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Suites of root traits differ between annual and perennial species growing in the field

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Summary

- Here, we tested whether root traits associated with resource acquisition and conservation differed between life histories (annuals, perennials) and families (Fabaceae, Asteraceae and Poaceae).
- Root topology, morphology, chemistry and mycorrhizal colonization were measured on whole root systems of 18 field-grown herbaceous species grown and harvested in central Argentina.
- Annuals differed from perennials in several root traits important in resource uptake and conservation. They exhibited higher specific root length (SRL), root nitrogen concentration (RNC) and mycorrhizal colonization but had lower root tissue density (RTD) than perennials. They did not differ in topology or construction cost. These differences were consistent among families. Families differed only in a few root traits known to be strongly associated with certain lineages such as topology and nitrogen concentration. There was a strong parallel between root traits and analogous leaf traits described in the literature for annuals and perennials.
- Our results suggest the existence at the root level of an acquisitive vs conservative syndrome consistent among families similar to that previously reported for above-ground traits.

Key words: annuals, Argentina, mycorrhizas, perennials, plant functional traits, root morphology, root topology, taxonomic families.

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Introduction

A growing body of theoretical (Grime, 1977; Chapin, 1980; Westoby, 1998) and empirical work (Grime *et al.*, 1997; Reich *et al.*, 1997; Díaz *et al.*, 2004; Wright *et al.*, 2004) points to the existence of a fundamental trade-off between rapid acquisition of resources and conservation of resources in plant species. Accordingly, annual species and fast-growing species in general should maximize resource acquisition, whereas perennial and slow-growing species should maximize resource conservation (Grime, 1977; Chapin, 1980; Poorter & Garnier, 1999; Aerts & Chapin, 2000). Leaf studies comparing annuals and perennials grown either in the laboratory or in the field largely support this theory: annual species are characterized by a set of leaf traits enabling high carbon acquisition (high specific leaf area and nitrogen concentration) while perennials are characterized by leaf traits associated with persistence and defence (high tissue density and thickness) (Garnier, 1992; Garnier & Vancaeyzeele, 1994; Garnier *et al.*, 1997).

If root systems are subjected to the same trade-off as leaves, parallel leaf and root trait patterns are expected, with root traits maximizing below-ground resource acquisition in annuals, and resource conservation and root persistence in perennials. The available information on roots for annuals and perennials is extremely limited. However, there is some evidence indicating that suites of correlated root traits are linked to plant growth strategies. Generally high specific root length (SRL) and high root nitrogen concentration (RNC) are found in fast-growing species; these traits are associated with high respiration rate of fine roots, likely reflecting metabolic activity related with

Table 1 List of root traits measured and their presumed or demonstrated functional significance

Traits	Abbreviations (units)	Functional significance	Key references
Topological index[a]	–	Soil exploration and exploitation efficiency (+) Water transport (–)	Fitter (1985)
Proportion of root length with diameter < 0.2 mm	Fine roots (% root length)	Rate of nutrient uptake (+) Root longevity (–)	Eissenstat (1992); Ryser (1998)
Specific root length	SRL (m g^{-1})	RGR (+) Root respiration (+) Rate of nutrient and water uptake (+) Root elongation rate (+) Root turnover (+)	Eissenstat (1992); Ryser (1998)
Root tissue density	(RTD) (g cm^{-3})	Longevity (+) Resistance to herbivores, drought (+) Root decomposition rate (–)	Eissenstat & Yanai (1997); Ryser (1998)
Root diameter (mm)		Rate of nutrient uptake (–) Longevity (+) Storage (+) Transport of water (+) Penetration force in soil (+)	Eissenstat (1992); Ryser (1998)
Root nitrogen concentration	RNC (%)	Root respiration (+) Root growth (+) Root longevity (–) Root decomposition rate (+)	Reich *et al*. (1998); Tjoelker *et al*. (2005)
Root construction cost	RCC (g glucose g^{-1})	Carbon invested in roots (+)	Poorter (1994)
Mycorrhizal colonization	(% root length colonized)	Nutrient uptake (+) Protection against pathogens (+)	Brundrett (1991); Newsham *et al*. (1995)

[a]High topological index indicates a herringbone branching system; low value a dichotomous, highly branched root system.
(+) and (–) indicate a positive or negative relationship between trait and function, respectively.

nutritional uptake and assimilation (Tjoelker *et al.*, 2005). Conversely, high root tissue density (RTD) and large root diameter favour root longevity, a syndrome usually found in slow-growing species (Ryser, 1996; Ryser, 1998; Craine *et al.*, 2001; Lavorel *et al.*, 2006) (Table 1). Other traits, such as root topology and symbiotic associations, which are often not taken into account in root trait studies, might differ between annual and perennials species. For example, Fitter's model predicted that annual species will tend to have more dichotomous (highly branched) root system topology than perennials because these root systems are cheaper to construct but less efficient in terms of the acquisition of mobile resources than a herringbone root system (Fitter & Stickland, 1991). In addition, dichotomous root systems as well as roots with high SRL usually have low level of mycorrhizal colonization or derive little benefit from mycorrhizas (Brundrett, 1991; Newsham *et al.*, 1995).

Comparative root trait studies have mostly involved seedlings grown in the laboratory under high-nutrient conditions (Fitter *et al.*, 1988; Gross *et al.*, 1992; Ryser, 1996, 1998) or adult plants grown in common experimental gardens (Craine *et al.*, 2001; Tjoelker *et al.*, 2005). Although these works are valuable in order to document genetically based trait differences among species, it is well known that root traits depend on ontogeny and on the soil chemical and physical conditions encountered by the plants when growing in their natural habitat (Robinson & Rorison, 1988; Jackson & Caldwell, 1989; Fitter, 1994; Hodge, 2004). For woody species, the patterns in morphological traits (SRL and root diameter) between fast- and slow-growing species were the same for seedlings and field-grown plants of the same species, while this was not the case for root physiological traits (phosphorus uptake and root respiration) (Comas *et al.*, 2002; Comas & Eissenstat, 2004). For herbaceous species, whether conclusions drawn from laboratory seedlings or experimental garden plants are likely to be valid for field-grown plants remains to be tested. The purpose of the present study was to examine whether root traits related to topology, morphology, chemistry and symbiotic associations, differ between annual and perennial herbaceous species growing in the field.

We chose annual and perennial species belonging to contrasting plant families (Asteraceae, Fabaceae and Poaceae) in order to assess the consistency across major families of the possible root traits associations detected for each life history. Fabaceae, Poaceae and nonlegume forbs (usually, although not exclusively Asteraceae) are often considered as distinct 'functional types' (e.g. Hooper & Vitousek, 1997; Hector *et al.*, 1999; Tilman *et al.*, 2001). However, recent studies based on above-ground traits show no clear evidence to support the classification of Asteraceae, Fabaceae and Poaceae into different a priori resource-use functional types (Craine *et al.*, 1999; Díaz *et al.*, 2004). At the root level, these families are known to differ. Poaceae have an adventitious, dense root system consisting of many fine, fibrous axes (Čiamporová *et al.*, 1998) with low nitrogen (N) concentration compared with forbs (Tjoelker *et al.*, 2005). By contrast, dicotyledons are characterized by a taproot system providing these species the ability to penetrate deeply into the soil (Lauenroth & Gill, 2003). Fabaceae, because of their symbiotic association with N-fixing bacteria, show higher RNC (Del Pozo *et al.*, 2000; Tjoelker *et al.*, 2005), reduced tissue longevity and higher root respiration rate (Tjoelker *et al.*, 2005). We therefore considered whether consistent root traits associations related to the resource acquisition–conservation trade-off could still be detected within contrasting families.

Our objectives were to: (1) test whether root traits differed between annual and perennial species and among three major plant families – Asteraceae, Fabaceae and Poaceae; (2) identify root traits that best express differences in resource-use strategies among species; and (3) find out whether those root trait syndromes were consistent with those reported in the literature for leaf traits. Eight root traits related to topology, morphology, chemical composition and mycorrhizal colonization were examined for 18 field-grown species from central Argentina. These traits were selected because of their putative or demonstrated relationship with resources acquisition and conservation (Table 1). The first hypothesis tested was that annual root systems had traits known to be more efficient for resource acquisition because they depend more on rapid exploitation of resources than on efficiency in their use; by contrast, perennial root systems should show more conservative traits in terms of resource uptake, use and retention. It was thus hypothesized that annuals have a more dichotomous architecture, higher SRL, lower RTD, higher RNC and lower mycorrhizal colonization than perennials. The second hypothesis was that, as previously found for above-ground traits, root traits should differ more between life histories than among plant families in terms of the resource acquisition-conservation trade-off.

Materials and Methods

Species selection and collection

Eighteen common herbaceous species from central Argentina were studied. The species were selected in order to include species with contrasting life histories (annuals and perennials) and belonging to three major and widespread plant families (Asteraceae, Fabaceae and Poaceae). Three species within each life history/family combination were chosen (Table 2). The plants were collected in natural and seminatural areas of the Córdoba mountains (*c.* 30–31 S, 64°30′–64°35′ W; altitude 880–2000 m). The study area is characterized by dry and relatively cold winters with most rainfall heavily concentrated in the warm season. Annual rainfall and mean temperature ranges from 720 to 840 mm and from 11 to 8.2°C at the lowest and highest sites, respectively. Soil characteristics from highest to lowest sites, respectively, averaged 2–10% C, 0.10–0.60% total N and pH 5.3–7. Phytogeographically, the vegetation belongs to the Mountain Chaco District (Luti *et al.*, 1979) with mountain grassland dominating in highest sites and secondary mountain shrubland in lowest sites.

For each species, at least 16 well-developed individuals grown in unshaded areas (i.e. not under tree cover) and in relative isolated position, were carefully dug up with a pickaxe to a depth of 30 cm at the beginning of the flowering season. Atypically large or small individuals were avoided. Root systems were placed into containers filled with deionized water. They were separated from the shoots and cleaned using a fine stream of water in order to remove soil and organic matter particles. Unattached roots which could have been broken during sampling and processing or which could came from another individual or species were removed. Among the whole set of root systems harvested, only 16 apparently undamaged root systems were conserved for further measurements. Roots and nodules of Fabaceae were carefully separated. For each species, four individual root systems were stored in ethanol 50% (v : v) at 4°C for later architectural and morphological analyses, four other replicate root systems were oven-dried at 60°C for 72 h for later chemical analyses and the remaining eight root systems of all species were used to assess mycorrhizal colonization.

Root topology and morphology

Roots were stained with methylene blue (5 g l^{-1}) in order to increase contrast during scanning. They were rinsed with water to remove adhering stain not incorporated by the roots. Root systems of each species were poured over a mesh panel (21 × 29.7 cm) submerged in water and spread out with the help of tweezers to minimize overlap. Root systems were not scanned in water to prevent reflection and parallax errors; they were transfer from water to an acetate sheet. The mesh panel with the spread root system was carefully lifted out of the water, placed onto a filter paper, and covered by a transparent acetate sheet and a plastic cover. The whole batch (the mesh panel, roots, acetate sheet and plastic cover) was then turned upside down. Following this, the roots were removed from the mesh panel and transferred onto the acetate sheet by wiping carefully the mesh. The acetate sheet with the spread root system was immediately scanned on a flatbed scanner at a resolution of 600 dpi (Acer Scan 300F, 6684 03A). The software DELTA-T SCAN (Delta-T Devices, Burwell, Cambridge, UK.) was used to determine total root length, volume, diameter, root diameter distribution and the number of tips. Modal diameter, the diameter associated with the greatest amount of root length in each root system, was used rather than average

Table 2 Root traits of 18 annual and perennial herbaceous species growing in the field in central Argentina

Species	Family	Topological Index [a/E(a)]		Fine roots < 0.2 mm) (% root length)		SRL (m g^{-1})		RTD (g cm^{-3})		Modal Diameter (mm)		RNC (%)		RCC (g glucose g^{-1})		Mycorrhizal colonization (% root length)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Annual species																	
Bidens pilosa L.	Asteraceae	1.29	0.17	26.89	4.12	59.83	4.00	0.091	0.010	0.24	0.02	0.85	0.07	1.13	0.06	0.63	0.05
Bromus catharticus Vahl	Poaceae	1.76	0.18	27.24	2.30	49.70	3.13	0.113	0.014	0.22	0.02	0.49	0.05	1.03	0.05	0.71	0.07
Medicago lupulina L.[a]	Fabaceae	1.07	0.09	9.98	1.70	45.32	3.70	0.047	0.004	0.37	0.086	2.41	0.12	1.21	0.01	0.74	0.04
Melilotus albus Desr.[a]	Fabaceae	0.53	0.08	10.16	0.97	28.29	3.38	0.058	0.006	0.43	0.12	1.69	0.61	1.12	0.39	0.31	0.04
Muhlenbergia peruviana (P Beauv.) Steud.	Poaceae	1.17	0.05	30.73	6.06	197.79	8.29	0.044	0.008	0.30	0.07	1.09		1.14	0.38	0.31	0.06
Tagetes minuta L.	Asteraceae	1.17	0.14	11.01	1.59	44.84	2.83	0.081	0.011	0.30	0.03	1.03	0.10	1.17	0.04	0.71	0.09
Vicia graminea Sm.	Fabaceae	1.01	0.08	5.49	0.45	11.00	1.22	0.070	0.007	0.62	0.12	2.89	0.11	1.19	0.03	0.36	0.05
Vulpia myuros (L.) Gmel.	Poaceae	1.13	0.15	45.38	5.02	268.91	21.83	0.044	0.003	0.19	0.01	0.75	0.04	1.00	0.04	0.36	0.05
Zinnia peruviana (L.) L.	Asteraceae	1.39	0.12	32.14	3.32	43.98	8.49	0.136	0.009	0.24	0.02	0.83	0.07	1.22	0.01	0.39	0.05
Perennial species																	
Adesmia bicolor (Poir.) DC	Fabaceae	0.86	0.04	21.83	2.84	10.09	2.15	0.104	0.015	0.27	0.02	2.24	0.11	1.06	0.08	0.15	0.02
Eustachys retusa (Lag.) Kunth	Poaceae	2.85	0.36	30.25	3.94	29.25	0.60	0.159	0.016	0.18	0.02	0.38	0.05	1.36	0.04	0.48	0.04
Hypochaeris argentina Cabrera	Asteraceae	0.49	0.08	33.09	4.82	11.60	1.08	0.142	0.025	0.22	0.02	0.37	0.03	1.05	0.05	0.47	0.07
Noticastrum marginatum (Kunth) Cuatrec.	Asteraceae	1.52	0.22	8.65	0.92	11.85	1.32	0.157	0.007	0.38	0.08	0.76	0.04	1.38	0.11	0.36	0.05
Paspalum dilatatum Poir.	Poaceae	1.64	0.13	5.26	0.91	18.37	1.58	0.102	0.004	0.59	0.06	0.62	0.09	0.93	0.09	0.61	0.06
Stipa eriostachya H.B.K.	Poaceae	1.74	0.30	17.25	3.69	19.56	2.60	0.168	0.020	0.37	0.10	0.37	0.05	1.24	0.10	0.20	0.01
Stylosanthes guianensis (Aubl.) Sw.	Fabaceae	0.75	0.12	18.54	1.97	3.94	0.46	0.196	0.052	0.25	0.01	1.15	0.15	1.25	0.05	0.13	0.04
Taraxacum officinale Weber ex F.H. Wigg.	Asteraceae	1.12	0.10	18.75	3.01	3.87	0.36	0.129	0.025	0.67	0.09	0.58	0.10	1.09	0.01	0.24	0.06
Trifolium repens L.	Fabaceae	1.61	0.18	11.69	1.93	90.10	25.86	0.041	0.007	0.33	0.07	2.47	0.18	1.18	0.04	0.55	0.07

SRL, specific root length; RTD, root tissue density; modal diameter, the size of the diameter that occurs most; RNC, root nitrogen concentration; RCC, root construction cost. Nomenclature follows Zuloaga *et al*. (1994) and Zuloaga & Morrone (1999).

[a] Can behave as annual or biennial. High topological index indicates a herringbone branching system, low value a dichotomous branching system.

root diameter because root diameter within samples had a non-normal left-skewed distribution. After scanning, each root system was oven-dried at 60°C for 72 h and weighed.

The topology of the root system was analysed using Fitter's (1985) terminology. The magnitude (μ) is the number of external links (i.e. root tips in the root system). The altitude (a) is the number of links in the longest unique path from an external link to the base link. [E(a)] is the expected altitude and was calculated from the magnitude (μ) of each species, on the assumption of random patterns of root branching. The algorithm for E(a) estimation was taken from Werner & Smart (1973). We described root topology using the ratio of altitude divided by expected altitude [a/E(a)]. This topological index was suggested by Fitter (1985) since it enables comparisons of branching patterns of different sizes. It indicates the extent to which branching deviates from a random growth model. A value of 1 indicates a random branching structure, < 1 indicates a largely dichotomous system and values > 1 a herringbone root system structure where branching is largely confined to a main axis. For Asteraceae and Fabaceae, a and μ were measured on the whole root system; for those of Poaceae, which are composed of multiple equivalent lateral root clusters, a and μ were measured on the longest axis.

The SRL was calculated as the ratio of total root length to root dry mass, RTD as the ratio of root dry mass to root volume and the percentage of fine roots as the ratio of root length with a diameter of less than 0.2 mm to the total root length.

Chemical analyses

Total root carbon (C) and N concentrations were determined on ground material using an elemental analyser (EA 1108; Carlo Erba Instruments, Milan, Italy). Four replicates per species, consisting of four denodulated whole root systems, were used, with the exception of very small species (*Mulhenbergia peruviana*) for which two root systems were pooled together to obtain two replicates.

Root construction cost (RCC), expressed in g glucose g^{-1}, was evaluated only on 13 species because we could not collect enough material from *Bromus catharticus*, *Muhlenbergia peruviana*, *Trifolium repens*, *Vicia graminea* or *Vulpia myuros*. The number of replicates ranged from one to four according to the material available. The RCC was determined by the method proposed by Vertregt & Penning de Vries (1987) and modified by Poorter (1994):

$$\mathrm{RCC} = (-1041 + 5.077 \times \mathrm{C}/(1000 - \mathrm{Min})] \times [(1000 - \mathrm{Min})/1000] + [5.325 \times \mathrm{N_{org}}/1000]$$

(C, Min and N_{org} are the concentrations of carbon, minerals and organic nitrogen, respectively, expressed in mg g^{-1}). We assumed that $N_{org} = N$, because root nitrate concentration has been found to be negligible in legumes (Gebauer *et al.*, 1988); in nonlegume species data on root nitrate concentration are scarce, but leaf nitrate concentration is negligible when leaf N is below 29 mg g^{-1} (Garnier & Freijsen, 1994). Since in this study root N was extremely low (< 10 mg g^{-1}), we assumed that nitrate concentration was insignificant. The mineral concentration was calculated as:

$$\mathrm{Min} = \mathrm{Ash} - \mathrm{AA} \times 30$$

(Ash is the ash content, in mg g^{-1}, and AA × 30 is the ash alkalinity, in mEq g^{-1}, multiplied by the mass of carbonate (g^{-1})). Ash content was determined after combustion of each sample in a muffle furnace at 550°C for 6 h. Ash alkalinity was determined acidimetrically (Poorter & Villar, 1997). A good correlation was found between construction costs and root carbon concentration ($r = 0.87$, $P < 0.001$). Therefore, in order to have a complete data set, we used the predicted RCC for all species as a surrogate for construction costs.

Mycorrhizal colonization

All thin roots (diameter < 2 mm, without apparent suberin) were cleared and stained (Grace & Stribley, 1991). They were then mounted on semi-permanent slides in polyvinyl–lactic acid–glycerol (PVGL) (Omar *et al.*, 1979). All the mycorrhizal structures belonged to the arbuscular mycorrhizal (AM) type. The root endophyte quantification was made according to the magnified intersection method (McGonigle & Fitter, 1990), using a compound microscope at ×150. The number of mycorrhizal structures (hyphae, vesicules and arbuscules) was scored for 100 intersections per individual.

Statistical analyses

The results were analysed by ANOVA using the general linear model (GLM) procedure. Data that did not meet the assumption of normal distribution were log- or square root-transformed. The model included two main factors: family and life history (annual/perennial), the interaction family × life history and species nested within family × life history, in order to account for differences among species in each combination of family and life history. The effects of family, life history and the family × life history interaction were tested against the mean square of species nested within family × life history. The effect of species nested within family × life history was tested against the mean square of residual. The log-transformed root biomass was included as a covariate in the analysis, when significant (i.e. for SRL) to account for the possible size-related effects. Differences among families and life histories were tested using the least significant difference (LSD) test. Statistical analyses were performed with STATGRAPHICS PLUS (Manugistics, Rockville, MA, USA). Differences at $P < 0.05$ were considered clearly significant whereas differences between $P = 0.10$ and $P > 0.05$ were considered as marginally significant to allow for large variation typical of field data.

Results

The topological index [a/E(a)] did not show any significant difference between annual and perennial species (Table 2, Fig. 1a). However, it differed marginally among families ($P = 0.056$). It was higher for Poaceae (especially perennial ones), indicating a more herringbone topology (sparsely branched) compared with Fabaceae and Asteraceae, which showed a more dichotomous root topology (highly branched) (Fig. 1a). The proportion of root length with diameter less than 0.2 mm, ranged from 5% (*Paspalum dilatatum*) to 45% (*Vulpia myuros*) (Table 2, Fig. 1b). Overall, it did not show clear differences between annuals and perennials and between families. However, as indicated by the marginally significant life history × family interaction, the proportion of fine roots of Fabaceae was lower in annuals compared with perennials, while the reverse was observed for Poaceae.

The SRL showed large variation among species, ranging from 3.9 (*Taraxacum officinalis*) to 269 m g^{-1} (*Vulpia myuros*) (Table 2, Fig. 2a). On average, annuals showed significantly ($P < 0.01$) higher values than perennials, especially for Asteraceae and Poaceae. There was no significant SRL difference among families. Annuals showed significantly lower RTD than perennials and there was no family effect on this trait (Fig. 2b). A marginally significant life history × family interaction was found for the modal root diameter; root diameter of Asteraceae and Poaceae was higher in perennials than in annuals, while the reverse was observed in Fabaceae (Fig. 2c).

On average, annual species had 34% higher RNC than perennials (Fig. 2d). As expected, being N-fixers, members of the Fabaceae had a RNC about three times higher than the other two families. There was no significant difference in RCC between life histories or families (Fig. 2e). Mycorrhizal colonization was significantly higher for annual species than for perennials while no difference between families was observed (Fig. 2f).

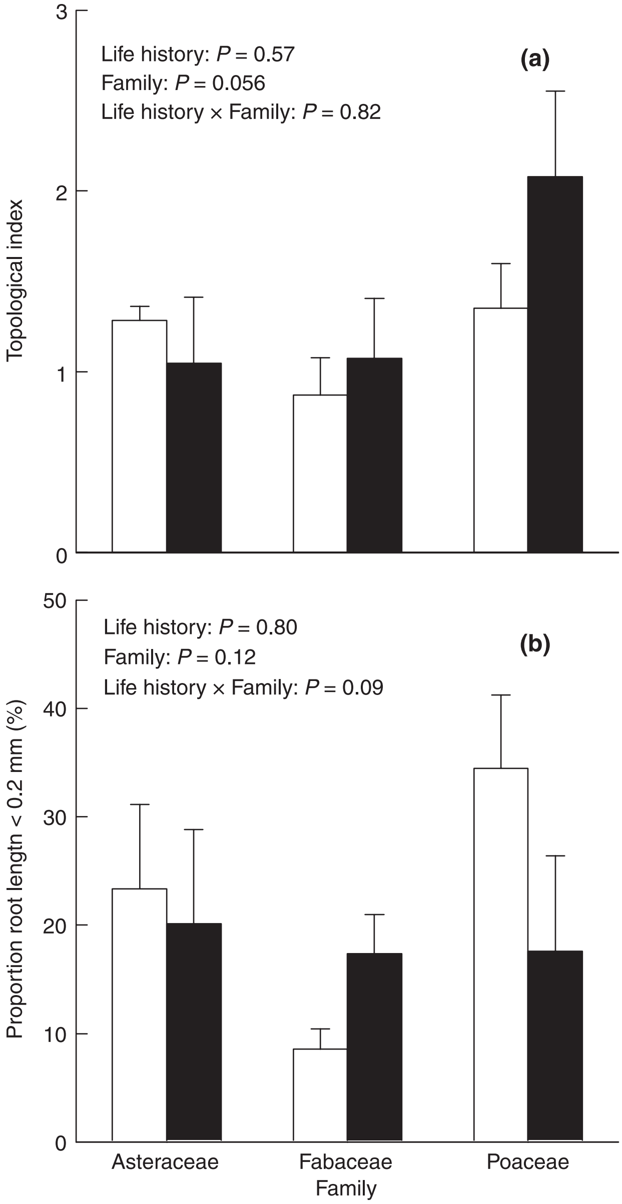

Fig. 1 (a) Topological index calculated as altitude divided by expected altitude and (b) proportion of fine root (diameter < 0.2 mm) of annual (open bars) and perennial (closed bars) species belonging to three plant families: Asteraceae, Fabaceae and Poaceae. High topological index indicates a herringbone branching system; low value a dichotomous branching system. Bars are means ± SE ($n = 3$ species for each combination of life history and family and $n = 4$ individuals per species). The *P*-values of two way ANOVA are given on panels for life history and family effects and their interaction.

Discussion

Root traits and life history

We found that annual and perennial species growing in the field differed significantly in terms of several root traits. Perennial species, compared with annuals, had lower SRL, higher RTD, lower RNC and mycorrhizal colonization and coarser root systems, except Fabaceae. These results suggest that perennials have traits that demonstrate a more conservative strategy than annuals, since higher RTD, larger diameter and lower RNC have been associated with longer-lived roots (for RTD, Ryser, 1996, 1998; for root diameter Eissenstat, 2000; Gill *et al.*, 2002; for RNC Weaver, 1958; Craine *et al.*, 2002; Tjoelker *et al.*, 2005). By contrast, annuals roots were characterized by a high SRL, which is usually associated with rapid rates of root elongation (Eissenstat, 1992), high relative growth rate (Reich *et al.*, 1998; Comas *et al.*, 2002; Comas & Eissenstat, 2004), and high nutrient and water uptake capacities (Graham & Syvertsen, 1985; Reich *et al.*, 1998). This is consistent with the higher RNC found in annuals, which has been shown to reflect high nutrient uptake capacity (Pregitzer *et al.*, 1998) and high metabolic activities (Tjoelker *et al.*, 2005). Our results

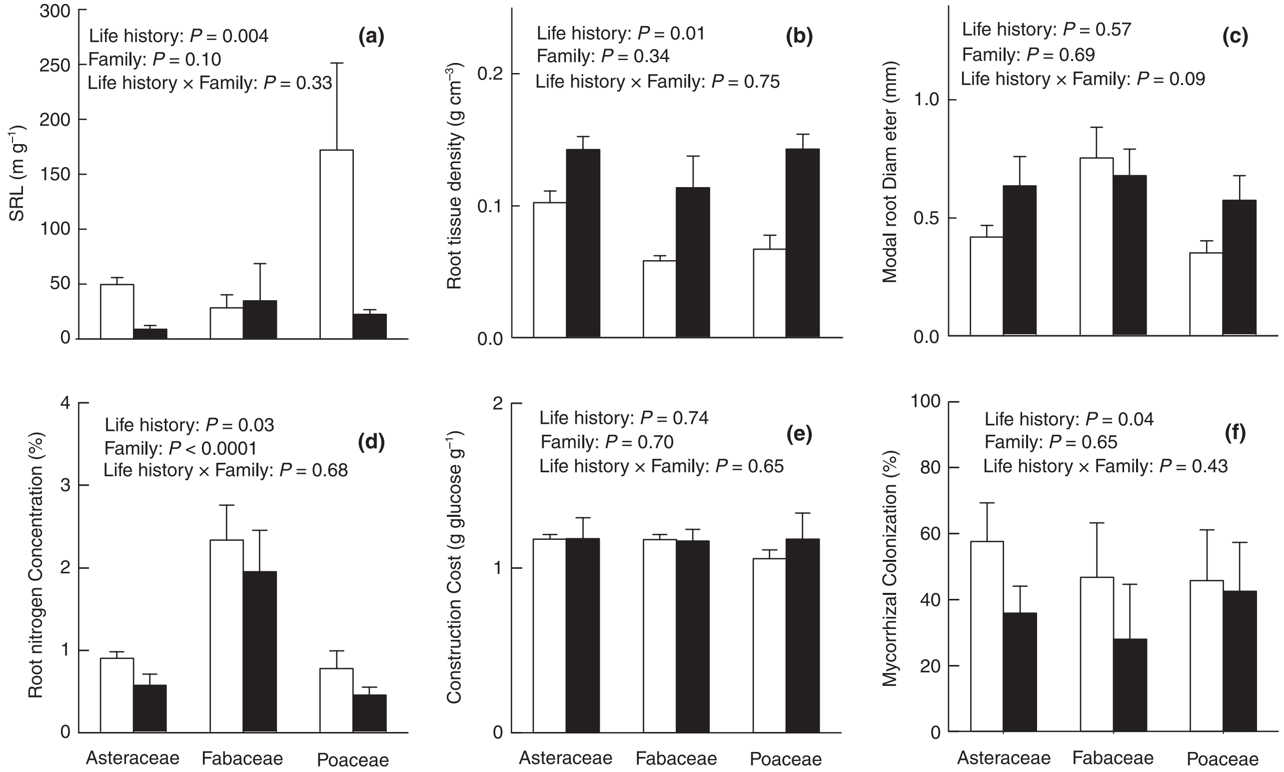

Fig. 2 (a) Specific root length (SRL), (b) root tissue density, (c) modal root diameter, (d) root nitrogen concentration, (e) root construction cost and (f) percentage of mycorrhizal colonization of annual (open bars) and perennial (closed bars) species belonging to three plant families: Asteraceae, Fabaceae and Poaceae. Bars are means ± SE (n = 3 species for each combination of life history and family and n = 4 individuals per species). The P-values of two way ANOVA are given on panels for life history and family effects and their interaction.

for field-grown species confirm those reported in a common experimental garden study, where annuals had higher SRL, lower root tissue density and lower root diameter than perennials (Craine *et al.*, 2001). For these three morphological traits, differences between life histories observed in controlled garden conditions are thus maintained in the field, suggesting that they are robust to genotypic and phenotypic plasticity. This should be tested more rigorously by comparing root traits measured for the same species both in the laboratory, common garden and in the field, as previously done for leaf traits (Garnier & Freijsen, 1994; Poorter & Garnier, 1999; Cornelissen *et al.*, 2003). Mycorrhizal colonization was higher in annuals compared with perennials; this result was unexpected since species with fine root systems and high SRL are supposed to be less dependent on mycorrhizal associations than coarse root systems (Brundrett, 1991; Hetrick *et al.*, 1991; Eissenstat, 1992; Newsham *et al.*, 1995). Nonetheless, mycorrrhizal colonization data should be interpreted with caution. First, mycorrhizal colonization is root age dependent; the greater colonization observed in annuals might thus reflect the fact that these species start developing earlier in the season than perennials. Second, the percentage of arbuscular mycorrhizal colonization does not always correlate with mycorrhizal benefit to the plant and/or extraradical mycelium in soil (Smith & Read, 1997). Whether the observed higher mycorrhizal colonization of annuals is consistent over the whole life cycle and whether it represents a benefit for plants in terms of higher resource acquisition requires further testing.

The root trait differences found between annuals and perennials were consistent among the three major families analysed, suggesting a general trade-off of adaptive value, which might reflect different strategies to grow and survive in contrasted habitats. In annuals, which generally occurred in disturbed habitats, high SRL and RNC might have been selected in order to maximize root surface area and root growth, enabling a rapid exploitation of resources which is crucial to grow fast and to complete their life cycle in a short period of time. By contrast, in perennials, the diversion of resources to thick roots and high-density tissues probably reflect an adaptation to survive and to monopolize space in environments where the competition is strong.

Despite the differences summarized above, annuals and perennials did not significantly differ in topology and construction costs, which contradicts our predictions. Fitter's (1985, 1987, 1991) topological model predicted that perennials have a herringbone topology while annuals have a dichotomous root system. This was found in a study comparing 12 herbaceous seedling species grown under controlled conditions (Gross

Table 3 Summary of the differences between analogous leaf and root traits in annual (A) and perennial (P) herbaceous species, reported in various studies (third column)

Traits	Pattern	References
Specific root length	A > P	Craine *et al*. (2001), this study
	A = P	Gross *et al*. (1992)
Specific leaf area	A > P	Pitelka (1977), Smith *et al*. (1987), Joffre (1990), Muller and Garnier (1990), Garnier (1992), Roumet *et al*. (1996), Garnier *et al*. (1997)
Root tissue density	A < P	This study, Craine *et al*. (2001)
Leaf tissue density	A < P	Garnier and Laurent (1994)
Root diameter	A < P	Craine *et al*. (2001), this study except Fabaceae
Leaf thickness	A < P A = P	Evans (1964), Sant (1969), van Arendonk & Poorter (1994), Garnier & Laurent (1994)
Root nitrogen concentration	A > P	This study
Leaf nitrogen concentration	A > P	Garnier & Vancaeyzeele (1994), Roumet *et al*. (1996), van Arendonk and Poorter (1994), Benech Arnold *et al*. (1992)
Root construction cost	A = P	This study
Leaf construction cost	A = P	Navas *et al*. (2003)

Each individual study refers to a particular set of species. In a majority of studies, differences between traits of annuals and perennials were tested using an analysis of variance. A > P, A = P and A < P indicates that the trait considered has, respectively, a higher, similar and lower value in annual than in perennial species.

et al., 1992). However, this was not confirmed by the present study. Plant maturation and field conditions may affect topology. A higher plasticity in topology has been reported under conditions of increased soil resource availability (Fitter, 1994; Taub & Goldberg, 1996; Arredondo & Johnson, 1999; Dawson *et al.*, 2003). Although annual and perennial roots differed largely in their tissue density, chemical composition and mycorrhizal colonization, RCC remained similar among life histories. Similarly, Martínez *et al.* (2002) did not observe significant differences in RCC between life forms, families or deciduous and evergreen species. The lack of difference in RCC between ecologically contrasting group of species is consistent with studies addressing leaf construction cost (LCC). No evidence of interspecific differences in LCC was found between herbaceous annuals and perennials (Navas *et al.*, 2003) or between fast- and slow-growing species (Poorter, 1994). One possible explanation for the constancy in RCC across life histories could be found in the chemical composition of roots. Annuals had a higher N concentration than perennials, suggesting that a higher proportion of the energy allocated to root construction is used for 'expensive' proteins, which are associated with rapid ion uptake and assimilation. Perennials, with thicker and denser root tissues, might allocate similar amount of energy to other 'expensive' compounds such as lignin, phenols or waxes that are related to resistance and defence (Poorter, 1994; Eissenstat & Yanai, 1997; Martínez *et al.*, 2002). As a consequence total RCC is similar between the two life histories.

Patterns between root traits and leaf traits

When our results are viewed in conjunction with previous studies relating differences in leaf traits between annual and perennial species (Garnier, 1992; Gross *et al.*, 1992; Garnier & Laurent, 1994; Garnier & Vancaeyzeele, 1994), it appears that root traits follow similar patterns to those observed for leaf traits (Table 3). Both leaves and roots of annuals have higher N concentration and specific area or length and lower tissue density and thickness/diameter. These results are consistent with the positive relationships reported in the literature between root and leaf traits, such as N concentration, tissue density (Ryser, 1996; Craine *et al.*, 2001; Craine & Lee, 2003), longevity and respiration rate (Tjoelker *et al.*, 2005). However, contrasting with our conclusions, SRL and specific leaf area (SLA) have often been reported as weakly related or unrelated to each other (Tjoelker *et al.*, 2005). The generality of these relationships at broader scales remains uncertain. For example, at a global scale, only N concentrations were correlated between leaves and roots; while there was no overall relationship between leaves and roots in thickness/diameter, tissue density or lignin concentrations because of environmental constraints (Craine *et al.*, 2005). The general pattern found in this study, between root traits measured on field-grown species and leaf traits reported in the literature, deserves further study where analogous leaf and root traits could be measured simultaneously on different annual and perennials species. Overall, however, the present study suggests that the morphology and physiology of leaves and roots are closely coupled, and that the general acquisition vs conservation trade-off previously reported for above-ground tissues (Garnier, 1992; Díaz *et al.*, 2004) also applies to roots.

Root traits and plant families

Families differed only in two traits: root topology and RNC. The main differences observed were related to the topology of

Poaceae and the higher RNC of Fabaceae. The patterns of root traits in Asteraceae were often intermediate between those of Poaceae and Fabaceae. In particular, Poaceae showed a more herringbone (sparsely branched) root system compared with the members of the two other families studied, agreeing with previous studies reporting a more herringbone topology in monocotyledons than in dicotyledonous (Fitter *et al.*, 1988; Fitter & Stickland, 1991; Taub & Goldberg, 1996; but see Gross *et al.*, 1992). Herringbone root systems are predicted to be more efficient in the acquisition of nutrients over large soil volume since there is less overlap between depletion zones (Fitter *et al.*, 1988; Fitter & Stickland, 1991; Dunbabin *et al.*, 2004). They are also predicted to be more costly because they require a larger diameter (Fitter, 1991). Our results did not support this last assumption since the root construction cost was similar for the three families. In turn, Fabaceae showed the highest RNC, likely owing to their symbiotic association with N-fixing bacteria. These associations have been reported to lead to high tissue N concentration both in roots and leaves (Gebauer *et al.*, 1988; Del Pozo *et al.*, 2000).

The facts that (1) the two traits (topological index and RNC) that did differ among two or the three families are known to be strongly associated to certain lineages and thus probably subjected to strong phylogenetic constraints and (2) contrasting families did not differ in several traits directly linked to resource uptake and use (SRL, tissue density, RCC and mycorrhizal colonization), are in accordance with previous studies of above-ground traits. On a large transregional species set, Asteraceae, Fabaceae, and Poaceae did not differ substantially in terms of their above-ground traits associated with resource acquisition and conservation (SLA, leaf area, thickness and toughness) (Díaz *et al.*, 2004). Our results add weight to their suggestion that Asteraceae, Fabaceae, and Poaceae cannot always be considered fundamentally different functional types a priori.

Conclusion

The present study shows that annuals differed from perennials in terms of several key root traits, even across families with contrasting rooting strategies. Annuals displayed root traits associated with enhanced resource acquisition via the presence of low-density roots with high nitrogen concentration, while root traits of perennials demonstrated enhanced root persistence via the presence of thick, dense root systems. Whether the higher arbuscular mycorrhizal colonization in the annual species studied here represents any advantage in a resource acquisition remains to be tested. These results suggest the existence of a fundamental trade-off between acquisition and conservation of resources within the root system level, analogous to that previously found for shoot traits of annuals and perennials plants grown under controlled conditions (Garnier, 1992).

In the case of above-ground traits, Díaz *et al.* (2004) and Garnier *et al.* (2004) have shown that traits of locally dominant species influence ecosystem functioning. Following the same line of reasoning, some plausible links could be established between primary productivity, nutrient cycling and retention, and trophic transfer to decomposers and the local dominance of acquisitive or conservative root traits. These issues deserve further study.

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