Leading dimensions in absorptive root trait variation across 96 subtropical forest species

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Received: *24 January 2014*
Accepted: *3 April 2014*

New Phytologist (2014) **203:** 863–872
doi: 10.1111/nph.12842

Key words: branch order, root anatomy, root architecture, root foraging strategies, trait economics.

Summary

- Absorptive root traits show remarkable cross-species variation, but major root trait dimensions across species have not been defined.
- We sampled first-order roots and measured 14 root traits for 96 angiosperm woody species from subtropical China, including root diameter, specific root length, stele diameter, cortex thickness, root vessel size and density, mycorrhizal colonization rate, root branching intensity, tissue density, and concentrations of carbon and nitrogen ([N]).
- Root traits differed in the degree of variation and phylogenetic conservatism, but showed predictable patterns of cross-trait coordination. Root diameter, cortex thickness and stele diameter displayed high variation across species (coefficient of variation (CV) = 0.51–0.69), whereas the stele:root diameter ratio and [N] showed low variation (CV < 0.32). Root diameter, cortex thickness and stele diameter showed a strong phylogenetic signal across species, whereas root branching traits did not, and these two sets of traits were segregated onto two nearly orthogonal (independent) principal component analysis (PCA) axes.
- Two major dimensions of root trait variation were found: a diameter-related dimension potentially integrating root construction, maintenance, and persistence with mycorrhizal colonization, and a branching architecture dimension expressing root plastic responses to the environment. These two dimensions may offer a promising path for better understanding root trait economics and root ecological strategies world-wide.

Introduction

Absorptive roots and their associated mycorrhizal fungi are the primary organs for plant resource acquisition from soils (Robinson *et al.*, 2003), and play key roles in carbon (C) and nutrient cycling as a consequence of their fast turnover and high nutrient concentrations (Pregitzer *et al.*, 2002; Xia *et al.*, 2010). A limited number of studies have shown that absorptive roots show large variation in their functional traits across species and biomes (Pregitzer *et al.*, 2002; Tjoelker *et al.*, 2005; Roumet *et al.*, 2006; Withington *et al.*, 2006; Holdaway *et al.*, 2011). Elucidating the patterns of, and factors controlling, these trait variations may provide critical insights into the geographical distribution of plant communities and their adaptations to the changing global environment.

Several general patterns of root trait variation have emerged from previous studies. First, different traits show different degrees of variation, with morphological and architectural parameters (such as specific root length (SRL), root diameter, and branching density or ratio) showing greater cross-species variation than chemical parameters (such as root nitrogen (N) concentrations; Comas & Eissenstat, 2009; Chen *et al.*, 2013). This pattern suggests that the morphology and architecture of absorptive roots can change markedly across species or across environmental conditions, whereas concentrations of vital biological elements such as N may stay relatively constant (Li *et al.*, 2010). Secondly, root form seems to be related to critical aspects of root evolution and root function, such that basal families from subtropical and tropical regions tend to have greater root diameters, lower branching densities, and higher mycorrhizal dependence (Baylis, 1975; St John, 1980; Fitter, 2004; Comas *et al.*, 2012). More recently, root morphology has also been linked to root lifespan (McCormack *et al.*, 2012) and foraging strategies (e.g. degree of plasticity in response to nutrient patches (Adams *et al.*, 2013)). Thus, root morphology and architecture may represent a major axis along which the form and function of absorptive roots of different species vary. Consequently, major dimensions of root trait axes represented by parameters of root form should exist, yet no such dimensions have been clearly defined.

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The goal of this work was to quantify the range of variation for key root morphological, anatomical and chemical traits and to extract major dimensions of root trait variation if they emerged. Specifically, we hypothesized that: (1) root morphological and anatomical traits would form a major root trait axis because marked cross-species variation in root morphology (Roumet *et al.*, 2006; Withington *et al.*, 2006; Comas & Eissenstat, 2009; Holdaway *et al.*, 2011) and anatomy (Guo *et al.*, 2008) has previously been observed; (2) as absorptive roots are branching structures (unlike leaves) (Pregitzer *et al.*, 2002; Xia *et al.*, 2010), how intensively first-order roots branch from a second-order root may represent another dimension of root trait variation indicating to what degree first-order roots of different species proliferate (Hodge, 2004) under average soil conditions for each species at a given site; and (3) variation in root morphology and anatomy should be closely related to mycorrhizal colonization rates in arbuscular mycorrhizal (AM) species because root diameter and cortex area directly determine rates of mycorrhizal colonization in AM species (Brundrett, 2002).

To test these hypotheses, we measured 14 root traits for the first-order roots, including root morphology, architecture, anatomy, chemistry and mycorrhizal colonization, across 96 angiosperm woody species (with > 70% being trees) from tropical and subtropical forests in China. We chose the first root branch order as our sampling unit because this branch order has been identified as the most important absorptive root tissue which shows only primary development and does not undergo secondary growth at any point in the root life history, and has the most rapid turnover and highest metabolic activity in all tree species examined to date (Pregitzer *et al.*, 2002; Guo *et al.*, 2008; Valenzuela-Estrada *et al.*, 2008; Xia *et al.*, 2010). Thus, this study focused on cross-species trait variations for the first-order roots only (but to assess branching pattern, we also used second-order roots), and differed from previous root trait studies that used the entire fine-root pool (all roots < 1 or 2 mm) as the sampling unit. We also constrained our sampling to subtropical and tropical woody angiosperms because this functional group contains species with comparable anatomical structures but encompasses diverse phylogenetic lineages, allowing us to obtain a better understanding of trait correlations and economics from a phylogenetically informed perspective (Comas *et al.*, 2012; Chen *et al.*, 2013).

Materials and Methods

Study site and root collection

A total of 96 angiosperm woody species were selected from tropical and subtropical forests in southern China. We sampled several key clades of common species such as magnoliids, fabids, and lamiids. Details of these sites and information on the species sampled are presented in Supporting Information Tables S1 and S2. Root samples were collected in July and August 2010 following the procedure described in Guo *et al.* (2008). For each species, at least three mature plants were chosen. Surface soil (0–20 cm) at the base of the trees was carefully excavated to expose the main lateral roots. Root branches with intact terminal branch orders were cut and samples of the branches including > 5 g of total fresh biomass of first-order roots (to allow sufficient biomass for measuring biomass-related chemical and morphological parameters) were collected. Subsamples of the roots from each tree were gently washed in deionized water to remove soil adhering to roots. These samples were immediately put into plastic tubes filled with formalin-aceto-alcohol (FAA) solution (90 ml of 50% ethanol, 5 ml of 100% glacial acetic acid and 5 ml of 37% methanol) for later anatomical measurements. The remaining samples were transported in plastic bags in a cooler to the laboratory and frozen at −20°C until subsequent morphological and chemical analyses.

Root trait measurements

More than five intact root branches were taken for morphologic measurement. Here we focused on the most distal roots, or the first-order roots (Pregitzer *et al.*, 2002). The first-order roots can be classified into pioneer and fibrous roots, but only the latter, which are primarily responsible for water and nutrient uptake (Zadworny & Eissenstat, 2011), were included in our analysis. Root diameter and length were determined using a ×40 stereomicroscope with an ocular micrometer (± 0.025 mm). Root tissue density (RTD) was calculated as the ratio of root dry mass to its volume assuming that a root was a cylinder. Specific root length (SRL) was calculated as the root length divided by its dry mass. The root branching ratio was calculated as the number of first-order roots divided by the number of second-order roots (as previously reported in Chen *et al.*, 2013). We also calculated root branching intensity as the number of first-order roots per centimeter of second-order roots to allow for comparisons with the literature (such as Comas & Eissenstat, 2009). Subsamples of roots in each species were cleaned and oven-dried at 60°C for 24 h and were ground to fine powder, and their C and N concentrations were determined using an elemental analyzer (Vario EL Cube; Elementar, Hanau, Germany).

For root anatomical indices, we randomly chose 20 first-order root segments from intact branches composed of the first three branch orders, which were fixed in the field in FAA solution for each species, with these segments coming from individual roots of all three tree individuals. The anatomical procedure is often a trial-and-error process and we could not guarantee that all segments would be successfully sectioned and imaged; thus, we often chose > 20 segments and randomly chose 20 from all successful segments for our anatomical trait estimation. These root segments were quickly immersed in a sequence of alcohol solutions for dehydration before being embedded in paraffin (Guo *et al.*, 2008). In the region of maturation as commonly defined in the literature, roots were cut into sections 8 μm thick. These sections were then stained with safranine-fast green, with the cortex staining green and the stele staining red, and were then photographed using a compound microscope (Axioscop 20; Carl Zeiss, Jena, Germany). The anatomical structures were measured using ImageJ (NIH Image, Bethesda, MD, USA), including traits such as root diameter, vessel diameter, stele diameter and cortex thickness. The ratio of stele diameter to root diameter was then

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calculated. As there were many vessels in a root segment, vessel diameter was expressed as the average across all vessels (Fan *et al.*, 2012). Vessel density was calculated as the number of vessels per unit stele cross-sectional area (Long *et al.*, 2013).

Mycorrhizal colonization was determined by carefully examining root anatomical structures. The appearance of coils (or arbuscules) in cortical cells indicated colonization by AM fungi (Brundrett, 2004). Ectomycorrhizal (EM) fungi were identified by visual observation of the fungal sheath or Hartig net. The rate of mycorrhizal colonization (MC) was calculated as the number of roots colonized by mycorrhizal fungi divided by the total number of roots examined for a species (Guo *et al.*, 2008).

The construction of plant phylogeny

Plant genomic DNA of each species was extracted from leaves, which were collected at the same time as the roots were sampled (Table S3). We determined *rbcL* and *matK* sequences (chloroplast gene fragments) for each species (Table S4). After model selection using jModelTest (Posada, 2008), a phylogenetic tree was constructed using neighbor joining (NJ), maximum likelihood (ML), and Bayesian approaches, respectively. In the phylogenetic analyses, tree branch length was set to be proportional to the difference in divergence time between neighbor clades. Divergence time was estimated using beast 1.7.1 (Drummond *et al.*, 2012) with fossil calibration points (Table S5) from Magallóan & Sanderson (2005) and Magallón & Castillo (2009). Detailed information can be found in Methods S1.

Data analysis

For each root trait, we calculated its mean value, minimum, maximum and coefficient of variation (CV). Pairwise trait relationships were assessed using Pearson's correlations in spss 11.3 (SPSS Inc., Chicago, IL, USA). In addition, linear regressions were used to examine the relationship between root diameter versus cortex thickness and between root diameter versus stele diameter. The slopes of the two regressions were compared using standardized major axis (SMA) in smart (version 2.0; Falster *et al.*, 2006). For AM species, piece-regression was performed to assess the relationship between root diameter and mycorrhizal colonization. We chose the piece-wise regression for two reasons: first, in our previous work (Chen *et al.*, 2013) we found that many root morphological traits changed nonlinearly across clades; and secondly, we detected statistical break-points in analysis *post hoc*, and we chose the break-points with the best fit; thus, the exact location of the break-point chosen may vary with changes in data points. Multiple trait relationships were analyzed by principal component analysis (PCA) in canoco software for Windows 4.5 (Microcomputer Power, Ithaca, NY, USA).

To quantify the influence of evolutionary history on each trait, Blomberg's K statistic (Blomberg *et al.*, 2003) was calculated in the R 3.0.0 statistical platform (R Development Core Team, 2013) with the R package *picante*. A larger K value indicates greater phylogenetic conservatism for a trait. We also performed Abouheif's test (Abouheif, 1999; Pavoine *et al.*, 2008) for detecting phylogenetic signal using the R package *adephylo*. In addition, trait relationships were analyzed after correcting for shared evolutionary histories (phylogenic independent contrasts (PICs)) using the R package *picante*.

Results

Range of variation in different root traits

Across the 96 species, there was 14-fold variation in first-order root diameter, ranging from a minimum of 72.59 µm in *Macaranga sampsonii* to a maximum of 1009.63 µm in *Endospermum chinense* (Fig. 1), with an overall CV% of 58.4%. Species in basal angiosperm families such as Magnoliaceae and Lauraceae generally had first-order roots with large root diameter (Fig. 1). A similar range of variation was found for cortex thickness and stele diameter; cortex thickness had a CV of 69.1% and stele diameter a CV% of 51.1%. By contrast, the variation in the ratio between stele diameter and root diameter was low (23.4%; Table 1). Also, the variation in vessel diameter (36.3%) was much smaller than that in vessel density (67.6%). The CV of root [N] (31.9%) was lower than that of other traits except for root [C] (9.8%). SRL had the largest cross-species variation (CV = 68.5%) among morphological traits.

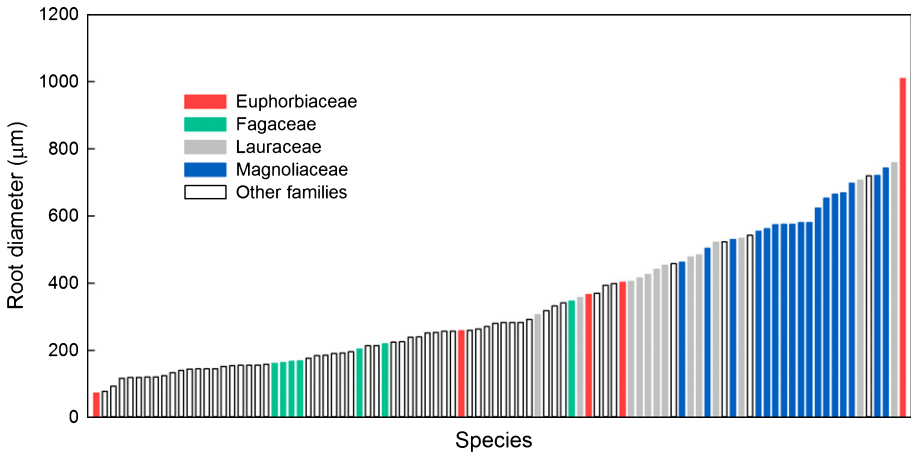

Fig. 1 Root diameter of first-order roots ranked in ascending order of 96 woody species in tropical and subtropical forests in southern China.

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Table 1 Summary of the 14 first-order root traits for 96 subtropical woody species in southern China

Root trait (abbreviation)	Units	Mean	Median	Min[1]	Max[2]	CV%[3]
Root diameter (Diam)	µm	342.82	281.85	72.59	1009.63	58.4
Cortex thickness (Cortex)	µm	218.65	169.70	26.13	674.81	69.1
Stele diameter (Stele)	µm	80.42	69.29	24.07	257.78	51.1
Stele to root diameter ratio (Stele : Diam)		0.25	0.25	0.13	0.51	23.4
Root length (Length)	mm	11.27	11.20	3.33	33.95	42.8
Root tissue density (RTD)	$g\ cm^{-3}$	0.35	0.33	0.13	0.72	38.2
Specific root length (SRL)	$m\ g^{-1}$	56.19	45.29	4.42	169.91	68.5
Root carbon concentration (C)	$mg\ g^{-1}$	466.8	463.8	323.4	590.1	9.8
Root nitrogen concentration (N)	$mg\ g^{-1}$	19.38	19.61	5.55	44.62	31.9
Root branching ratio (BrRatio)		2.87	2.54	1.21	10.10	47.3
Branching intensity (BrIntensity)	cm^{-1}	1.49	1.22	0.44	7.37	67.1
Vessel density (VesDens)	mm^{-2}	4508.89	3841.06	1095.33	18683.67	67.6
Vessel diameter (VesDiam)	µm	5.53	5.32	1.94	9.56	36.3
Mycorrhizal colonization (MC)	%	62.76	64.10	7.50	100.00	40.4

[1]Min, the minimum value of the trait across the 96 species.
[2]Max, the maximum value of the trait across the 96 species.
[3]CV%, the coefficient of variation.

Influence of plant phylogeny on root traits

Phylogenetic trees constructed by three different methods (NJ, ML and Bayes) had similar topologies. Of the 14 root traits examined, 12 traits were phylogenetically conservative as indicated by Blomberg's *K* values (Table 2), the index that assesses the amount of phylogenetic signal in a biological trait to allow comparisons of different traits across different phylogenetic trees for the purpose of discovering possible general patterns of relative evolutionary lability across trait types (Blomberg *et al.*, 2003). *K* values of diameter-related traits except vessel density were all significant and from high to low values were: root diameter ($K = 0.397$), cortex thickness ($K = 0.307$), SRL ($K = 0.176$), stele diameter ($K = 0.175$), MC ($K = 0.131$), and ratio of stele to root diameter ($K = 0.054$). RTD ($K = 0.114$) and root [N] ($K = 0.107$) showed intermediate phylogenetic signals, while neither the root branching ratio nor vessel density showed a significant phylogenetical signal ($P > 0.05$). For vessel traits, only vessel diameter showed a relatively strong phylogenetic signal ($K = 0.070$; $P < 0.01$). We also analyzed the effect of phylogeny on root traits using Abouheif's test (Fig. S1), and found similar results.

Table 2 Blomberg's *K* values for 14 root traits

Root traits	Blomberg's *K*	*P*
Root diameter	0.397	0.001
Cortex thickness	0.307	0.001
Specific root length	0.176	0.001
Stele diameter	0.175	0.001
Mycorrhizal colonization rate	0.131	0.001
Root tissue density	0.114	0.001
Root length	0.112	0.001
Root nitrogen concentration	0.107	0.001
Vessel diameter	0.070	0.002
Branching intensity	0.055	0.045
Root carbon concentration	0.054	0.046
Stele to root diameter ratio	0.054	0.033
Root branching ratio	0.046	0.108
Vessel density	0.045	0.078

The significance level was set at 0.05.

Trait correlations

Several root anatomical parameters strongly covaried with root diameter (Table 3). Specifically, cortex thickness and stele diameter both increased linearly with root diameter ($R^2 = 0.98$ and $R^2 = 0.85$, respectively; *P*-values < 0.001; Fig. 2), with the slope for cortex thickness much steeper than that for stele diameter ($P < 0.01$ in SMA analysis). These patterns were particularly strong for AM species (Fig. S2). In addition, the ratio between stele diameter and root diameter was negatively correlated with root diameter (Table 3). For the root vessel-related parameters, vessel diameter was positively correlated but vessel density was negatively correlated with root diameter. Root diameter was also strongly correlated with morphological traits such as SRL and RTD but weakly correlated with branching intensity and unrelated to root branching ratio (Table 3). Root [N] was significantly but weakly ($R = 0.22$) correlated with root diameter. Across all species, the MC rate was positively and strongly correlated with both root diameter and cortex thickness (Table 3). Across 96 species, 83 species were colonized by AM fungi, eight by EM fungi and five by other types of fungus (Table S2). For these AM species, piece-wise regression for MC and root diameter showed a much lower slope when root diameter was > 463.5 µm ($R^2 = 0.62$; $P < 0.001$; Fig. 3). Correlations were similar when using original data and PICs (Table 3).

Multivariate ordination

The PCA using original trait data showed that the first two trait axes accounted for 48.3% and 12.1% of total variation, respectively (Fig. 4a), with diameter-related parameters scoring high on

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Table 3 Coefficients of Pearson's correlation for pairwise traits with original data (upper-right diagonal) and phylogenetically independent contrasts (lower-left diagonal)

	Root diameter	Cortex thickness	Stele diameter	Stele to root diameter ratio	Root length	RTD	SRL	[C]	[N]	Root branching ratio	Branching intensity	Vessel density	Vessel diameter	MC rate
Root diameter		0.99**	0.92**	−0.56**	0.64**	−0.56**	−0.79**	−0.10	0.22*	−0.16	−0.37**	−0.52**	0.79**	0.66**
Cortex thickness	0.96**		0.89**	−0.60**	0.60**	−0.59**	−0.78**	−0.09	0.24*	−0.19	−0.38**	−0.51**	0.78**	0.66**
Stele diameter	0.56**	0.38**		−0.26*	0.65**	−0.50**	−0.75**	−0.09	0.17	−0.10	−0.32**	−0.55**	0.76**	0.62**
Stele to root diameter ratio	−0.20	−0.37**	0.54**		−0.24*	0.44**	0.42**	0.07	−0.23*	0.16	0.23*	0.13	−0.48**	−0.36**
Root length	0.36**	0.38**	0.04	−0.15		−0.35**	−0.59**	−0.14	0.02	−0.11	−0.52**	−0.37**	0.46**	0.39**
RTD	−0.42**	−0.45**	−0.20*	0.12	−0.31**		0.39**	−0.02	−0.36**	0.18	0.26**	0.34**	−0.57**	−0.34**
SRL	−0.36**	−0.32**	−0.34**	0.15	−0.19	0.02		0.03	−0.14	0.04	0.29**	0.55**	−0.70**	−0.63**
[C]	0.15	0.03	0.40**	0.40**	0.06	−0.20*	−0.04		0.17	−0.09	−0.03	−0.08	−0.04	−0.05
[N]	0.27**	0.37**	0.04	−0.24*	0.05	−0.50**	0.16	0.02		−0.05	−0.07	−0.10	0.21*	0.01
Root branching ratio	−0.13	−0.11	−0.03	−0.21*	−0.17	0.09	−0.29**	−0.36**	0.13		0.86**	−0.01	−0.14	−0.11
Branching intensity	−0.20*	−0.19	−0.03	−0.14	−0.49**	0.14	−0.13	−0.32**	0.06	0.87**		0.13	−0.29**	−0.20
Vessel density	−0.16	−0.08	−0.34**	−0.23*	0.00	−0.04	0.34**	−0.34**	0.14	0.08	0.05		−0.48**	−0.52**
Vessel diameter	0.36**	0.27**	0.50**	−0.05	−0.23*	−0.07	−0.50**	0.04	−0.06	0.23*	0.35**	−0.40**		0.49**
MC rate	0.25*	0.16	0.28**	0.21*	−0.17	0.09	−0.06	0.33**	−0.31**	−0.51**	−0.29**	−0.45**	0.26**	

Correlations were significant at: **, $P = 0.01$; *, $P = 0.05$.
[C], root carbon concentration; MC, mycorrhizal colonization; [N], root nitrogen concentration; RTD, root tissue density; SRL, specific root length.

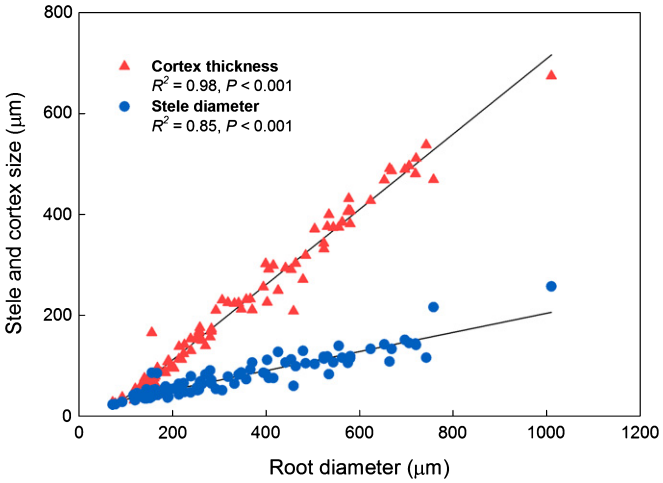

Fig. 2 Positive correlations between root diameter and cortex thickness (red) or stele diameter (blue) for the first-order roots across 96 woody species.

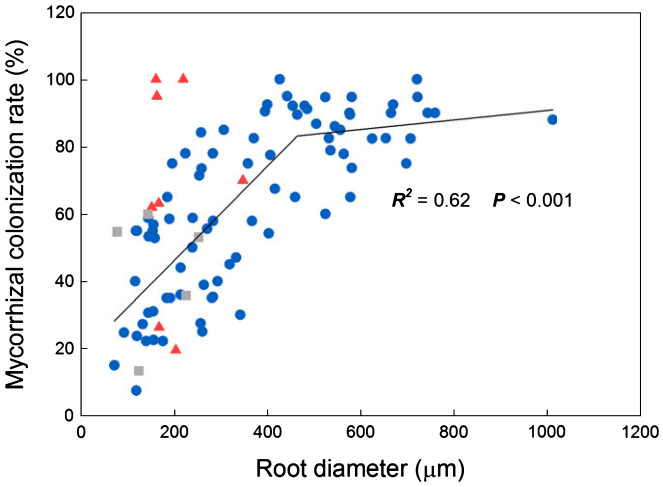

Fig. 3 The relationships between first-order root diameter and mycorrhizal colonization rate in species with different mycorrhizal types. Blue circles, species colonized by arbuscular mycorrhizal (AM) fungi; red triangles, species colonized by ectomycorrhizal (EM) fungi; gray squares, species colonized by types of fungus other than AM and EM fungi. Piece-wise regression was used for AM species with a break-point diameter of 463.5 μm ($P < 0.001$).

the first axis and two branching parameters scoring high on the second axis (Table S6). Most species in Magnoliaceae and Lauraceae were clustered and separated from species of other families (such as Fagaceae; Fig. 4b). Species from Euphorbiaceae scattered on both axes (Fig. 4b).

Discussion

Leading dimensions of root functional trait variation

Absorptive roots are not simple, discrete structures such as leaves or needles; rather, they have a complex branching architecture, and form intimate associations with mycorrhizal fungi. This

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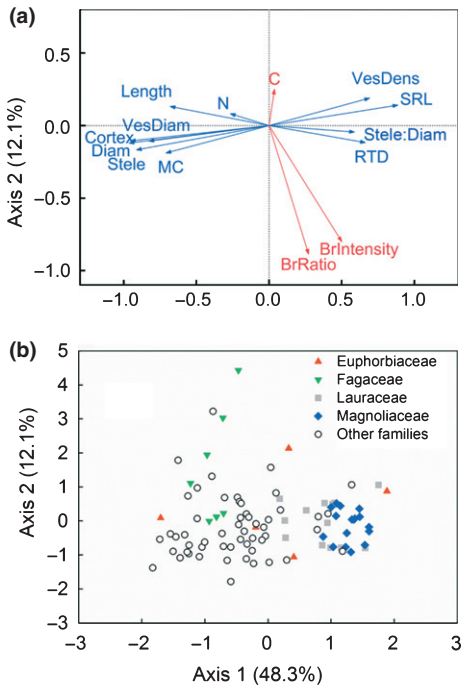

Fig. 4 Principal component analysis (PCA) for root traits in 96 species using original trait data. (a) Trait loadings biplot; (b) species distribution in the two-dimensional trait space. See Table 1 for explanation of abbreviations.

hierarchical structural complexity has hindered our understanding of absorptive root trait variation because it is very difficult to identify which roots are truly absorptive and what unit is functionally identical for cross-species trait comparisons (Guo *et al.*, 2008; Xia *et al.*, 2010). Here we focused on the first-order (on a stream-based ordering system) roots, which are the most distal and metabolically active component of the woody root system (Pregitzer *et al.*, 2002; Xia *et al.*, 2010). The choice of this branch order allows us to better understand cross-species root trait variation. Our results clearly suggest that the 14 root traits we measured showed different degrees of variation and were segregated in two major trait dimensions.

The first dimension was dominated by root diameter-related traits, including root diameter, cortex thickness, stele diameter, vessel diameter, and MC (Fig. 4a), all of which were highly correlated with each other (with all Pearson's correlation coefficients > 0.62; Table 3). The second dimension, largely independent of the first dimension (i.e. forming an orthogonal axis to the first axis; Fig. 4a), was dominated by the root branching ratio and branching intensity, the two parameters that were either weakly correlated (in the case of branching intensity, all correlation coefficients < 0.40 with the exception of the correlation with root length) or not correlated (in the case of branching ratio) to all parameters on the first axis (Table 3). The identification of these two dimensions supported our first two hypotheses. These two largely independent dimensions represent two important aspects of the adaptation of roots to their environment and offer new, fundamental understanding of root functioning in resource foraging and acquisition, and root trait evolution.

The first dimension, or diameter-related dimension, describes the coordinated variation among root diameter, cortex thickness, stele diameter, and MC rate. The degree of coordination among these traits is very high, such that thick roots always have thicker cortex and stele, particularly in angiosperms, which show large variation in diameter (Fig. 2), and higher MC rates (Fig. 3). The tight linkage among root diameter, cortex thickness and stele diameter (Fig. 2) should be particularly useful for predicting how the root cortex and stele would vary with root diameter across other angiosperms species. Moreover, these size-related root traits are among the most variable traits we measured here (all with a CV > 50%; Table 1); they are also phylogenetically conservative (Table 2), showing limited convergence (Fig. S1). Clearly, variation in diameter, the associated anatomical structure, and the degree of mycotrophy is the most significant way in which the first-order roots of different subtropical tree species in China are differentiated.

Variation of root diameter-related traits across species may represent distinct strategies of root construction, maintenance, and persistence. First, species with thicker first-order roots devote more C and nutrients per unit area (or length) to root construction. This type of species (termed 'magnoid type' by Baylis (1975)) may seem less efficient in producing root surface area. However, the loss of surface area resulting from this thick root strategy can be compensated by increased MC (Fig. 3), and consequently increased density of extramatrical hyphae (although this has yet to be verified). Thus, species with thick first-order roots may have no less total surface area per unit root mycorrhizal biomass in comparison with species with thin roots. Also, given the large cortex space conferred by thick roots (Fig. 2), the association between mycorrhizal fungi and thick roots may be particularly strong (Brundrett, 2002), contributing to the relative evolutionary conservatism of both root diameter and MC rate (Table 2), particularly in ancestral families (e.g. close clustering of Magnoliaceae species in Figs 4b, S3).

Secondly, species with magnoid-type roots should have higher [N] as a consequence of their larger proportion of cortex because the majority (> 70%) of root N is in the cortex (D. L. Guo, unpublished; Fig. 2). Indeed, we found a positive yet weak correlation between root diameter and root [N] (Table 3), a finding similar to that of Holdaway *et al.* (2011). Thus, it would be interesting to determine in the future if root respiration also relates positively to root diameter for first-order roots across a large number of species as a result of the relatively close link between [N] and root maintenance respiration (Reich *et al.*, 2008; but see Bouma *et al.*, 2001, in which citrus (*Citrus paradisi* Macf.) had a root diameter twice as great as that of apple (*Malus domestica* Borkh.), yet the two species had similar root respiration rates). It should be noted that MC is high in most species studied here, so that maintenance respiration would have to be quantified for roots and mycorrhizal fungi at the same time. If AM species generally tend to have low hyphal biomass within the root, as suggested by Ouimette *et al.* (2013), then mycorrhizal respiration may mainly originate from extrametrical hyphae. Therefore, we would need a better understanding of both the standing biomass and turnover of extramatrical hyphae or, better, a direct measurement of hyphal respiration. Hyphae can turn over on weekly

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time-scales (Hernandez & Allen, 2013) but roots usually turn over on annual time-scales (Withington *et al.*, 2006; McCormack *et al.*, 2012). The maintenance costs incurred by hyphae may be reduced if plants produce and maintain hyphae only when nutrient and water uptake rates are high. Hyphal turnover of different species should be a profitable avenue of future research in understanding root–fungal trait economics.

Thirdly, compared with the thin-root strategy, the apparent disadvantage of building thick roots with less root surface area per unit biomass may be further compensated by living longer, and by having better chemical defense and thus less tissue loss as a result of herbivory. It is possible that the total return of nutrient and water uptake may be the same relative to root lifespan between thin- and thick-root species. In fact, there may be two distinct strategies of nutrient uptake for species of different root morphologies: the fast strategy, or high uptake rate over a short lifespan (which corresponds to small root diameter), and the slow strategy, or low uptake rate over a long lifespan (which corresponds to a thick root diameter; Eissenstat *et al.*, 2000; Bouma *et al.*, 2001), although other strategies may also exist. If the positive correlation between root diameter and root lifespan found in McCormack *et al.* (2012) is broadly true, then we may finally be able to link root morphology and chemistry with root lifespan in a trait economics framework analogous to the leaf mass per area lifespan relationship reported for leaf economics traits (Wright *et al.*, 2004).

The second dimension, represented by branching intensity and branching ratio, may be critical for nutrient foraging in the soil. Root branching is a key trait determining root plastic responses to nutrient patches. Many studies have shown that roots branch extensively into nutrient-rich patches (so-called morphological plasticity; Drew & Saker, 1975, 1978; Pregitzer *et al.*, 1993; and reviewed by Hodge, 2004). This local proliferation and enhanced nutrient uptake in diverse natural ecosystems may be critical for species competition (Jackson & Caldwell, 1989; Jackson *et al.*, 1990; Robinson *et al.*, 1999). In our study, branching intensity (the number of first-order roots per cm of second-order roots) ranged from 0.44 to 7.37 first-order roots per centimeter of second-order roots, with an overall CV of 67.1% (Table 1). The wide variation in branching intensity across species may be an indicator of large inter-specific and inter-individual differences (although the inter-individual differences were not as strong as the inter-specific differences; Table S2) in the plasticity of the absorptive root system, sensitivity to patchy and pulsed nutrient supply, and competitive capacity. For example, species with a high branching intensity may be capable of rapid proliferation into nutrient and water patches, conferring on them a competitive advantage in relatively nutrient-poor environments.

In addition to branching intensity, we also used branching ratio as a measure of root architecture. Branching ratio is the number of first-order roots per second-order root without considering the length of the second-order roots. This parameter ranged from 1.2 to 10, with an overall variation of 47%, also suggesting quite large variability across species (Table 1). Moreover, in contrast to some degree of correlation between branching intensity and root diameter (which was driven by the correlation of both parameters to root length), branching ratio was unrelated to root diameter in Pearson's correlation with both original data and PIC data (Table 3), suggesting that this trait can vary independently of root diameter-related indices (Fig. 4a).

Both branching intensity and branching ratio had small Blomberg's K values and showed weak phylogenetic conservatism (Table 2), suggesting the strong influence of environmental factors (possibly soil nutrient and water conditions) on root branching. Holdaway *et al.* (2011) found that branching intensity, defined as the number of tips divided by the total length of the first two to three branch orders, was negatively correlated with soil available phosphorus (P) and N across species and sites, suggesting that higher branching intensity may be required at low-fertility sites.

Mechanisms underlying major trait dimensions

The two major dimensions of root traits reported may be explained by several underlying mechanisms. From a biophysical perspective, absorptive roots composed of the first two to three branch orders can only vary in two major ways: the thickness of an individual root segment, and the branching intensity, because any root branch can do only one thing: occupy and divide a soil volume of limited size. As root diameter is strongly related to root length (Table 3 in this study; Chen *et al.*, 2013), thick first-order roots are also longer, and thick-root species should occupy the same area with much less dense roots than thin-root species. Also, the space between individual roots on a root branch may be thoroughly exploited by extramatrical hyphae (Fig. S4).

From an ecological perspective, species differ in growth rates, competitive ability and dominance in natural ecosystems, and variation in root form may contribute to the competitive abilities of different species. In the cold-desert plant community of the Great Basin in the USA, the invader species *Agropyron desertorum*, also a superior competitor in the system, had greater rooting densities, which was mainly attributable to having thinner roots rather than having higher root biomass (Eissenstat & Caldwell, 1988a,b). In addition, the ability to branch out in nutrient patches can be important for competition (Robinson *et al.*, 1999), and there are only a few ways in which root proliferation can be achieved: producing many lateral roots (probably the most likely strategy for species with thin and densely branched roots; Johnson *et al.*, 2008), many root hairs (which also seems to occur mostly in thin-root and less mycotrophic species (Baylis, 1975)), or abundant mycorrhizal hyphae (the most likely strategy for species with thick and sparsely branched roots).

From an evolutionary perspective, a trend of decreasing root diameter and increasing root branching from more primitive species to more modern species has been observed (Baylis, 1975; Fitter, 1991; Comas *et al.*, 2012; Chen *et al.*, 2013). Baylis (1975) reported that modern plants with 'magnolioid' roots, that is, thick, sparsely branched root systems, are associated with the primitive family Magnoliaceae, and this was supported by our data (Fig. 1). Also, more modern families are associated with thin first-order roots (Comas & Eissenstat, 2009; Comas *et al.*, 2012; Chen *et al.*, 2013). This thinning trend in first-order root form appears to coincide with an increasingly drier global environment

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and local habitats since the mid-Cretaceous (Comas *et al.*, 2012; Chen *et al.*, 2013). Thus, selection pressures such as water supply patterns may be instrumental in creating the large differences in root form among species of different lineages and for maintaining these differences in the present environment. Additionally, between the two main clades in the phylogenetic tree, we observed contrasting values for PC1 based on Abouheif's metric, and for traits such as root diameter, stele diameter and cortex thickness (Fig. S1). These contrasting patterns across different phylogenetic clades may provide a basis for inferring how different angiosperm groups altered their functional traits during species divergence and evolution.

Future directions: testing hypotheses related to root trait dimensions

Our studies clearly point to a number of testable hypotheses for the future. First, root lifespan, a critical but difficult-to-measure root trait, may be hypothesized to be positively related to root diameter. As already discussed, compared with building thin roots, building thick roots of the same length would carry higher construction costs, leading to a lower nutrient uptake rate per unit time per unit biomass, and thus a longer root lifespan may be necessary for a net gain of nutrients equal to that of thin roots. One caveat in testing this hypothesis is that the lifespan–root diameter relationship may be mediated by other plant traits such as plant growth rate and wood (tissue) density (McCormack *et al.*, 2012).

Secondly, hypotheses linking root diameter with strategies of nutrient uptake can be tested so that the functional significance of root trait variation can be better understood. For example, slow and fast strategies were found to be associated with a long and a short root lifespan, respectively, and root lifespan was negatively associated with root diameter (Eissenstat *et al.*, 2000). Determining whether these patterns are general is a high-priority goal for future research. Moreover, how mycorrhizal fungi are involved in the lifespan–root diameter relationship deserves attention in light of our observation that the MC rate increased with root diameter and then leveled off at a root diameter of *c.* 470 μm (Fig. 3). Does this suggest that we may assume a constant level of colonization for species with coarser roots (e.g. first-order root diameter > 470 μm) and that the root lifespan of these species is less plastic because of the lack of substantial changes in mycorrhizal colonization rate, thus a lack of mycorrhizal influence on root lifespan.

Last but not least, a better understanding of the root–mycorrhizal association needs to be achieved. The present finding that thick roots had higher colonization rates confirms earlier work on British flora (Peat & Fitter, 1993), suggesting that this is a common pattern. Yet we still lack mechanistic understanding and elucidation of the functional significance of this pattern. For example, does a high MC rate in thick roots serve mainly nutrient uptake functions, or alternatively other key functions such as defense? It has been shown that the root MC rate may be negatively correlated to extramatrical hyphae production (fig. 2a,b in Maherali & Klironomos, 2007). In addition, Resendes *et al.* (2008) found that mycorrhizal colonization and nonmycorrhizal fungal colonization were mutually exclusive in the first 25 d of root life, suggesting that mycorrhizal fungi may be an important factor preventing nonmycorrhizal fungi from colonizing roots. The relationship between root form and fungal identity/abundance may be an area of great importance for both theoretical and practical endeavors, as recognized by Newsham *et al.* (1995).

As a consequence of the lack of knowledge of the broad-scale patterns of root trait variation, we still lack consensus on which root traits to choose in a root trait study. Our results and those of previous root trait studies (e.g. Pregitzer *et al.*, 2002; Tjoelker *et al.*, 2005; Roumet *et al.*, 2006; Withington *et al.*, 2006; Holdaway *et al.*, 2011) suggest that root morphology, anatomy, and chemistry are the basic parameters in any root trait study. These traits have several features: they are relatively easy to measure, having clear functional significance at the individual root and whole-plant levels, and have been linked to ecosystem-scale belowground processes, such as root production, mortality and decomposition, and to aboveground traits. Our study also suggests that consideration of anatomy may be essential for a better understanding of the linkage between root form and function. In future studies, root lifespan and nutrient uptake rates are urgently needed for broad comparisons and more comprehensive understanding of root functional traits.

Conclusions

By measuring 14 root traits on 96 species of diverse phylogeny, we found two leading dimensions of trait variation: a diameter-related dimension that may integrate root construction, and possibly maintenance and persistence, with MC, and a branching density dimension that may express differences in root plastic responses to environment. Knowledge of these two readily measured dimensions offers a promising path for understanding root trait economics and root ecological strategies.

The patterns and arguments presented here are only a peek into the tremendous diversity of root traits and strategies. Progress in recent decades supports a view that roots are complex structures and play a multifaceted role in plant functioning and ecosystem processes. Roots are at the same time structures of nutrient acquisition (Pregitzer *et al.*, 2002) and active resource foraging (Kembel & Cahill, 2005), hosts and organizers of mutualistic and nonmutualistic microbial communities (Brundrett, 2002), circuit breakers of the plant hydraulic system (Hacke & Sauter, 1996; Johnson *et al.*, 2012), stations of signaling and below–aboveground communication (Bais *et al.*, 2006; Parniske, 2008), and 'weapons' against competitors (Dybzinski *et al.*, 2011), to name just a few. Extraordinary structural diversity and plasticity in root form and function are needed to achieve the complex role that absorptive roots play, and our study represents a small but promising step toward a full understanding of this highly intriguing and critical plant organ.

Acknowledgements

We thank Shenglei Fu, Zhongliang Huang, Yuhong Liu, Xiaobao Deng and Mingxian Lin for their assistance in field

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sampling, and Fusheng Chen and Zhengquan Wang for assistance with root anatomy. We also thank the following field research stations and government agencies for their kind support: Dinghushan and Heshan Station for Subtropical Forest Studies of South China Botany Garden, Chinese Academy of Sciences, Xianhu Botanic Park of Shenzhen, Ailaoshan Station for Subtropical Forest Studies and Xishuangbanna Station for Tropical Forest Studies of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, and Jianfengling Nature Reserve Management Bureau. We also thank Luke McCormack for his valuable comments. This study was funded by the One-Hundred Talent Project of the Chinese Academy of Sciences (no. KZZD-EW-TZ-11), the National Basic Research Program of China (no. 2010CB950602), and the Natural Science Foundation of China (NSFC grant no. 31325006).

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Analysis of traits of first-order roots in the phylogenetic framework.

Fig. S2 The relationships between root diameter and cortex thickness and stele diameter with different mycorrhizal types.

Fig. S3 Piece-wise regression of mycorrhizal colonization rate predicted by root diameter of first-order roots across 96 tree species of different families (break-point = 470.4 μm; $P < 0.001$).

Fig. S4 Pictures showing absorptive roots and endophytic fungal hyphae. (a) *Cinnamomum camphora*; (b) *Michelia chapensis.*

Table S1 Characteristics of the six sampling sites

Table S2 Root traits of the 96 woody species in this study and their sampling sites (mean ± SE)

Table S3 Primer sequences used in this study

Table S4 Accession numbers for 96 subtropical woody species in South China

Table S5 Fossils information, prior distribution of fossil calibration nodes and the estimated divergence time

Table S6 The loading scores of traits of the first-order roots in the first two PCA axes

Methods S1 Construction of plant phylogeny.

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