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Seedling root anatomy and morphology: an examination of ecological differentiation with rainfall using phylogenetically independent contrasts

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Abstract We examined patterns of seedling root architecture, morphology and anatomy in Australian perennial plants chosen as phylogenetically independent contrasts (PICs) for rainfall in the areas they inhabit. Our objective was to assess whether there are consistent evolutionary patterns in structure of seedling root systems in species from different rainfall environments when examined across multiple evolutionary lineages. Seedlings were grown to a standardised developmental stage under controlled conditions. We found that seedling root systems of species restricted to low rainfall environments are characterised by greater proportional allocation to main root axis and have proportionally smaller main root axis diameter and areas of stele and xylem. Species of low rainfall environments also had higher specific root length (SRL) of the main axis, but lower SRL when the entire root system was considered. Seedling root system elongation rates were higher in species of high rainfall relative to those of low rainfall environments, paralleling expected differences in relative growth rate. The higher root system elongation rates in species of high rainfall environments were associated with greater numbers of growing tips in the root system, but not with differences in elongation rates of individual tips, relative to species of low rainfall environments.

Keywords Root architecture · Specific root length · Xylem · Root elongation · Root development

Introduction

The evolution of root system traits has not received much attention from comparative biologists. Studies that have examined root systems tend not to include explicit phylogenetic structure (Bauhus and Messier 1999; Eissenstat et al. 2000; Fahey and Hughes 1994; Fitter et al. 1991b; Fitter and Stickland 1991; Holmes and Rice 1996; Huante et al. 1992; Reich et al. 1998a, 1998b; Ryser 1996; Ryser and Lambers 1995; Taub and Goldberg 1996). Research to date demonstrates that characteristics of plant species' root systems reflect environmental conditions typical of a species' native environment and that these characteristics vary with developmental stage and growth conditions. Of course, root system characteristics are also likely to reflect the history of different evolutionary lineages.

Root systems may differ in: (1) architecture or topology, the spatial configuration and branching pattern respectively, which determine soil exploration capacity; (2) diameter, density and length relationships; and (3) anatomical traits such as allocation to vasculature and patterns of cell division and elongation – characteristics that determine ability to transport water and nutrients. The present study was designed to examine how seedling root architecture, morphology and anatomy vary among species from environments which differ in rainfall, and to determine whether these traits are correlated. We applied a phylogenetically independent contrast (PIC) design (Burt 1989) so that we could examine generality in patterns of seedling root characteristics in species of different rainfall environments across evolutionary lineages.

Architecture

Researchers have hypothesised that architectural traits of root systems of species from dry habitats should differ from those found in wetter habitats with regard to number and length of branches. Fitter and colleagues hypo-

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thesise that root architecture should be more herringbone-like (ribs coming off a spine) in dry or nutrient poor conditions and more dichotomously branched under better conditions (Fitter 1985; Fitter et al. 1991a, 1991b; Fitter and Stickland 1991; Taub and Goldberg 1996). Herringbone systems are also predicted to be associated with longer individual root branches (Fitter et al. 1991a, 1991b).

Diameter, density and length

Across species, there is a shift towards lower potential relative growth rates (RGR) in habitats not permitting fast potential RGR (Cornelissen et al. 1996; Garnier 1992; Garnier and Laurant 1994; Hunt and Cornelissen 1997; Poorter and Remkes 1990; Saverimuttu and Westoby 1996). Species with slow potential RGR typically have low specific leaf area ($\text{mm}^2 \text{mg}^{-1}$). The analogous belowground trait, specific root length (SRL, mm mg^{-1}), expresses the amount of root length achieved per unit root mass invested, and thus is an indicator of the absorptive surface produced per volume of root (Boot 1989; Huante et al. 1992; Lambers and Poorter 1992; Reich et al. 1998a, 1998b; Wright and Westoby 1999). Using a phylogenetically structured design, Wright and Westoby (1999) found that species typical of low rainfall environments had both lower SRL and lower potential RGR than species typical of higher rainfall environments. The decreases in SRL resulted from larger root diameter, most likely reflecting anatomical modifications to enhance ability to conduct water in arid conditions, or ability to penetrate dry soil.

Anatomy and development

Larger xylem vessels conduct water with less resistance than small ones (Poiseuille-Hagen law, conducting power of a vessel is proportional to the fourth power of the radius of the vessel, e.g. Castro-Diez et al. 1998; McCully 1995). Thus, species from environments where water is available only episodically might have larger xylem vessels, larger diameter roots, and lower SRL to maximise water uptake when water is available. However, large vessels may also be more prone to cavitation and embolism during freezing and water stress such that xeric species may achieve resistance to cavitation at the cost of hydraulic efficiency (Pockman and Sperry 2000). Thus, it is unclear whether the increased capacity for water conductance that can be gained from large xylem vessels outweighs the risk of decreased functionality from cavitation (Pockman and Sperry 2000). Across a wide range of species there is a weak but significant positive correlation between vulnerability to embolism and xylem vessel diameter (but see Alder et al. 1996; Tyree et al. 1994). Research to date indicates that low potential RGR species of arid zones may have smaller vessels in roots than species of mesic zones (e.g. Castro-Diez et al. 1998;

Huang and Eissenstat 2000; Shumway et al. 1993; Zimmermann 1978).

Relationships between architecture, growth and anatomy determine elongation and development of the entire root system. Elongation of the root system depends upon the number of meristems in the root system and the elongation rate of each of these meristems. The resulting patterns of elongation in seedling root systems are likely to determine colonisation and establishment ability of the species.

The present study was designed to address three specific questions:

1. Do architectural, morphological and anatomical characteristics differ in a consistent manner when species from environments with relatively high rainfall are compared to congeners from environments with lower rainfall?
2. Do the differences indicate consistent correlated divergences between variables across lineages?
3. Do observed shifts and correlated divergences support previous results regarding relationships among growth rates and SRL such that we can understand the source of differences in root system production rates?

Materials and methods

Species selection

Species were selected as PICs within genera contrasted by rainfall. High rainfall species came from coastal areas in New South Wales, Australia (1,000–1,250 mm rainfall year⁻¹). Low rainfall species were from the arid zone in western New South Wales (200–400 mm rainfall year⁻¹); latitude and elevation were similar. All species were perennial, and no ephemerals or biennials were included. Beyond these restrictions, no control was made for soil type or growth form. Potential species were selected according to the above distribution requirements, using Jacobs and Pickard (1981) and Harden (1990). PICs were then chosen depending on availability of field-collected seed from native seed supply companies and on germinability, resulting in a total of 11 PICs (Table 1). Sometimes more than one representative species from each rainfall level was included in each PIC, for a total of at least 16 species at each rainfall level.

Growth

In the laboratory, seeds were germinated in Petri dishes on moist filter paper. To stimulate germination some species were soaked in dilute bleach solution (2.5%) or boiling water, or their seed coats were nicked. The day following radicle emergence each seedling was planted into a free-draining plastic tree tube (5×5×12.5 cm) containing pasteurised river sand (2.5 h at 70°C) topped with 1 cm vermiculite. The smallest-seeded genera (*Callistemon* and *Melaleuca*) were germinated on sterilised fine sand and planted into free-draining 3×6-cm round tree tubes when the root was approximately 3 mm long. Plants were flushed with Aquasol solution (containing trace elements and 0.322 mM NH₄⁺, 3.786 mM NO₃⁻, 0.322 mM P, 1.150 mM K) every second day and surface watered on intervening days.

Plants were grown in a growth chamber with 12-h days (~450 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The day-time temperature was set at 24°C and the night-time temperature at 19°C. Pot locations within the chamber were rearranged every second day.

Table 1 Species included in study, organised by phylogenetically independent contrasts

Family	Genus	Species	Rainfall
Cesalpiniaceae	<i>Senna</i>	<i>S. aciphylla</i> ^a	High
		<i>S. artemisioides</i>	Low
		<i>S. pleurocarpa</i> ^a	Low
Fabaceae	<i>Bossiaea</i>	<i>B. heterophylla</i> ^a	High
		<i>B. scolopendria</i>	High
		<i>B. walkeri</i> ^a	Low
	<i>Pultenaea</i>	<i>P. blakelyi</i>	High
		<i>P. daphnoides</i> ^a	High
		<i>P. microphylla</i> ^a	Low
Mimosaceae	<i>Acacia</i>	<i>A. binervata</i> ^a	High
		<i>A. cognata</i>	High
		<i>A. aneura</i>	Low
		<i>A. brachybotrya</i> ^a	Low
Myrtaceae	<i>Eucalyptus</i>	<i>E. amplifolia</i> ^a	High
		<i>E. largiflorens</i> ^a	Low
	<i>Callistemon</i>	<i>C. salignus</i>	High
		<i>C. sieberi</i> ^a	High
		<i>C. brachyandrus</i> ^a	Low
	<i>Corymbia</i>	<i>C. eximia</i> ^a	High
		<i>C. gummifera</i>	High
		<i>C. terminalis</i> ^a	Low
	<i>Melaleuca</i>	<i>M. alternifolia</i>	High
		<i>M. hypericifolia</i> ^a	High
		<i>M. lanceolata</i> ^a	Low
		<i>M. uncinata</i>	Low
Chenopodiaceae	<i>Atriplex</i>	<i>A. cinerea</i> ^a	High
		<i>A. lindleyi</i> ^a	Low
		<i>A. nummularia</i>	Low
Proteaceae	<i>Hakea</i>	<i>H. gibbosa</i>	High
		<i>H. sericea</i> ^a	High
		<i>H. eyreana</i> ^a	Low
Sapindaceae	<i>Dodonaea</i>	<i>D. viscosa</i> ^a	High
		<i>D. lobulata</i> ^a	Low
		<i>D. peduncularis</i>	Low

^a Species used for anatomical measurements

Every effort was made to harvest plants at a standardised ontogenetic or developmental stage (e.g. Castro-Diez et al. 1998), in this case ~120 mm of main axis length (~55 mm for small-seeded species), marginally shorter than the depth of the growing tubes. Harvesting at a standard developmental stage across species was considered better than harvesting plants at a particular age but different developmental conditions. Though the plants may have been at very different developmental stages with regard to root system branching or aboveground form, they would at least have been exploring an equivalent depth of soil at this stage in their development. Any plant whose root tip was damaged or deformed or grew out of the pot was excluded. Because plants had to be harvested before they touched the bottom of the pot, there was a small amount of variation in final main root axis length. None of the species exhibited determinate growth of the main axis, nor did any of them develop cluster roots or nodules during the course of the study.

At harvest, roots were rinsed from the sand and fixed for 24 h in 3:1 ethanol:glacial acetic acid solution. Samples were then moved through an ethanol dehydration series, spending 24 h each in 30% and 50% ethanol and finally being stored in 70% ethanol.

Root architecture

The "root system" refers to the entire belowground portion of the plant. The magnitude of a root system is the number of external branches or number of root tips in the root system, hereafter called number of tips. The altitude of the root system is the longest single path (largest number of branches passed) through the root system (Werner and Smart 1973). A topological index of branching pattern can be calculated from altitude divided by expected altitude [$\alpha/E(\alpha)$], where the expected altitude is calculated on the assumption of random patterns of root branching given magnitude (Berntson 1995; Fitter 1985; Fitter et al. 1991b; Fitter and Stickland 1991). A value of 1 indicates a random branching structure, <1 indicates a largely dichotomous system and values >1 a herringbone root system.

Entire root systems of five plants per species were stained in Nile blue to increase contrast and scanned with a digital scanner. The computer program BranChing (Berntson 1992) was used to determine root length, number of root tips, altitude, and branching index of each root system for all study species. Following architectural analysis, scanned root systems were examined and plants with obvious developmental deformities excluded. Specifically, we checked whether root systems whose main axis was <85 mm (50 mm for small-seeded genera) were developmentally comparable to root systems of conspecifics (similar number of secondary roots, similar overall length of root system). Variation in main axis length arose because plants were harvested before the main axis reached the bottom of the pot. Of the shorter root systems, 18 were excluded from further analysis resulting in an average of four and minimum of three root systems per species.

Root anatomy and morphology

One high and one low rainfall species from each PIC was randomly selected for anatomical studies (Table 1). For all microscopy, tissues were embedded in standard tissue freezing medium, sectioned on a freezing microtome, and stained with Nile blue. Cross-sections were taken 5 cm above the root tip on the main root from each of five root systems of each species. On each section, root diameter, cross-sectional area, stele area, total xylem area, total number of xylem vessels, and the number and diameter of all xylem vessels >15 μ m were measured (see also, Castro-Diez et al. 1998; Wan et al. 1994). At the time of harvest there was no cambial activity in any of the root systems. All had both protoxylem and metaxylem vessels, at least some of which were fully open.

Sections were examined under a light microscope, and images saved to computer. All anatomical measurements were made on a PC using the public domain software Scion Image (available on the Internet at <http://www.scioncorp.com>) or on a Macintosh using NIH Image (available at <http://rsb.info.nih.gov/nih-image>).

SRL was determined by sub-sampling from the main axis and secondary branches of the root system. Five root systems were subsampled from each of the species used in the anatomical measurements. Between 50 mm and 80 mm each of main axis and secondary branch were sampled, dried at 80°C to constant mass, and weighed to the nearest 0.001 mg. SRLs for the main axis and secondary branches were determined separately from the subsamples; these data were used to estimate SRL for the entire root system, based on length of the main axis and secondary branches.

Root system elongation rate was calculated by dividing total root system length by the number of days post germination at harvest. Elongation rate of the main axis was calculated using the final length of the main axis only and the number of days post germination.

Analysis

Data for architectural and anatomical traits were analysed using a mixed model ANOVA in which genus and rainfall were main ef-

fects, with genus a random factor. When there was more than one species within a genus-rainfall combination, species were nested within genus and rainfall (full dataset of species' means available from the authors on request). Main effects of rainfall and genus were examined to determine whether there was a general pattern in the relationship of a trait to rainfall habitat of the species across lineages and whether lineages differed from one another. As main root axis length was the developmental character on which harvest time was determined, small variations in length could strongly influence results. Thus, where significant, length of the main root axis was included as a covariate in the analysis. Many of the architectural parameters are sensitive to the scale or stage of development within the root system; magnitude was included as a covariate in the analysis of architectural parameters. Variables were log transformed to meet the assumptions of normality as needed.

To examine correlated divergence from common ancestors we plotted correlated divergence graphs. We subtracted the mean value of a given trait in the high rainfall species from that of the low rainfall species in each PIC and plotted the resulting divergence values. This enables us to ask what the impact of a radiation or shift in habitat from high to lower rainfall is on a given set of traits, and whether the direction of divergence in these traits is consistent. In the plots, points that fall in the upper right quadrant indicate a positive shift in both attributes, moving from species maintaining natural populations in high rainfall to species occurring in low rainfall. Points that fall in the lower left quadrant indicate a decrease in both attributes associated with the shift from high to low rainfall. If points fall in both upper right and lower left quadrants, the data indicate that there is a positively correlated shift in the two traits, but that the direction of the shift with rainfall is not consistent. Points falling in the upper left and lower right quadrants indicate negatively correlated shifts between traits. These graphs can be tested against a zero-correlation null hypothesis by regression forced through the origin. Direction and size of shift both affect the likelihood of significance. Correlations across genus (tip correlations) were examined for all traits in which correlated change was considered. Such correlations provide insight into patterns at older evolutionary divergences. Tip correlations among species were not included because species were selected as contrasts rather than in a random manner.

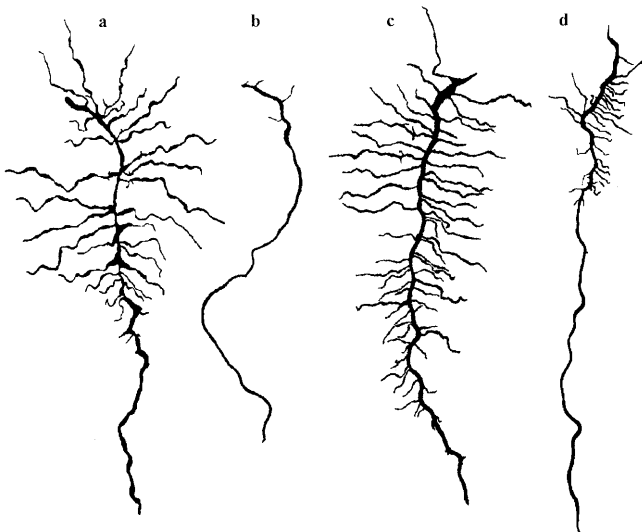


Fig. 1a-d Scanned images of root systems, all at same scale. **a** *Acacia cognata*, high rainfall; **b** *A. brachybotrya*, low rainfall; **c** *Hakea eyreana*, high rainfall; **d** *H. sericea*, low rainfall

Results

Single traits in relation to rainfall habitat of species

Architecture

In the majority of PICs, low rainfall species produced less total root length over the course of the study than did high rainfall species, even though harvests were standardised to a similar main axis length (Fig. 1, Fig. 2a). Because there were more secondary branches in the high rainfall species, the main axis represented a larger proportion of the total root system in species of low rainfall environments (Fig. 2b). When root elongation rate of the entire root system on a daily basis is compared, low rainfall species had lower rates of elongation than did high

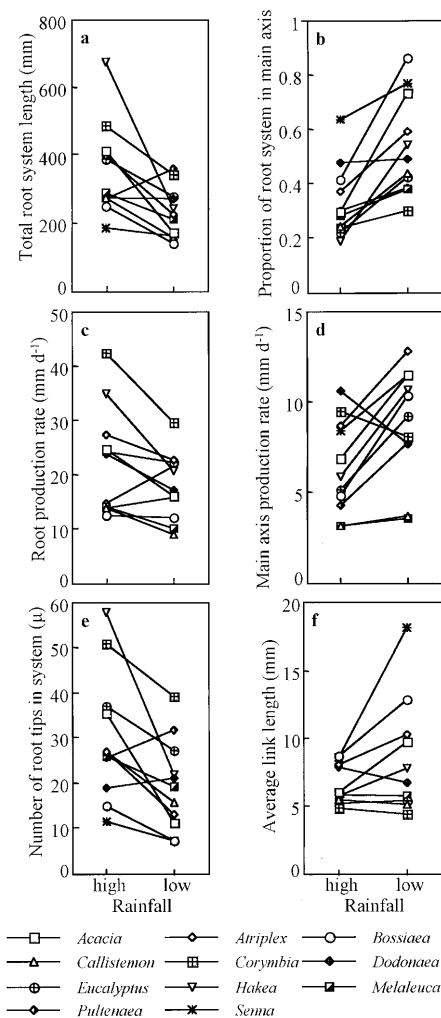


Fig. 2a-f Divergences in root architectural traits among phylogenetically independent contrasts (PICs) for rainfall. *Points joined by a line* represent separate generic contrasts. Values are averages for replicates of one or two species (see also Table 1). **a** Total root system length, **b** proportion of root system that is main axis, **c** root production rate per day (*d*) for whole root system, **d** rate of root production per day for main axis only, **e** number of root tips in the root system (magnitude, μ), and **f** average link length

Table 2 ANOVA for lineage (random) and rainfall (fixed) effects. *a/E(a)* Altitude divided by expected altitude, *n.a.* not applicable

Variable	Rainfall (<i>df</i> =1)		Lineage (<i>df</i> =10)		Species (<i>df</i> =22)		Main axis length (Covariate, <i>df</i> =1)		Error	
	MS	<i>P</i>	MS	<i>P</i>	MS	<i>P</i>	MS	<i>P</i>	<i>df</i>	MS
Main axis length (mm)	13		4,619	***	615	***	n.a.	n.a.	117	254
Total root system length (log, mm)	0.024		0.182	**	0.049	***	1.093	***	116	0.013
Root production rate (log, mm day ⁻¹)	0.090	**	0.231	***	0.027	**	0.879	***	116	0.012
Main axis production rate (log, mm day ⁻¹)	0.021	*	0.093	**	0.026	***	0.347	***	116	0.003
Proportion of root system in main axis (log)	0.025		0.198	**	0.049	***	0.119	**	116	0.013
Number of root tips (log)	0.257	***	0.648	***	0.114	***	1.126	***	116	0.021
Average link length (log, mm)	0.052	*	0.051		0.025	***	0.431	***	116	0.008
Altitude (log)	0.006		0.010		0.009	***	2.638	***	116	0.003
<i>a/E(a)</i>	0.012		0.278	*	0.104	***	5.435	***	116	0.036

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

rainfall species (Fig. 2c, Table 2); however, these same low rainfall species elongated the main root axis at a significantly higher rate (Fig. 2d, Table 2).

The lower root lengths and root system elongation rates in species from low rainfall habitats could reflect either fewer branches in the root system, or shorter individual branches. Low rainfall species usually had fewer branches, therefore fewer root tips (Fig. 2e, Table 2), but in the majority of PICs average link length was also greater at low rainfall (Fig. 2f, Table 2). The lower number of root tips in the root systems of the low rainfall species was reflected in lower altitude than for high rainfall species (Table 2). There was no consistent effect of rainfall on branching index (Table 2), though all species had indices >1 , indicating herringbone topology.

Morphology and anatomy

Based on results of previous studies, we expected the SRL of seedlings of low rainfall species to be lower than that of high rainfall species. We found a trend toward lower SRL (estimated for the entire root system) in low rainfall species (Fig. 3a, Table 3), but because three PICs did not follow this trend, (especially the fine-rooted *Callistemon*) the rainfall effect was only marginally significant ($P=0.06$). Even though root systems of species from low rainfall environments had lower SRL averaged over the entire root system, SRL of both the main axis and of secondary branches was on average higher in low rainfall members than high rainfall members of the PICs (Fig. 3b, c). This pattern resulted in a significant main effect of rainfall for main axis and secondary branch SRL (Table 3). Thus, the lower SRL at the full root system level was a reflection of the greater proportion of the root system that was the main axis in low rainfall species, together with a markedly higher SRL of secondary branches relative to the main axis.

Patterns in SRL are determined by both the diameter and tissue density of roots. Low rainfall species had smaller diameters and cross-sectional areas than did high

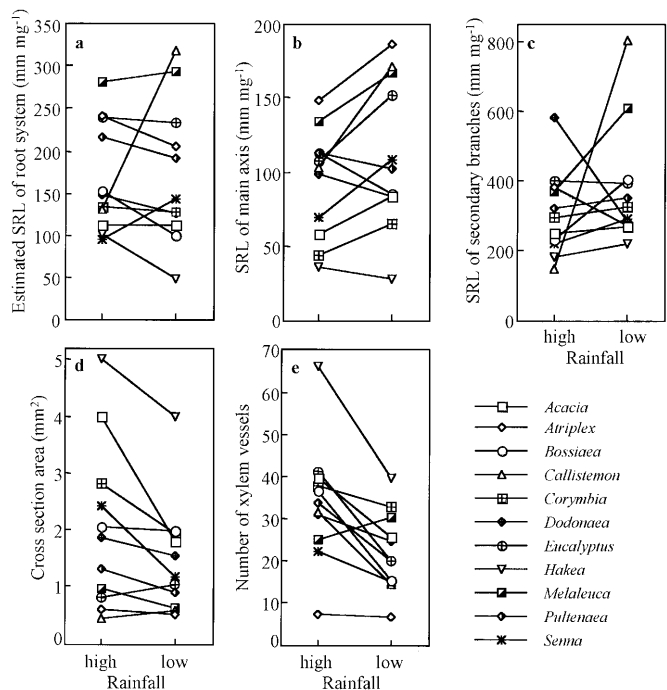


Fig. 3a–e Divergences in specific root length (SRL) and anatomical traits among phylogenetically independent contrasts for rainfall. **a** Estimated SRL for entire root system, **b** SRL measured for main axis only, **c** SRL measured for secondary branches of root system only, **d** cross-sectional area 5 cm behind root apical meristem, **e** number of xylem vessels at 5 cm behind root apical meristem

rainfall species (Fig. 3d, Table 3), which was reflected in lower stele area and lower total xylem area (but not a significant rainfall effect, Table 3). The ratios of stele to cortex area, stele to xylem area and xylem to cross-sectional area showed no consistent pattern across PICs, with roughly half of the PICs showing a decrease and half showing an increase between low and high rainfall members of each PIC (data not shown). These results suggest an overall reduction in root structures in species of low rainfall habitats, rather than different proportionality among components of the root cross-section.

Table 3 ANOVA for lineage (random) and rainfall (fixed) effects. SRL Specific root length

Variable	Lineage (<i>df</i> =10)		Rainfall (<i>df</i> =1)		Error	
	MS	<i>P</i>	MS	<i>P</i>	<i>df</i>	MS
SRL, main axis (mm mg ⁻¹)	16,552	***	10,097	***	95	828
SRL, secondary roots (log, mm mg ⁻¹)	0.129	*	0.374	*	83	0.062
SRL, estimated for entire root system (log, mm mg ⁻¹)	0.319	***	0.000		83	0.026
Cross-section area (log, μm ²)	0.780	***	0.394	***	85	0.032
Stele area (log, μm ²)	0.655	***	0.603	***	87	0.025
Xylem area (log, μm ²)	0.771	***	0.729	***	86	0.031
Number of xylem vessels (log)	0.396	***	0.781	***	88	0.029
Number of large xylem vessels+1 (log)	0.566	***	0.029		93	0.054
Potential flow through large xylem vessels (log)	1.297	***	0.084		78	0.133

P*≤0.05, *P*≤0.01, ****P*≤0.001

In addition to a decrease in xylem area, low rainfall species had fewer xylem vessels than high rainfall species (Fig. 3e, Table 3). However, flow through the xylem is limited not by number, but by the relative size of xylem vessels. When the area of the largest xylem vessel was compared for high and low rainfall species there was no consistent rainfall effect. Likewise, when total area was calculated for all large xylem vessels (>15 μm) there was no consistent pattern across PICs. Accordingly, when potential flow was estimated (sum of the radii to the fourth power) for the large xylem vessels, there was no consistent rainfall effect (Table 3).

Correlated divergences among variables

Architectural traits

We found consistent evidence of correlated divergences among traits. The low rainfall species had simpler root systems, showing significant correlated decreases between root length and number of tips in the root system (note the congregation of eight of the 11 points in the lower left-hand quadrant, Fig. 4a). A similar pattern was found between root length and altitude (Table 4). There was, however, no clear pattern of correlated divergence between total root length and average link length (Table 4), confirming that the difference in root length between high and low rainfall members of the PICs was associated with increased numbers rather than increased lengths of links in the root system. There was significant correlated divergence between the altitude and magnitude of the root system, as both traits were lower in low rainfall than high rainfall species (Fig. 4b). All tip correlations among architectural traits were significant at the genus level (Table 4).

Morphological and anatomical traits

Decreases in cross-sectional area were associated with decreases in the area of stele (and xylem) in eight of the

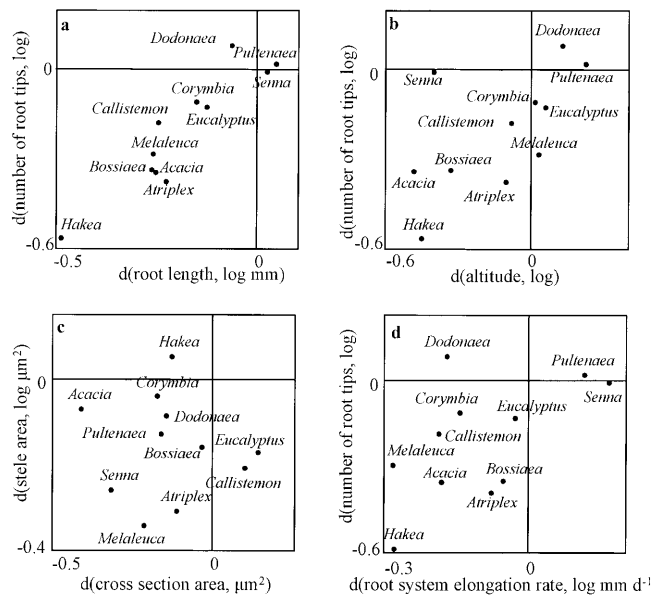


Fig. 4 Correlated divergences between **a** total root length and number of root tips in system (magnitude), **b** altitude of the root system and number of root tips, **c** cross-sectional area 5 cm behind root tip and stele area, and **d** elongation rate of the entire root system and number of root tips in root system. Changes are associated with the shift from high to low rainfall environments. Points are labelled with genus names for each PIC. Plots present differences in log-transformed values between low and high rainfall members of each PIC; see text for further explanation

11 PICs (Fig. 4c); these majority patterns did not result in significant regression statistics. Decreases in stele and xylem area were correlated, as were each of these traits with vessel number (Table 4). Differences in anatomical traits might be expected to correlate with changes in size of individual vessels; however, because size of the largest vessel and total area of all large vessels did not shift consistently with rainfall there were not strong patterns of correlated divergence. All combinations of anatomical traits exhibited significant positive tip correlations among genera (Table 4).

Table 4 Above the diagonals (*in italics*), coefficients for tip correlations at the genus level^a. Below the diagonals, significance values for correlated change analysis^b

	Root length (mm)	Magnitude	Altitude	Average link length (mm)	Cross-section area (µm ²)	No. vessels	Stele area (µm ²)	Xylem area (µm ²)
Root length (mm)		<i>0.913</i>	<i>0.935</i>	<i>-0.654</i>				
Magnitude	***		<i>0.994</i>	<i>-0.905</i>				
Altitude	**	***		<i>-0.871</i>				
Average link length (mm)	n.s.	n.s.	**					
Cross-section area (µm ²)						0.723	0.952	0.861
No. vessels					n.s.		0.779	0.931
Stele area (µm ²)					†	*		0.936
Xylem area (µm ²)					n.s.	***	**	
Largest vessel area (µm ²)					*	n.s.	n.s.	n.s.
Area large vessels (µm ²)					n.s.	n.s.	†	†
SRL, main (mg mm ⁻¹)					n.s.	n.s.	*	n.s.
SRL, secondary (mg mm ⁻¹)								
SRL, total (mg mm ⁻¹)	n.s.	n.s.	n.s.					
Axis elongation rate (mm day ⁻¹)	*							
System elongation rate (mm day ⁻¹)	***	**		n.s.				
	Largest vessel area (µm ²)	Area large vessels (µm ²)	SRL main (mm mg ⁻¹)	SRL total (mm mg ⁻¹)	SRL secondary (mm mg ⁻¹)	Axis elongation rate (mm day ⁻¹)	System elongation rate (mm day ⁻¹)	
Root length (mm)				-0.382		0.15	0.826	
Magnitude				-0.108			0.612	
Altitude				-0.17				
Average link length (mm)								-0.273
Cross-section area (µm ²)	0.857	0.852	-0.917					
No. vessels	0.558	0.658	-0.769					
Stele area (µm ²)	0.818	0.875	-0.963					
Xylem area (µm ²)	0.71	0.803	-0.897					
Largest vessel area (µm ²)		0.916						
Area large vessels (µm ²)	n.s.							
SRL, main (mm mg ⁻¹)					0.856			
SRL, secondary (mm mg ⁻¹)			†					
SRL, total (mm mg ⁻¹)								
Axis elongation rate (mm day ⁻¹)							0.649	
System elongation rate (mm day ⁻¹)						n.s.		

^a Above diagonal, $df=10$ for each comparison, $P \leq 0.05$ if $R > 0.576$, $P \leq 0.01$ if $R > 0.708$

^b Below diagonal, † $P \leq 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P \leq 0.001$, n.s. not significant; $n=11$ for all tests. All variables log transformed

prior to analysis. Empty cells represent comparisons between variables that were not considered

Determinants of SRL and elongation rates

SRL and elongation rates can be viewed as emergent traits, variation in which may be explained by interactive effects of anatomical, morphological and architectural traits. SRL of the main axis may be influenced by cross-sectional area, number of xylem vessels, and diameter of the root apical meristem; however, despite strong tip correlations (Table 4) and consistent rainfall effects, evidence for correlated change was weak. Only stele area showed significant correlated divergence with SRL of the main axis (Table 4). Thus, while the increase in SRL of the main axis with a shift from high to low rainfall was associated with a decrease in root diameter and associated traits, the correlation was more apparent when compared across genera than when compared within PICs.

The SRL of the entire root system or of the secondary branches may also be influenced by architectural traits of the root system. There were not, however, significant tip correlations between SRL of the entire root system and architectural traits, nor were there good indications of correlated divergence. There was a trend toward correlated change between SRL of the secondary branches and magnitude (seven of 11 PICs showed negative correlation, $P=0.28$, Table 4), suggesting that at the system level, higher SRL would be associated with increases in the number of growing root tips, and thus increases in the number of thinner secondary (tertiary etc.) branches.

At the whole root system level, we expected correlations between root system elongation rate and both the number of root tips (magnitude) and the elongation rate of these tips (each tip is a meristem). There was strong

evidence of correlated decreases in root system elongation rate and magnitude between high and low rainfall species (eight of 11 PICs, Fig. 4d). This correlation was also apparent as a tip correlation across genera (Table 4). High rainfall species had greater root system level elongation rates, but lower main axis elongation rates, resulting in a negative relationship between these traits (but not a significant regression statistic, Table 4). Thus within PICs, the faster system level elongation in high-rainfall species was driven by more branching (more tips), despite a slower elongation of the main axis, and possibly of each secondary branch as well. Across genera, there was a positive correlation between elongation rate of the main axis and elongation rate of the entire root system (Table 4).

Discussion

Our results demonstrated a suite of seedling root characters that show consistent and correlated shifts when comparing high and low rainfall species across a range of evolutionary lineages. When assessed with regard to SRL and elongation rates of the root system, the data support the results of previous studies, but add considerable detail to our understanding of root system development, function and evolution. At the whole root system level, species from low rainfall environments had lower SRL and lower root system elongation rates, but these patterns were driven by the proportion of thicker main to thinner lateral roots, rather than by shifts in diameters or anatomy of each type.

Species of low rainfall environments had lower SRL at the level of the entire root system primarily because they had fewer high-SRL secondary branches. SRL of the main axis never exceeded 200 mm mg⁻¹, whereas SRL of secondary branches ranged from 200 to 800 mm mg⁻¹. The influence of the main axis on the system average SRL was therefore very strong. The pattern observed in root system level SRL supports a wide body of theoretical and empirical work: the seedlings of species typical of low resource habitats have lower potential RGR and lower SRL (see references in Introduction). But, at the level of the individual axis, our results provide evidence that despite their slower growth rates, species of low rainfall environments produce thin, high SRL initial roots (most likely less dense and less expensive to produce per unit length) to maximise depth of soil explored. The pattern of SRL in the main axis in particular has obvious implications for water transport capacity and efficiency.

The number and size of the vascular elements, and the number and size of vascular junctions, influence both the potential for water flow and the efficiency with which it moves within the plant (Castro-Diez et al. 1998; Shumway et al. 1993; Wan et al. 1994; Zimmermann 1978). Having large xylem vessels decreases resistance to water flow and thus increases the flow potential; however, large vessels may be more prone to cavitation than

smaller vessels (Pockman and Sperry 2000; Tyree et al. 1994). Our results showed that total xylem area and vessel number was lower in the main root axis of low rainfall species than high rainfall species, in accordance with an overall downsizing of the main root axis in the low rainfall species. Despite these differences, there were not significant differences in cross-sectional area of the largest xylem vessel or in the total area comprised by large xylem vessels (>15 µm). This result may indicate that in seedlings of low rainfall species total xylem vessel number is decreased, but that the largest vessels are maintained, thereby minimising the impact of decreased root diameter on water flow potential. Low diameter, high SRL roots may maintain higher conductivity for water passage because there is less resistance to radial flow in these roots than in denser or thicker roots (Huang and Eissenstat 2000). Though we found that SRL of the main axis was greater in low rainfall species than high rainfall congeners, we found no consistent shifts in stele to cortex ratios with rainfall. Identifying such shifts, which may be more apparent in older root systems, could be critical to understanding function in arid versus mesic zone species.

Species of low rainfall environments also had lower system level root elongation rates. As species of low resource environments tend to have lower RGR, it follows that they would also have lower root production rates – at least on a mass basis. Our results suggest that they have lower production rates on a length basis as well. When the factors contributing to elongation rate are examined singly, it is the number of tips (magnitude) in the root system that shows correlated directional shift with elongation rate. Notably, the elongation rate of individual tips, estimated in our data as rate of elongation of the main root axis, did not correlate well with the system level elongation rate.

The major determinants of system level seedling root elongation rate are architectural or topological characteristics. As the number of root tips increased, so did the elongation rate of the system. The increase in magnitude was associated with a decrease in average link length. Notably, we found no consistent change in branching index with rainfall. All root systems were generally more herringbone than dichotomous in topology, but this may be expected in seedlings, and topological indices also may shift with developmental age (Glimskar 2000).

Evolutionary trends and tip correlations

The results of the present study demonstrate substantial differences between characteristics of seedling root systems in their first weeks of development, under benign conditions. Seedlings are likely to germinate under benign conditions (e.g. after rain or fire), but arid zone species in particular are likely to be exposed to harsh conditions relatively soon after germination. Thus, initial characteristics are likely to be critical to ultimate establishment and subsequent survival. Growth under

standardised benign conditions does not allow investigation of plasticity in these seedling root traits. However, to the extent that root systems respond plastically to growth conditions, the plastic response in low rainfall species, grown under benign conditions, should have shifted them towards greater similarity to high rainfall species. Consequently, comparisons under benign conditions should give a minimal or conservative measurement of the evolutionary differences between species that sustain populations in lower versus higher rainfall habitats.

Across a wide range of evolutionary lineages, species from low rainfall habitats produced seedling root systems that increased rooting depth at the expense of branches, root diameter, and vascular development, relative to species from higher rainfall habitats. Indications are that this tradeoff is made with minimal impact on potential flow through the xylem. Further, this pattern is counter-intuitive given lower elongation rates and lower SRL on a whole root system level. The vast majority of traits that showed significant correlated change across PICs also demonstrated significant tip correlations at the genus level, though the opposite was not always true. Thus, to a large extent trait divergences within genera along the rainfall gradient were also apparent between older evolutionary divergences, irrespective of the rainfall gradient.

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