has not received much support (Eissenstat & Yanai, 1997; Withington *et al.*, 2006; Hobbie *et al.*, 2010).

Identification of a 'leaf economics spectrum' also suggests convergence of leaf traits of coexisting species under similar environmental conditions, despite the great genotypic diversity among these species (Reich *et al.*, 2003). This is due to the strong environmental selection force on plant leaves. For example, in tropical forests, competition for light is intense so overstorey trees tend to have a narrow range of leaf thickness to optimize their light absorption (Wright *et al.*, 2004). Another example is the distinct pattern of leaf life history along temperature gradient, with deciduous angiosperms being distributed in colder environments that have a longer and more severe winter, whereas evergreen angiosperms occupy warmer environments that have shorter and more mild winters (Reich *et al.*, 1997). However, whether root traits are similar among coexisting species or, conversely, whether root traits vary strongly along a broad environmental gradient, is not clear due to the scarcity of reliable root trait data (Comas & Eissenstat, 2009).

The scarcity and reliability of root trait data is related to the approaches for dealing with the relationship between root structure and function. For root-soil interaction studies, we have often conveniently used diameter classes such as 0–2 mm to define the absorptive part of the root system for all species. This approach is problematic when comparing root traits across multiple species because different species have markedly different root diameters (Craine *et al.*, 2002; Pregitzer *et al.*, 2002; Guo *et al.*, 2008; Comas & Eissenstat, 2009). Recent studies linking root function with structure showed that plant root systems have multiple branch orders, and only the most distal one to three orders (a stream-based ordering system with the first order being the most distal roots) primarily serve resource acquisition functions (Pregitzer *et al.*, 2002; Guo *et al.*, 2008; Comas & Eissenstat, 2009). Additional evidence also showed that these distal branch orders have short life span and form ephemeral modules in the root system (Xia *et al.*, 2010). Thus, studies of root traits must be based on an appropriate sampling unit, which seems to be the most distal portions (lower branch orders) of the root system, because these orders, particularly the first order, should have the same function across species.

To better understand root trait variation and root adaptation in different habitats, attention to the evolutionary history of

root traits is required. Understanding root evolution is challenging because root fossils are rare (Raven & Edwards, 2001; Hao *et al.*, 2010). Existing root fossils do not allow us to trace how root traits diverged over time as has been done for leaf traits (Hickey & Doyle, 1977; Gleissberg & Kadereit, 1999; Bond & Midgley, 2003). An alternative method is to study the root traits of extant species in which traces of evolution along the divergence time series may be identified (Brodrribb & Feild, 2010). Previous studies have shown that late-divergent species on the phylogenetic tree usually have thinner and more branched root systems than species of basal clades (Baylis, 1975; St John, 1980; Pregitzer *et al.*, 2002; Comas & Eissenstat, 2009), but investigations across biomes are lacking. Consequently, broad patterns and potential drivers of root functional trait evolution are yet to be identified.

Here we sampled the first-order roots (confirmed to be the primary absorptive tissue of the entire root system; Guo *et al.*, 2008; Xia *et al.*, 2010) of 65 tree species in six forests from temperate to subtropical zones of China and measured the first-order root diameter, length, specific root length (SRL; length per unit dry mass), tissue density, branching ratio (number of first-order roots per second-order root) and root [N], as well as specific leaf area (SLA; area per unit dry mass) and leaf [N]. We aimed to test the following hypothesis: (1) consistent with patterns in leaf traits, first-order root traits would vary markedly from subtropical to temperate forests, but would converge within the same climate zone; (2) there would be tradeoffs between root traits, which would form a 'root economics spectrum' such that species with thicker roots should have lower [N] whereas species with thinner roots should have higher [N]; (3) root traits have been significantly modified during species radiation in geological time such that a marked trend in root traits can be identified in species with different divergence times.

MATERIALS AND METHODS

Study site

Portions of branching root systems and leaves were sampled from 65 tree species in six forest sites in China. These sites covered a latitudinal range from 21 to 40° N, a mean annual temperature (MAT) range from 4.8 to 22.4°C and a mean annual precipitation (MAP) range from 550 to 1948 mm. Across the six sites, soil nutrients varied markedly: total soil [N] ranged from 0.10 to 0.47% and total soil [P] from 0.01 to 0.08%. Thirty-five species were sampled at three tropical and subtropical sites (hereafter combined as subtropical sites, MAP = 1906.7 ± 32.5 mm, mean ± SE) and 30 species at three temperate sites (MAP = 616.7 ± 44.1 mm, mean ± SE); detailed site information is shown in the Supporting Information (Fig. S1, Tables S1 & S2). In our study range, the soil moisture was positively correlated with MAP (Fig. S2), thus precipitation may be considered as a proxy for soil moisture, particularly when comparing subtropical with temperate sites.

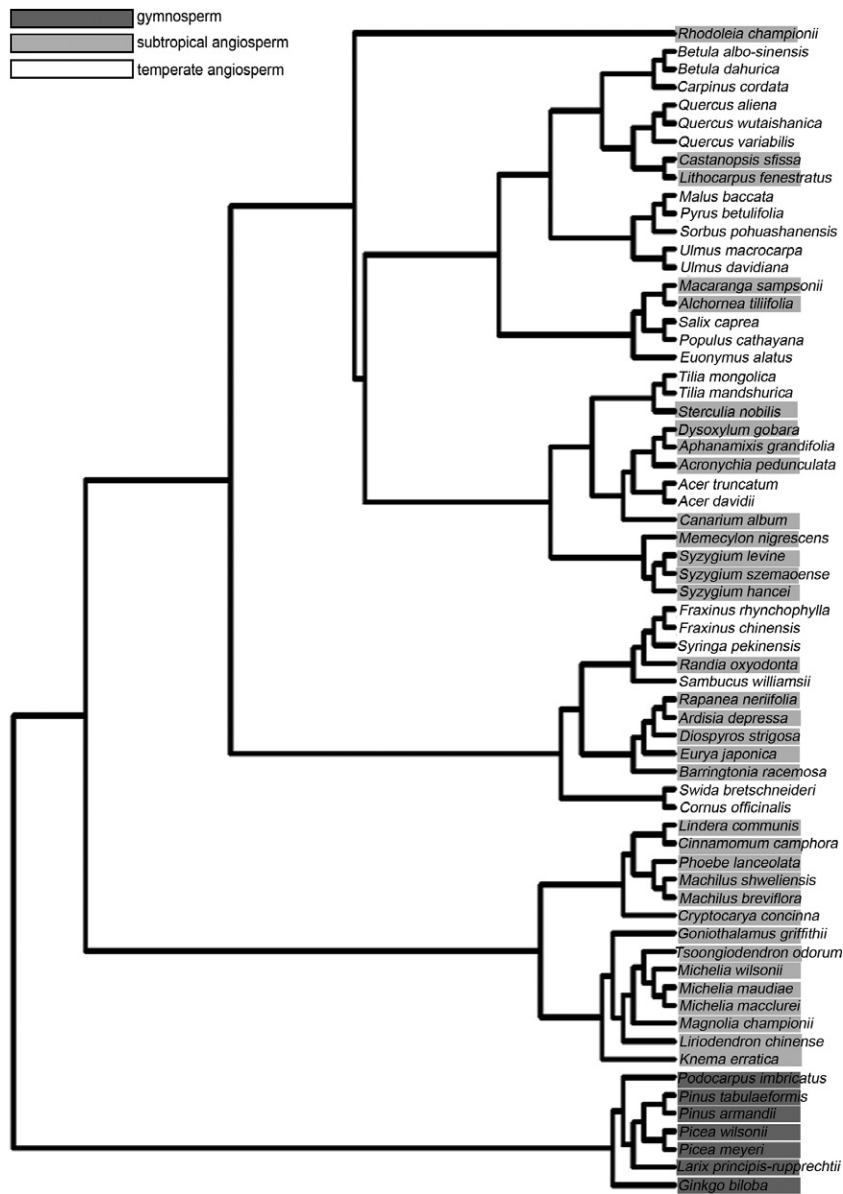


Figure 1 Phylogenetic tree of 65 species sampled in this study. All phylogeny structures follow those used by the APG III (Bremer *et al.*, 2009) and APweb (Stevens, 2001 onwards) and added phylogeny studies (Chanderbali *et al.*, 2001; Li & Conran, 2003; Potter *et al.*, 2007).

Species and phylogeny

The selected species represented a broad range of taxa, covering 51 genera, 30 families and 16 orders. Among the 65 species selected, 8 were gymnosperms and 57 were angiosperms (Table S2). All phylogeny structures follow those used by the APG III (Bremer *et al.*, 2009) and APweb (Stevens, 2001 onwards) and added specific phylogeny studies (Chanderbali *et al.*, 2001; Li & Conran, 2003; Potter *et al.*, 2007). Key clades of common tree species in China are well covered, including magnollids, rosids and asteroids of eudicots for angiosperms and pinales and ginkgopsida for gymnosperms. Species within the same clade were sampled from no fewer than three forest sites to encompass environmental variation (Fig. 1).

Moreover, we obtained divergence times of angiosperm families from Wikström *et al.* (2001) based on molecular clock theory using calibrated gene sequences and ACCTRAN

optimizations. We determined the geological time when a family and its nearest sister family diverged from their immediate ancestor as the divergence time for this family. However, in Wikström *et al.* (2001), divergence time was given for the divergence node between two adjacent related genera, rather than between families, so the divergence time of the earliest genus within a family in Wikström *et al.* (2001) was chosen to represent the divergence time of the family in our study. We also use the 'bladj' function in the software *Phylocom* (Webb *et al.*, 2008) with the node ages from Wikström *et al.* (2001) to adjust all the branch lengths in our phylogenetic tree.

Plant sampling and processing

For each species, the first five branch orders (the definition of branch orders followed Pregitzer *et al.*, 2002) were carefully excavated from 0–20 cm depth of soil. Our sampling focused on

the fibrous root system that is the dominant root type in the soil with mainly resource acquisition functions (Guo *et al.*, 2008) and short lifespan (Xia *et al.*, 2010). We did not sample the roots that can be labelled as first order at the time of sampling but may have potential to become coarse woody roots, the type of roots termed 'pioneer roots' (Zadworny & Eissenstat, 2011). Before excavation, three adult trees were identified for each species. Within a distance of 2 m to the stem of each tree, we used a specially constructed fork (with three teeth, each 20 cm long and 1 cm in diameter and having a pointed tip) to loosen the soil in the sampling area. Once root systems were exposed, target root branches were followed to the tree stem to confirm the identity of the species. Root branches were then cut from the main roots. Once collected, root branches were immediately put on ice in a cooler and transported to the laboratory within a few hours and frozen for later processing. In the laboratory, following careful cleaning of adhering soil particles and organic matter, the root branching networks were kept moist with deionized water (1°C) and dissected into different branch orders with distal root segments labelled as first order. More than 200 first-order root segments were collected for each tree of each species.

Mature leaves were collected from the top third of tree canopies. For most species, leaves were collected from the same trees from which roots were sampled. Leaf samples were put into an envelope and returned to the laboratory for further processing.

Trait measurement

The diameters of first-order roots were measured using a 40 × stereomicroscope with an ocular micrometer (± 0.025 mm). For root length, relatively short segments were measured using a 10 × stereomicroscope (± 0.1 mm), and relatively long segments were assessed using a measuring tape to the nearest 0.5 mm. Average root diameter and length for the measured roots were calculated for each tree and then for each species. The turgid tissue volume was calculated by treating the root segments as a circular cylinder. And the root branching ratio was calculated from the total number of first-order roots divided by the number of second-order roots that bore them. To measure leaf traits, a punch was used to cut leaves into small pieces of an identical area (i.e. disks with a diameter of 2 cm) for broad-leaved samples and whole lamina pieces were chosen for coniferous samples, and all leaf pieces were scanned and area measured. All lamina pieces and root segments were dried at 60°C for 72 h and then weighed (± 0.0001 g). Oven-dried root and leaf samples were ground to a fine powder using a SPEX 8000-D mixer mill (SPEX, Edison, NJ, USA). Ground roots and leaves were subsampled for ash determination (550°C for 4 h). Specific root length (SRL) was calculated from total root length divided by dry root mass, and tissue density from mass divided by turgid tissue volume, and both indices were expressed on an ash-free dry mass basis. SLA was calculated from leaf area divided by leaf biomass. Finally, tissue [N] of both roots and leaves was determined using an elemental analyser (Vario EL III Universal CHNOS Elemental Analyzer, Elementar, Hanau, Germany), expressed on an ash-free mass basis. For first order

roots, 'ash' content varied from 5–13% with a grand mean of 7.8% across all species; the ash content included both metals in plant tissue and adhering soil particles.

Data analysis

Summary statistics of all leaf and root traits were performed for all species. Moreover, all species were separated into three groups, namely subtropical angiosperms, temperate angiosperms and conifers. The significant effects of group on root and leaf traits were tested with ANOVA. Additionally, a principal components analysis (PCA) was performed on the correlations among traits, and species from the three groups were ordered by their score on the first two principal components. Also, the variation patterns of all traits were analysed in the three groups. In addition, the coefficient of correlation of each pair-trait was calculated with all species pooled together with phylogenetic independent contrasts (PICs, Felsenstein, 1985), which provided an estimate of correlation of a pair of traits with the influence of phylogeny removed.

Piecewise regression (Toms & Lesperance, 2003) analysis of traits predicted by divergence time at the family level was conducted to examine the trends and thresholds (if existing) of root and leaf trait variation in geological time. The piecewise regression was performed in R (2011, version 2.14, R Foundation for Statistical Computing, <http://www.r-project.org>) with settings such that two lines with significantly different slopes were joined at an unknown breakpoint with minimum fitted residuals. In addition, we performed linear PIC regression with all measured traits against the divergence time in order to test the dependence of trait variation on divergence time without the influence of phylogeny. We did not include gymnosperms in these regressions as they evolved in a different lineage and differed markedly in root anatomy from angiosperm species (Guo *et al.*, 2008). All other statistics and significance tests were conducted using spss (2001, version 13.0, SPSS Inc., Chicago, IL, USA).

RESULTS

Trait variation across and within climate zones

For angiosperms, first-order roots of subtropical species were on average thicker (root diameter of 0.38 ± 0.04 vs. 0.25 ± 0.02 mm, $P < 0.05$) and branched less (branching ratio of 2.96 ± 0.31 vs. 4.52 ± 0.20 , $P < 0.001$) than temperate species (Table 1, Fig. 3). However, root [N] showed no significant difference between these two groups (2.04 ± 0.10 vs. $1.97 \pm 0.09\%$, $P = 0.62$), whereas subtropical species had significantly lower leaf [N] than temperate species (1.86 ± 0.10 vs. $2.36 \pm 0.14\%$, $P < 0.05$) (Table 1). In comparing with all angiosperm species, first-order roots of coniferous species were significantly shorter (2.77 ± 0.53 vs. 6.34 ± 0.65 mm, $P < 0.001$) and had lower root [N] (1.49 ± 0.09 vs. $2.01 \pm 0.07\%$, $P < 0.001$), but did not differ significantly in diameter (0.29 ± 0.01 vs. 0.33 ± 0.02 mm, $P = 0.19$) and branching ratio (5.79 ± 2.23 vs. 3.66 ± 0.22 , $P = 0.39$). Difference in SLA (82.7 ± 7.75 vs. 162.7 ± 6.84 cm² g⁻¹,

Table 1 Summary statistics of first order root and leaf traits among coniferous, subtropical and temperate angiosperm species. Different lowercase letters in mean values indicated significant differences among the three species groups ($P < 0.05$).

Traits	Angiosperm subtropical species				Angiosperm temperate species				Coniferous species				
	<i>n</i>	mean	SE	CV (%)	<i>n</i>	mean	SE	CV (%)	<i>n</i>	mean	SE	CV (%)	
Root	Diameter (mm)	35	0.38 ^a	0.04	56.39	24	0.25 ^b	0.02	41.02	6	0.29 ^b	0.01	8.41
	SRL (m g ⁻¹)	35	68.1 ^{ab}	10.5	90.11	24	71.6 ^a	5.6	38.36	6	45.9 ^b	4.4	23.56
	Length (mm)	35	7.60 ^a	0.90	69.35	24	4.55 ^b	0.78	83.43	6	2.77 ^b	0.53	46.56
	TD (g cm ⁻³)	35	0.32 ^{ab}	0.04	72.56	24	0.44 ^a	0.05	57.92	6	0.30 ^b	0.04	30.80
	BR	29	2.96 ^a	0.31	55.13	23	4.52 ^b	0.20	21.27	6	5.79 ^{ab}	2.23	94.27
	[N](%)	29	2.04 ^a	0.10	25.33	20	1.97 ^a	0.09	20.49	6	1.49 ^b	0.09	14.29
Leaf	SLA (cm ² g ⁻¹)	23	160.5 ^a	9.9	30.34	20	165.0 ^a	9.6	27.83	5	82.7 ^b	7.75	24.46
	[N] (%)	24	1.86 ^a	0.10	25.16	23	2.36 ^b	0.14	26.95	6	1.36 ^c	0.09	15.27

SRL, specific root length; TD, tissue density; BR, branching ratio; [N], nitrogen concentration; SLA, specific leaf area. *n* = sample size.

$P < 0.001$) and leaf [N] (1.36 ± 0.09 vs. $2.09 \pm 0.09\%$, $P < 0.05$) were also detected between angiosperms and conifers (Table 1).

PCA identified three significant axes (eigenvalue > 1) of variation. The first component was mainly associated with species differences in diameter and length in first-order roots, and the subtropical group showed a greater spectrum of variation than the temperate group. The second component was associated primarily with leaf traits (leaf [N] and SLA), and generally separated the temperate angiosperms from the conifers. The third component was mainly associated with root [N] and tissue density (Table 2, Fig. 2).

Subtropical angiosperms had greater variation in root morphology than temperate angiosperms (Table 1). For root diameter, the coefficient of variation (CV) for subtropical angiosperms was higher than temperate ones (56.39 vs. 41.02%, Table 1), mainly because a number of subtropical species had very coarse roots (Fig. 3). SRL also exhibited much higher variation in subtropical than in temperate forests (CV = 90.11 vs. 38.36%, Table 1). Similarly, root branching ratio in subtropical angiosperms exhibited wider variation than temperate angiosperms (CV = 55.13 vs. 21.27%).

Compared with root traits, variation in leaf traits was smaller, particularly for subtropical angiosperms: CVs for root morphology traits were at least 25% greater than SLA (Table 1). However, variation in tissue [N] was similar between roots and leaves (Table 1).

Trait correlation with PICs

When calculating the correlation of coefficient with PICs across all species, we found that SRL was strongly and negatively correlated with diameter ($P < 0.05$), but not with tissue density. Root diameter (and length) was significantly correlated with branching ratio ($P < 0.05$). However, root diameter and SRL were not correlated with root [N]. Between leaves and roots, we did not find significant correlation in either [N] or morphology (SRL versus SLA) (Table 3).

Table 2 Reports from principal components analysis on the six root traits and two leaf traits, including the proportion of variation explained (top table) and loading scores of traits on each component (bottom table).

Component	Eigenvalue	Proportion	Cumulative
1	2.84	0.36	0.36
2	1.61	0.20	0.56
3	1.17	0.15	0.71
4	0.87	0.11	0.82
5	0.64	0.08	0.90
6	0.42	0.05	0.95
7	0.33	0.04	0.99
8	0.10	0.01	1.00

Variable	Component 1	Component 2	Component 3
Diameter	-0.56	-0.12	0.01
SRL	0.44	-0.10	0.40
Length	-0.51	-0.16	-0.00
Tissue density	0.34	0.13	-0.51
Branching ratio	0.28	-0.14	-0.25
Root [N]	0.15	-0.46	0.60
Leaf [N]	0.11	-0.60	-0.29
SLA	-0.05	-0.58	-0.30

[N], nitrogen concentration; SLA, specific leaf area; SRL, specific root length.

Trait evolution

Angiosperms of more ancient families (the families that diverged earlier in the evolutionary history of plants) had thicker and longer first-order roots than those of more recently diverged families. Piecewise regression analyses showed that the decrease in first-order root diameter and length with divergence time was faster and highly significant from 120 to 64 million years ago (Ma), but disappeared afterwards (Fig. 4). Moreover, SRL, branching ratio and [N] of first-order roots increased significantly with divergence time before *c.* 60–70 Ma. The piece-

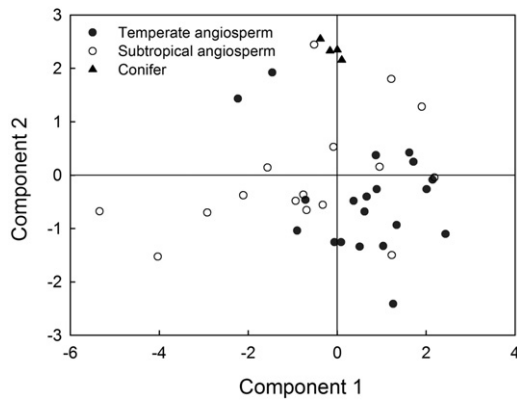


Figure 2 Score plot of species from three groups in the principal components analysis. Component 1 was mainly related to first-order root diameter and length; component 2 was mainly related to leaf [N] and specific leaf area (SLA).

wise regression explained 57% of the variations in diameter ($P < 0.001$), 32% of the variations in SRL ($P < 0.05$), 36% of the variations in length ($P < 0.05$), 21% of the variations in branching ratio ($P = 0.05$) and 27% of the variations in root [N] ($P = 0.05$) at the family level (Fig. 4). The linear PIC regression analysis showed that without the influence of phylogeny, divergence time at family level still significantly explained (though to a lower degree) the variation of diameter ($r^2 = 0.19$, $P < 0.05$), as well as SRL ($r^2 = 0.23$, $P < 0.05$) and branching ratio ($r^2 = 0.24$, $P < 0.05$) of first-order roots (Fig. 4).

DISCUSSION

Species with thick and thin first-order roots coexist in the subtropical zone

This study focused on trait variation of the most ephemeral roots, classified as first-order roots where they have no other branches, probably serving mainly water and nutrient acquisition functions. We had predicted that first-order root traits would vary markedly from subtropical to temperate forests but would converge within the same climate zone, a pattern similar to that found in leaf traits. Yet surprisingly, a much higher variation in root morphology was found within the subtropical zone (Table 1, Figs 3 & S3), despite the similar mean annual temperature and precipitation within this zone (Tables S1). This large variation in root morphology, particularly in root diameter, appears to be driven by the existence of species with very thick first-order roots in the subtropical zone co-occurring with species with thin roots (Fig. 3). If we excluded those species with a root diameter > 0.5 mm, the CV of diameter in the subtropical zone was much smaller ($CV = 43.72\%$), and similar to that in temperate zone ($CV = 41.02\%$; Table 1). Earlier studies have reported that basal angiosperms (species from families like Magnoliaceae) tend to have thicker roots (Baylis, 1975; St John, 1980) thus the occurrence of species with thick roots may be a reflection of the similarity in habitat con-

ditions between the current subtropical zone and the ancient environment where basal angiosperms originated (Baylis, 1975; St John, 1980; Feild *et al.*, 2009). Meanwhile, some subtropical species with thin roots have appeared, probably because thin roots have lower construction costs than thick roots (Eissenstat, 1992) and are more plastic in growth proliferation (Eissenstat, 1991), which may confer certain advantages in phosphorus-poor subtropical soils if nutrient supply is spatially or temporally heterogeneous.

By contrast, the smaller variation in root morphology among temperate species suggests that the soil environment of temperate forests may have constrained the occurrence of a thick-root strategy. Temperate species displayed a much narrower range in root traits than the subtropical group (Table 1, Fig. 2) with only 2 out of 30 species having a root diameter greater than 0.5 mm (Fig. 3). Moreover, these coarse-root species tend to be found in landscape positions of high water availability, such as toe slopes and valleys. We speculate that thin roots are probably better equipped to cope with the generally lower and temporally more variable soil moisture content in the temperate zone (precipitation at our subtropical sites was three times that of the temperate sites; Table S1, Figs S2 & S3). The lower construction costs for thinner roots of temperate species would allow greater flexibility (or plasticity) in root growth (construction) and death, giving them advantages in seasonally more variable environments associated with temperature and moisture supply. Moreover, the thin-root temperate species tend to have higher branching ratio (Tables 1 & 3), which might increase small-scale foraging and nutrient absorption in the more arid temperate zone (Fitter, 1994). By contrast, environmental pressure such as water stress for first-order roots may be weak in the subtropical zone, which would have allowed for the existence of a greater number of thick-root species. When colonized by mycorrhizal fungi [mostly arbuscular mycorrhizal (AM) fungi], species with thick first-order roots may be highly effective in nutrient (particularly P) uptake. Indeed, thicker roots usually have both a higher cortex area which is preferred by AM fungi (Brundrett, 2002) and larger vessels (D.G., unpublished data), which can be advantageous in water transport. Therefore, thick roots may remain a successful strategy in humid subtropical forests. Although we do not yet fully understand the patterns of species radiation, it is likely that temperate species experienced a shift from the hot and humid conditions of their origins (Cox & Moore, 2005) to conditions of much higher variation in temperature and moisture. These changes possibly served as a strong environmental filter (Westoby & Wright, 2006), leading to many of the thick-root species failing to survive in habitats where droughts and low temperatures are common.

Does the predicted 'root economics spectrum' exist?

Our study did not support a 'root economics spectrum' analogous to the 'leaf economics spectrum' (Wright *et al.*, 2004). For example, we did not find that thinner roots had higher [N] using either PIC correlations (Table 3) or simple Pearson correlations

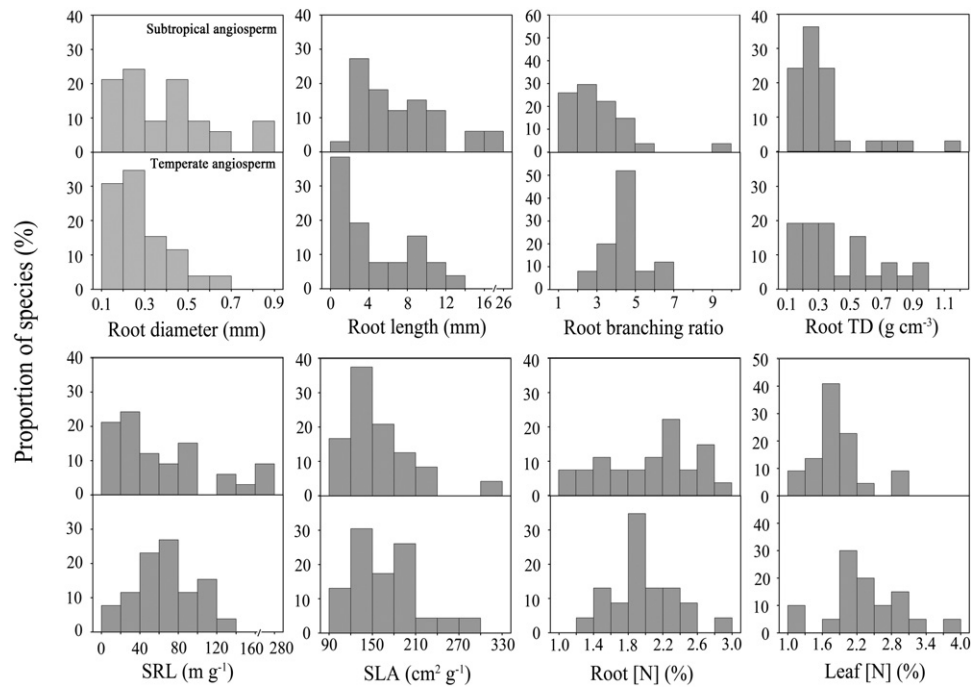


Figure 3 Frequency distribution of morphological and chemical traits for first-order roots and leaves. For each plant trait, the upper panel is for subtropical angiosperms and the lower panel for temperate angiosperms. TD, tissue density; SRL, specific root length; SLA, specific leaf area.

Table 3 Correlation coefficients among root and leaf traits for all species with phylogenetic independent contrasts.

	Diameter	SRL	Length	Tissue density	Branching ratio	Root [N]	Leaf [N]
SRL	-0.72**						
Length	0.70**	-0.42**					
Tissue density	-0.46**	0.22	-0.31*				
Branching ratio	-0.38*	0.25	-0.38*	0.06			
Root [N]	-0.15	0.27	-0.23	-0.14	0.16		
Leaf [N]	0.31*	-0.36*	0.22	-0.10	-0.08	0.17	
SLA	-0.06	0.28	0.07	0.04	0.13	-0.02	0.11

Correlations significant at ** $P < 0.001$ and * $P < 0.05$.

[N], nitrogen concentration; SLA, specific leaf area; SRL, specific root length.

of raw data (data not shown). Although we did not find significant PIC correlations between leaf [N] and SLA either (Table 3), we did find significant ahistorical correlations between leaf [N] and SLA. Therefore, in some cases, the leaf 'economics spectrum' partly resulted from phylogenetic effects, but the 'root economics spectrum' as hypothesized did not occur in our data set regardless of the phylogenetic effects.

Root [N] of first-order roots is mainly in cortical tissues because xylem is composed mainly of nutrient-poor cells (Li *et al.*, 2010). A study of root anatomy across 23 temperate trees reported that in the first-order roots, the proportion of cortical cross-sectional area to whole root cross-sectional area is relatively consistent across angiosperm species, despite the variation in whole-root diameter among these species (Guo *et al.*, 2008). If the same is true in our study, such that species with thick and

thin first-order roots have a similar proportion of cortical cells, then their root [N] will likely be similar and thus uncorrelated with the root diameter (or SRL).

The strong correlations between leaf [N], photosynthetic rate and SLA (Reich *et al.*, 1999) across species may not have equivalent parallels in roots. In leaves, photosynthetic capacity is strongly related to Rubisco concentration, which is strongly correlated with leaf [N]. In addition, leaves with a long life span are typically thick, have low SLA and low [N]. But in roots no single enzyme is mainly controlling resource acquisition, nitrogen may be found in both organic and inorganic forms in the root and, most importantly, absorptive surface area may be acquired by either roots directly or by the hyphae of mycorrhizal fungi. Across diverse plant lineages, therefore, there may be multiple solutions to resource acquisition in relatively moist, infertile

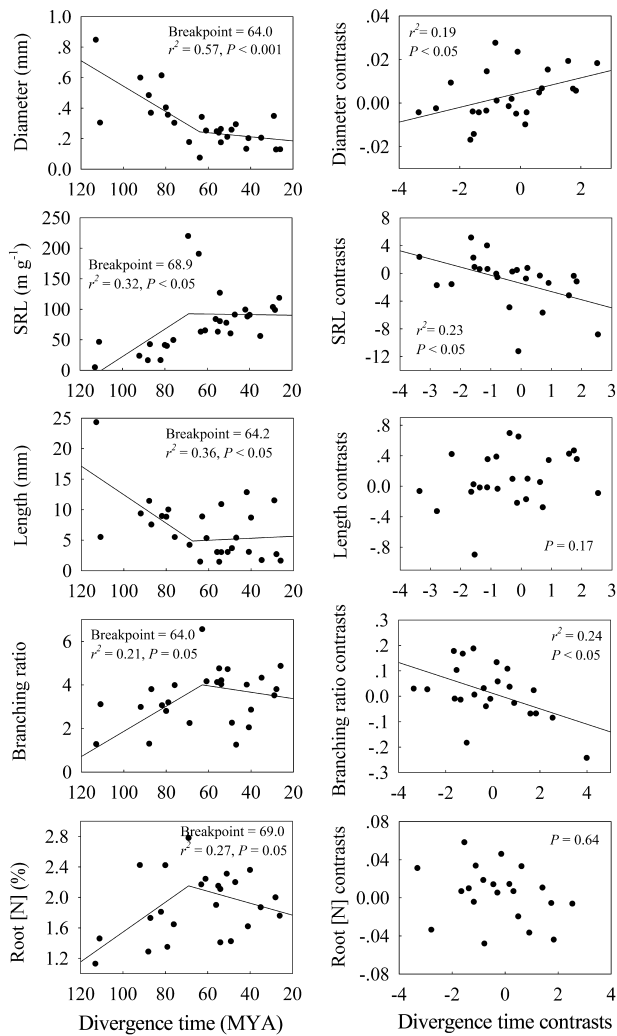


Figure 4 Piecewise (left panels) and phylogenetic independent contrast (PIC) (right panels) regression analysis of first-order root traits and divergence time. Significant piecewise regressions predicted by divergence time at family level were only detected for diameter ($r^2 = 0.57$, $P < 0.001$), specific root length (SRL) ($r^2 = 0.32$, $P < 0.05$), length ($r^2 = 0.36$, $P < 0.05$), branching ratio ($r^2 = 0.21$, $P = 0.05$) and root [N] ($r^2 = 0.27$, $P = 0.05$). Significant breakpoints ($P < 0.05$) are shown in the diagrams (million years ago, Ma). For PIC regressions, only diameter ($r^2 = 0.19$, $P < 0.05$), SRL ($r^2 = 0.23$, $P < 0.05$) and branching ratio ($r^2 = 0.24$, $P < 0.05$) of first order roots showed significant evolution trend in our study.

environments. While a strong correlation of root [N] and nitrate uptake has been observed as a function of root age in grape (Volder *et al.*, 2005), such patterns have not been observed across species. Similar to this study, in a comparison of 25 co-occurring temperate trees there was wide variation in root diameter but little variation in root N concentration (Comas & Eissenstat, 2009). These observations suggest that root [N] is not linked with root morphology in the same way as described in leaves. A major limitation to progress in understanding ‘root economics’ is the limited data on root life span needed to iden-

tify the traits associated with long- and short-lived roots (but see McCormack *et al.*, 2012). Nonetheless, evidence is accumulating that single vectors of variation linking root morphology to nutrient acquisition or life span are likely to remain elusive.

Root trait modification during angiosperm radiation

By analysing root traits of extant species of different origins, we estimated patterns of root trait evolution as species radiated in geological time. We estimated that from 120 to 64 Ma, a period corresponding to the mid to late Cretaceous, the diameter of first-order roots decreased (Fig. 4). Fossil records showed that during the Cretaceous (around 145–65 Ma), a remarkable adaptive radiation of the angiosperms occurred (Hickey & Doyle, 1977; Wing & Boucher, 1998; McElwain *et al.*, 2005), and evolutionary changes in root morphology found in our study seemed to coincide with the angiosperm radiation.

Additionally, the smaller dependence of diameter (or SRL) on divergence time in the PIC regression than in the piecewise regression (Fig. 4) suggested a strong phylogenetic influence of plant lineages (or ancestors) on the diameter (or SRL) of newly diverged families. However, the correlation between diameter (or SRL) with divergence time remained significant when the influence of phylogeny was removed (Fig. 4). These patterns may be related to changes in global climate and species distributions in the Cretaceous. After emergence in the late Jurassic, angiosperms went through early evolution and diversification in the Cretaceous, during which CO_2 progressively decreased (McElwain *et al.*, 2005). In order to cope with lower atmospheric CO_2 concentration, plants might have increased their stomatal conductance at the expense of increased water loss (Mueller *et al.*, 2010). In addition, global precipitation decreased in the early Cretaceous, compared with the earlier periods (Frakes, 1979), further increasing the selection pressure on plants to promote water absorption and reduce water loss (McElwain *et al.*, 2005; Brodrribb & Feild, 2010; Mueller *et al.*, 2010). Producing root systems with smaller diameter and higher branching density may have helped plants build and shed roots at lower cost when exposed to periodic water stress. The evolution of root morphological traits identified here may have occurred synchronously with evolution of some leaf traits such as leaf stomatal density (Woodward, 1987) and vein density (Brodrribb & Feild, 2010) because modification of both root and leaf traits are critical ways for plants to successfully survive and reproduce in drier habitats.

There are some uncertainties in our results and interpretation. First, a limited sample size did not allow us to thoroughly analyse the variation within the same family or genus; such an analysis would require a much larger number of species. Second, we did not consider root trait variation for a given species across environmental gradients, which would allow us to examine trait plasticity. Root morphology of a given species may vary little with large changes in annual precipitation (Leuschner *et al.*, 2004) but vary markedly along temperature and nitrogen gradients (Ostonen *et al.*, 2011). Systematic investigation across

environmental gradients would improve our understanding of the range in plasticity of different root traits.

CONCLUSIONS

By sampling first-order roots and analysing their traits over a broad range of phylogeny and habitats, we demonstrated several novel findings regarding patterns of root trait variation, correlation and evolution. First, we found large morphological variation of roots in subtropical zones where species with both very thick and thin first-order roots coexisted, probably as the result of weaker environmental selection pressure on roots than on leaves. Second, root [N] was uncorrelated with root morphology, in contrast with the correlation of leaf [N] with leaf morphology, suggesting that the underlying tradeoffs in roots may be distinctly different from those in leaves. Finally, root diameter might have decreased strongly from mid to late Cretaceous as newly diverged species colonized drier habitats during angiosperm radiation of this geological period. These findings demonstrate that patterns of root trait variation and evolution are distinct from those in leaves, presumably because of different selection pressures above and below ground.

ACKNOWLEDGEMENTS

We thank Dr Zhengquan Wang, Wenjing Chang, Shasha Chen, Ye Jin, Ang Li and Lijing Peng for assistance in the field and laboratory. We appreciated Dr Mingyao Ai, Shaopeng Wang and Shuang Liang for their help in data analysis. We also thank the editors and anonymous referees for their valuable comments on the early version of the manuscript. The funding for this work came from the National Science Foundation of China (nos 40971030 and 31021001) and One-Hundred Talent Project of Chinese Academy of Sciences (no. KZZD-EW-TZ-11).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Figure S1 Distribution of the sampling sites in woody vegetation area of China.

Figure S2 Soil water content positively and significantly correlated with annual precipitation in our study range (temperate and subtropical China).

Figure S3 Box-plots of diameter, specific root length (SRL), length, tissue density, branching ratio and nitrogen concentration ([N]) of first-order roots and leaf [N] and specific leaf area (SLA) from six sample sites with different mean annual precipitation (MAP).

Table S1 Sampling site information.

Table S2 Taxonomic list and sampling sites of the 65 tree species.

BIOSKETCH

The research interests of **Weile Chen** centre on below-ground ecology, emphasizing plant roots and mycorrhizal fungi. In this paper, D.G. designed the study, W.C., H.Z. and D.G. collected the data, W.C. performed the analysis, W.C. and D.G. wrote the first draft of the manuscript, and all authors contributed substantially to revision.

Editor: Arndt Hampe