Oecologia (2011) 165:321–331
DOI 10.1007/s00442-010-1806-y

PHYSIOLOGICAL ECOLOGY - ORIGINAL PAPER

Root traits explain different foraging strategies between resprouting life histories

Susana Paula · Juli G. Pausas

Received: 4 May 2010 / Accepted: 29 September 2010 / Published online: 20 October 2010

Abstract Drought and fire are prevalent disturbances in Mediterranean ecosystems. Plant species able to regrow after severe disturbances (i.e. resprouter life history) have higher allocation to roots and higher water potential during the dry season than coexisting non-resprouting species. However, seedlings of non-resprouters have a higher survival rate after summer drought. We predict that, to counteract their shallow-rooting systems and to maximize seedling survival, non-resprouters have root traits that confer higher efficiency in soil resource acquisition than resprouters. We tested this prediction in seedlings of less than 1.5 months old. We select 13 coexisting woody species (including both resprouters and non-resprouters), grew them in a common garden and measured the following root traits: length, surface, average diameter, root tissue density (RTD), specific root length (SRL), surface:volume ratio (SVR), specific tip density (STD), tip distribution in depth, internal links ratio (ILR), and degree of branching. These root traits were compared between the two resprouting life histories using both standard cross-species and phylogenetic-informed analysis. Non-resprouters showed higher SRL and longer, thinner and more branched laterals, especially in the upper soil layers. The external links (i.e. the most absorptive root region) were also more abundant, longer, thinner and with higher SVR for non-resprouters. The results were supported by the phylogenetic-informed analysis for the root traits most strongly related to soil resource acquisition (SRL, SVR and branching pattern). The seedling root structure of non-resprouters species allows them to more efficiently explore the upper soil layer, whereas seedling roots of resprouters will permit both carbon storage and deep soil penetration.

Keywords Drought · Fire · Mediterranean-type ecosystems · Root branching · Root morphology

Introduction

Resource availability and disturbances have been regarded as the major factors driving plant functioning (Grime 1979; Westoby 1998; Lavorel and Garnier 2002), and traits associated with these two factors define the range of strategies for plant coexistence (Ackerly 2004). In Mediterranean conditions, the topsoil water content during the dry season is drastically reduced, sometimes to less than 1% (Lossaint and Rapp 1978; Puigdefábregas et al. 1996; Martínez-Vilalta et al. 2003; Padilla and Pugnaire 2007), which is very close to the permanent wilting point estimated for xerophytes (Larcher 1995). Consequently, plant traits related to water uptake are of paramount importance for explaining plant persistence in Mediterranean-type ecosystems (Valladares et al. 2004). Moreover, the productivity and aridity levels of Mediterranean ecosystems provide dry fuel loads that promote recurrent fires during

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Communicated by Fernando Valladares.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-010-1806-y) contains supplementary material, which is available to authorized users.

S. Paula
CEAM Centro de Estudios Ambientales del Mediterráneo,
Charles R. Darwin 14, Parc Tecnològic, 46980 Paterna,
València, Spain

J. G. Pausas (✉)
CIDE Centro de Investigaciones sobre Desertificación (CSIC),
Camí de la Marjal s/n Apartado Oficial, 46470 Albal,
València, Spain
e-mail: juli.g.pausas@uv.es

the dry season (Pausas 2004; Pausas and Bradstock 2007). There are two main mechanisms for post-fire persistence at population level coexisting in Mediterranean-type ecosystems: (1) the regeneration of the above-ground biomass after being 100% scorched (i.e. resprouting), and (2) the recruitment of new individuals from a fire-resistant seed bank (Pausas et al. 2004). Each of these mechanisms is strongly associated with vegetative and reproductive functional traits, and define two contrasted life histories (Keeley and Zedler 1978; Pausas et al. 2004; Pausas and Verdú 2005): resprouters and non-resprouters.

Resprouting species need to allocate more resources to below-ground organs, where energetic reserves are stored to sustain regrowth (e.g., Bowen and Pate 1993; Schutz et al. 2009). Consequently, they show lower shoot:root ratio than coexisting non-resprouting species (Pate et al. 1990; Bell et al. 1996; Verdaguer and Ojeda 2002; Silva and Rego 2004; Schwilk and Ackerly 2005); this allows resprouters access to more reliable deep water throughout the year (Hellmers et al. 1955; Davis et al. 1998; Bell et al. 1996; Silva et al. 2002; Ackerly 2003; Guerrero-Campo et al. 2006). In contrast, non-resprouting species are subjected to seasonal changes in water availability because of their smaller and shallower roots. In fact, the decreasing water potential of non-resprouters during the dry season is seldom observed in resprouting species (Davis et al. 1998; Clemente et al. 2005; Gratani and Varone 2004; Jacobsen et al. 2007; Pratt et al. 2007a). As a consequence of exposure to stronger seasonality, non-resprouters have drought resistance mechanisms. At leaf level, they have higher water use efficiency and leaf mass area ratio (Ackerly 2003; Knox and Clarke 2005; Paula and Pausas 2006; Pratt et al. 2007a). For roots and stems, the low vulnerability to cavitation shown by non-resprouters has been described as a mechanism for water stress tolerance in both adults (Davis et al. 1998; Jacobsen et al. 2007; Pratt et al. 2007a) and seedlings (Pratt et al. 2008). Higher water stress tolerance at the seedling stage is specially relevant in species in which population persistence relies exclusively on seedling recruitment (non-resprouters) and explains their higher survival rates under summer drought (Keeley and Zedler 1978; Zammit and Westoby 1987; Frazer and Davis 1988; Davis et al. 1998; Enright and Goldblum 1999; Pratt et al. 2008). Furthermore, seedlings of non-resprouters show greater water transport efficiency (Pratt et al. 2010) allowing for faster growth and earlier maturity (Pausas et al. 2004). The lack of evidence relating water stress resistance and rooting depth in seedlings (Frazer and Davis 1988; Pratt et al. 2008) suggests that the correlation between the hydraulic architecture and the life history constitute a functional syndrome appearing early in plant development and not as a consequence of differences in water availability at the seedling stage.

Root structure is strongly correlated with physiological traits responsible of the plant's water status (Hernández et al. 2010), and it has been suggested as a promising candidate for explaining the differences in hydraulic architecture between life histories in seedlings (Pratt et al. 2010). One of the most relevant root traits related with soil exploration and resource uptake is the specific root length (SRL; the root length achieved per unit of root biomass invested), which in turn depends on root diameter and/or tissue density (Wright and Westoby 1999; Nicotra et al. 2002). Roots with high SRL have a high surface:volume ratio (SVR) for the same carbon investment, and this maximizes the root–soil interface and hence the root absorption potential (Larcher 1995). Moreover, thin roots with high SRL offer less resistance to the radial flow of water, thus increasing radial conductivity (Huang and Eissenstat 2000). In addition, plants with high SRL tend to show higher root hydraulic conductance per leaf unit surface area (Pemán et al. 2006) or per stem cross-section area (Hernández et al. 2010). Consequently, plants with high SRL (considering either the whole root system or only fine roots) show high uptake rates of water (Eissenstat 1991), nitrogen (Reich et al. 1998) and phosphorus (Comas et al. 2002).

Intensively branched roots, which have abundant root tips, are highly efficient for water transport because of their low overall distance from the tips to the root crown (Fitter 1986). Tips are the root parts with the highest absorptive capacity due to the presence of root hairs and non-lignified tissues, whereas the main functions of older roots are anchorage, storage and transport (Fahn 1985; Wells and Eissenstat 2003; Guo et al. 2008; Valenzuela-Estrada et al. 2008). Consequently, the number of tips per unit of root biomass (i.e. specific root tip density; STD hereafter) is a relative measure of allocation to the most absorptive tissues, which is particularly relevant in woody plants.

Our hypothesis is that, to counteract their lower root allocation and maximize seedling survival, non-resprouting species have root traits conferring higher efficiency in soil resource acquisition than coexisting resprouters. Specifically, we predict that, in comparison to the root system of resprouters, the root system of non-resprouters should have: (1) longer and thinner roots and higher surface area per unit of root biomass (i.e. higher SRL and SVR values) and/or (2) more tips (per unit of root biomass) and higher branching. To test our predictions, we grew seedlings of several Mediterranean woody species in a common garden, measured their root traits, and compared them between resprouting abilities. We used a common garden approach to minimize environmental heterogeneity that could affect root structure (Goss 1977; Padilla et al. 2007). Since root traits are strongly affected by the species' phylogenetic affiliation (Fitter and Stickland 1991; Nicotra et al. 2002;

Guerrero-Campo et al. 2006), and resprouting ability is phylogenetically aggregated in the Mediterranean Basin flora (Pausas and Verdú 2005), our hypothesis was also tested taking into account the phylogenetic relatedness among the species.

Materials and methods

We selected 13 shrubs co-occurring in fire-prone areas of the SE Iberian Peninsula, including species with post-fire resprouting ability (8 species) and species that lack the ability to resprout (5 species; Fig. 1). Resprouting ability was assigned on the basis of the BROT database (Paula et al. 2009) and personal experience; we considered resprouters those species that regrow after 100% removal of their above-ground biomass. We restricted our study to shrubs because: (1) root traits, like biomass allocation, depth, lateral spread and branching pattern vary with growth form (Gross et al. 1992; Canadell et al. 1996; Schenk and Jackson 2002), and (2) shrubs are the most abundant and most relevant growth form in the functioning of Mediterranean fire-prone ecosystems. All species are common in the region (Bolòs and Vigo 2001) and they represent the most frequent genera of woody species in the Mediterranean basin. In this region, there are less non-resprouting than resprouting species, and they are represented in fewer lineages (Pausas and Verdú 2005). Consequently, the number of studied non-resprouters was both lower and represented by fewer families.

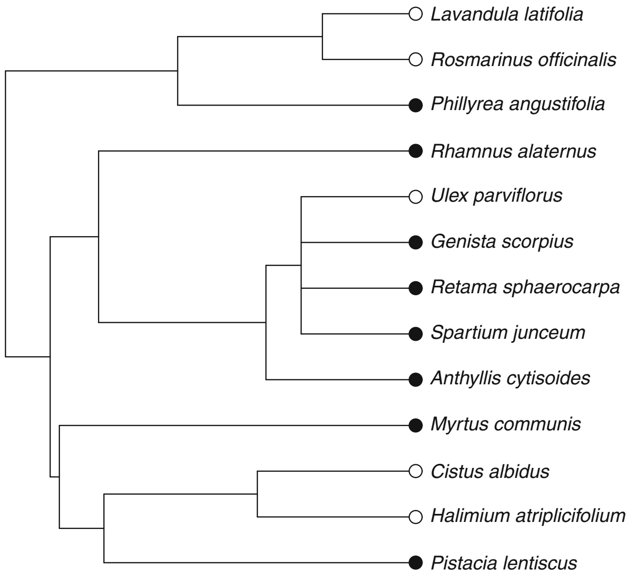

Fig. 1 Phylogenetic relationship among the species considered, including the resprouting life-history (*closed symbols* resprouter, *open symbols* non-resprouter)

Seedling growth and sampling

Seeds were provided by local seed banks, purchased from an accredited commercial collector, or collected by the authors. In all cases, they were collected from wild populations from SE Spain, except for *Lavandula latifolia* seeds, which were from a cultivated population in the same area (Table S1 in the Electronic Supplementary Material, ESM).

Seeds were sown in Petri dishes with paper as substrate. When necessary, the seeds were scarified (with acid or sand paper) to stimulate germination (see Table S1 in the ESM). The Petri dishes were placed in germination chambers in a light and temperature regime that maximized seed germination for each species (at 20°C in darkness for all species, except for *Lavandula latifolia*, the seeds of which were exposed to alternate 8 h light:16 h dark). Germination was monitored every day.

Germinated seeds were sown in 18.8-l, 40-cm-deep pots (23.5 × 20 × 40 cm) with drainage holes. For the growth medium, we used a substrate with loamy to sandy-loamy texture (USDA 1951), basic pH (pH in H_2O 1:2.5 = 8.28) and a moderate level of carbonates (28.5%), which corresponds to the typical forest soil in the Mediterranean basin (Rubio et al. 1997). We extended a fine layer of vermiculite on the surface of this substrate to ameliorate stressful conditions and increase seedling survival. Pots were arranged in a garden shed to form a grid of 4 × 77 positions; all species had individuals in all 4 rows, randomly allocated to the 77 positions.

Although we initially sowed one to three seeds in the middle of each pot, when the seedlings were established, we only allowed a single seedling to grow. Seedlings were watered with micro-diffusers when needed. The harvesting time was based on a standardized developmental stage (cf. Cornelissen et al. 2003); specifically, seedlings were sampled 1 week after the total expansion of the first pair of true leaves. Nevertheless, the age at sampling did not differ between resprouting abilities (mean ± SD 42 ± 10 days for resprouters and 43 ± 8 days for non-resprouters; $P = 0.560$). When the root sampling took place, there were no differences in soil humidity (measured in all pots with a ThetaProbe Soil Moisture Sensor-ML2x; Delta-T Devices, Cambridge, England) between the pots with resprouters and those with non-resprouters ($P = 0.417$) at any depth interval ($P = 0.194$; soil moisture measured at 5-cm intervals).

To extract the root systems, we first cut one side of the pot and then carefully washed the roots. Rooting depth was also measured during the extraction. The root of each seedling was introduced into a 2-l container with deionized water, and the samples were kept cold until arrival at the laboratory. The root systems were separated from the shoots, spread on a glass tray (A3-size) and scanned (400 dpi; Bouma et al. 2000; Himmerlbauer et al. 2004) using a flat-bed scanner (Epson Expression 1680, Model G780B; Seiko Epson, Nagano, Japan) with a light transparency unit (Epson Transparency Unit, model EU-35; Seiko Epson). Root measurements were conducted using WinRhizo image analysis software version 2002c (Regent Instruments 2002). To detect pale roots, we used the Lagarde method, which establishes the threshold for converting grey images into binary (black–white) images (Regent Instruments 2002). Finally, the above- and the below-ground biomass was oven-dried and weighed (separately).

Root measurements

A root system can be described as a mathematical tree, in which each unbranched root portion enclosed between either two branching points or one branch and one tip is called a "link" (Fitter 1987; Berntson 1994). For each link, WinRhizo provides its length, diameter, surface area and the branching angle (i.e. the angle between each link and the link attached to it in the longitudinal plane; see Regent Instruments 2002 for details). We used these variables to calculate the volume of each link, assuming they were cylindrical. We assigned to each link a branching order on the basis of the developmental sequence (i.e. increasing the order from the base of the root to the tip; Fig. 2). We used similarity in diameter to assign two consecutive links to the same root branch and therefore to the same branching order (Regent Instruments 2002). Additionally, links were classified as external, when they finished in an apical meristem (tip), or internal, if not. The number of internal links is an indicator of the number of branches (Fitter 1987).

Length, surface area and volume of the whole root system were calculated as the sum of the lengths, areas and volumes from all links, and the average diameter of the whole root system as the average of the mean diameter for all their links weighted by the length of each link. All our plants developed a well-defined main axis (i.e. tap root; see examples in Fig. S1 in the ESM) and all lateral roots had the same color and similar appearance. Therefore, we calculated the total length, total surface area and average diameter for the main axis (composed of links of zero order) and for the overall lateral roots (with link orders greater than zero). Given that roots ending in a meristem are the most absorptive root region (Guo et al. 2008; Valenzuela-Estrada et al. 2008), we also performed the analyses using the external links (excluding the main axis). The specific root length (SRL) was calculated as the ratio between the length of the root system and its dry weight. Root tissue density (RTD) was calculated by dividing root dry weight by its fresh volume.

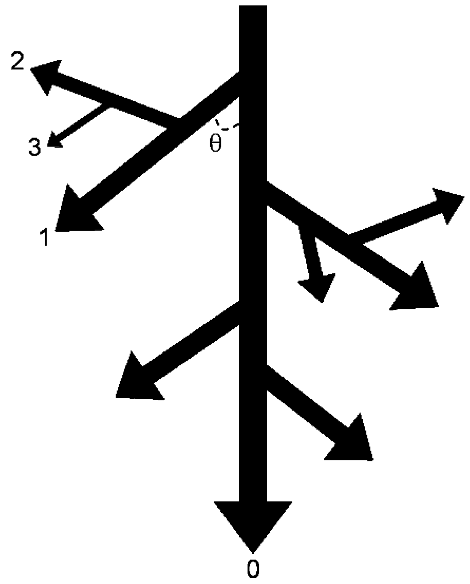

Fig. 2 Schematic diagram of a root system with branches of the same order drawn with lines of the same thickness. *Numbers* correspond to the branching order (assigned in developmental order sequence) and θ to the branching angle

To study the pattern of the absorptive potential in depth, we counted the number of tips at each 5-cm interval in the images. In addition, as an indicator of lateral spread, we used the length of the first order branches (which account for 92% of the total length of lateral roots in the studied seedlings) and their branching angle, estimated as the angle between the main axis and the first link of each branch attached to it (Fig. 2).

Branching patterns were quantified by: (1) the specific tip density (STD; the number of tips per the corresponding root dry weight), (2) the proportion of internal links in the main axis in relation to the total number of internal links (ILR; as an indicator of the branching location), and (3) the frequency of each branching order (hereafter, branching degree). Topological indexes sensu Fitter et al. (1991) were not considered because they often fail to discriminate the branching patterns in seedlings (Gross et al. 1992; Nicotra et al. 2002; see Padilla and Pugnaire 2007); this is probably due to the fact that at the seedling stage most allocation is devoted to the main axis.

Statistical analysis

We used 9–18 individuals per species for the comparison of biomass allocation (Table S1 in the ESM). When roots reached the bottom of the pot, they changed in diameter and branching pattern, and thus they were excluded from the remaining analyses (Table S1 in the ESM). The frequency of excluded plants was equal for both resprouting abilities ($P = 0.799$). *Anthyllis cytisoides* was discarded in the comparisons of tip distribution in depth, branching pattern, first order branch length and external link morphology, because we failed to obtain enough seedlings with the whole root system.

Root parameters were compared statistically between resprouters and non-resprouters (hereafter, R+ and R−, respectively) using mixed models and including species as a random factor nested in resprouting ability. For normal distributed data, we used linear mixed models and tested by analysis of variance; variables were transformed when necessary to meet normality and homoscedasticity requirements. We used generalized linear mixed models (GLMM) with a Poisson error distribution and log link function for counts (tips in depth) and a binomial error distribution and logit link function for binary variables (the presence of a given branching order; see below); then, differences between resprouting abilities were tested by an analysis of deviance (McCullagh and Nelder 1989). The GLMM analyses were performed with the lme4 library of the R package (Bates and Maechler 2009).

When analysing root length, surface area and diameter, we included root biomass as a covariate in the model because the range of root dry weight are different between resprouting abilities. In addition, preliminary analyses showed that root dry weight, which showed large variability, explained a significant proportion of the variability of these root traits ($P > 0.001$; see Table 1 and Fig. S2 in the ESM). For the remaining variables (ratios, number of tips per depth interval, branching angle and branch frequency), root dry weight was not included in the analyses. An alternative covariate could have been seed mass as it is correlated with plant size and seedling survival (Leishman et al. 1995; Moles and Westoby 2004); however, resprouters and non-resprouters do not differ in seed mass when a wide range of species are considered (Pausas and Verdú 2005). In our dataset, non-resprouters tend to have smaller seeds than resprouters ($P = 0.011$; seed size data at species level only). However, seed mass explained a low proportion of the biomass variability either for the whole plant ($r^2 = 0.29$, $P = 0.072$) or for the roots ($r^2 = 0.30$, $P = 0.052$). In addition, seed mass did not explain a significant variability in root length ($r^2 = 0.01$, $P = 0.809$) or surface area ($r^2 = 0.10$, $P = 0.283$), whereas root dry weight did (Table 1). Therefore, root dry weight was a preferred covariate.

All seedlings developed first-order branches (i.e. branches growing directly from the main axis; Fig. 2 and Fig. S1 in the ESM) and most also had second-order branches (84%), whereas higher branching orders were rare (27, 6 and 1% for third-, fourth- and fifth-orders, respectively). Consequently, we analysed two branching categories: second-order and higher, the latter being the presence or absence of a third-, fourth- or fifth-order branch.

In order to understand the role of common ancestry in shaping the root traits of our species, all the statistical analyses were also performed considering the phylogenetic relatedness of the studied species. For this purpose, we assembled a phylogenetic tree using the megatree of angiosperm families (Davies et al. 2004), grafted our species onto it, and adjusted branch lengths (Wikström et al. 2001) with the help of the Phylomatic software implemented in Phylocom 3.41 (Webb and Donoghue, 2005). *Anthyllis* was then separated from the other Fabaceae based on Wojciechowski et al. 2004; no information was available for further separating the remaining Fabaceae and thus they were considered as a polytomy. We then tested, for each root parameter (species mean values), the differences between resprouting abilities (with root dry weight as covariate when needed; see above) by means of a generalized estimating equation (GEE). This procedure uses a GLM approach incorporating the phylogenetic relatedness among species as a correlation matrix in the model (Paradis and Claude 2002). To account for individual variability (within species), all analyses were also performed for 100 randomly generated values following a normal distribution with the observed mean and SD values of each variable for each species. Thus, the phylogenetically-informed results are expressed using the P value of the species mean trait, and the proportion of significant cases ($P < 0.05$) when considering the individual variability. All phylogenetically-informed analyses were perfomed with the APE library of the R package (Paradis et al. 2004).

Results

Biomass allocation

Root systems of R+ tended to be heavier (mean ± SD 26.3 ± 21.4 mg) than those of R− (11.3 ± 8.7 mg), but, due to the great variability, the differences were only marginally significant ($P = 0.081$; root biomass log-transformed; Fig. 3). A similar pattern was found for above-ground biomass (43.2 ± 34.6 mg for R+ and 19.6 ± 17.3 mg for R−; $P = 0.088$; shoot biomass log-transformed), and thus there were no differences in the shoot:root ratio (1.77 ± 0.50 for R+ and 1.70 ± 0.21 for R−; $P = 0.993$; response variable log-transformed). Rooting depth did not differ between resprouting abilities either (27.9 ± 6.2 cm for R+ and 28.6 ± 5.9 cm for R−; $P = 0.924$). Phylogenetic analyses did not support the differences between resprouting abilities in any of these variables (i.e., $P \geq 0.05$ for more than the 90% of the phylogenetic analyses comparing resprouting abilities).

Table 1 *P* values for the analyses of covariance comparing morphological root traits between resprouting abilities (*R*) including root dry weight (*RW*) as covariate, performed by both cross-species and phylogetically-informed analyses

	Non-phylogentic			Phylogenetic		
	RW	R	R × RW	RW	R	R × RW
Total length						
Root system	<0.001	0.006 (R−)	0.588	0.014 (82)	0.696 (8)	0.374 (6)
Main axis	<0.001	0.043 (R−)	0.444	0.587 (42)	0.317 (8)	0.220 (9)
Laterals	<0.001	0.016 (R−)	0.847	0.018 (78)	0.746 (6)	0.431 (6)
External links	<0.001	0.031 (R−)	0.582	0.022 (76)	0.852 (4)	0.544 (3)
Total surface						
Root system	<0.001	0.012 (R−)	0.963	0.002 (98)	0.813 (6)	0.486 (2)
Main axis	<0.001	0.820	0.500	0.048 (94)	0.822 (5)	0.818 (5)
Laterals	<0.001	0.020 (R−)	0.757	0.006 (91)	0.496 (3)	0.277 (3)
External links	<0.001	0.060	0.538	0.008 (90)	0.782 (2)	0.551 (3)
Average diameter						
Root system	<0.001	0.007 (R+)	0.479	0.028 (96)	0.886 (10)	0.510 (9)
Main axis	<0.001	0.046 (R+)	0.300	0.018 (99)	0.900 (7)	0.662 (7)
Laterals	<0.001	0.267	0.128	0.004 (93)	0.097 (6)	0.149 (7)
External links	<0.001	0.035 (R+)	0.800	0.018 (98)	0.378 (8)	0.686 (7)

For non-phylogenetic analyses, species was included in the model as a random factor nested within resprouting ability. When $P < 0.05$, the corresponding resprouting ability with the highest value is indicated in parentheses (*R+*, resprouters, *R−* non-resprouters). For phylogentically-informed analyses, the proportions of analyses that were significant ($P < 0.05$) when using randomly generated data are shown in parentheses. Root diameter was root-square transformed prior to the analyses; the other variables and the covariate were log-transformed

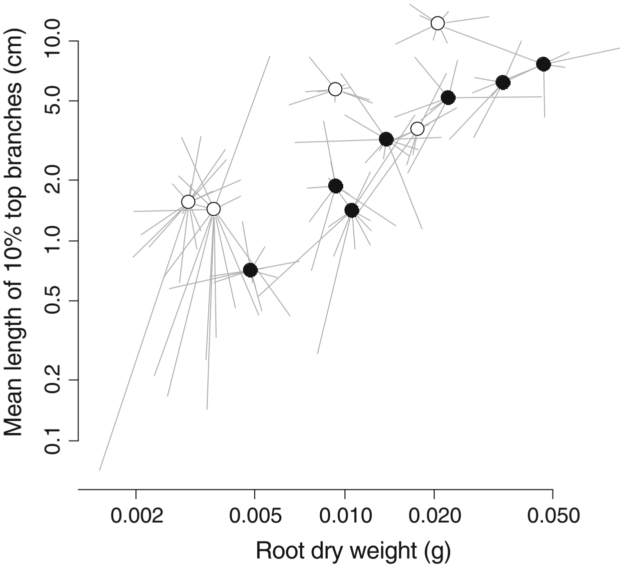

Fig. 3 Relationship between the root dry weight and the mean length of the 10% longest first order branches (log10 scale for both axes) for resprouting (R+ *closed symbols*) and non-resprouting (R− *open symbols*) species. Intra-specific variability is indicated by segments emerging from each symbol (mean species value). The difference in the length of the 10% top first order branches between resprouting abilities were significant for the cross-species analysis ($P = 0.030$) but not for the phylogenetically-informed analysis ($P = 0.376$)

Root traits

R− species had longer roots than R+ species for a given root biomass (Table 1), and, consequently, they had higher specific root length (SRL; Table 2, Fig. 4). These differences were mostly due to differences in the total length of lateral roots rather than to differences in the main axis (Table 1; Fig. S2 in the ESM). Differences in the total length of lateral roots were not explained by differences in the number of first-order branches (i.e. number of internal links of the main axis; $P = 0.199$), but by differences in the length of individual branches, since both the length of the longest first-order branches, and the mean length of the top 10% longest first-order branches (the latter corresponding to 7 ± 3 axes per plant) were higher in R− ($P = 0.006$ and $P = 0.030$, respectively; Fig. 3). The branching angles of the first-order branches were similar ($P = 0.922$) for R+ (69.9 ± 5.7°) and for R− (70.2 ± 6.8°), suggesting that the differences in first-order branch length reflect differences in lateral spread. Total length of external links was also higher for R− (Table 1; Fig. S2 in the ESM). Such differences were not explained by differences in the average length of the external links ($P = 0.973$) but by the higher abundance of external links per gram of root biomass of R− (i.e. STD), and, consequently, the higher lateral branching (see below).

Table 2 Mean (±SD) values of root biomass-independent traits for resprouters (R+) and non-resprouters (R−), and *P* values of the corresponding non-phylogenetic (*Non-phy*) and phylogenetic (*Phy*) comparisons

	R+	R−	Non-phy	Phy
Specific root length (SRL cm g^{-1})	61.4 ± 18.3	130.1 ± 36.1	<0.001	0.011 (62)
Root tissue density (RTD g cm^{-3})	0.13 ± 0.04	0.16 ± 0.08	0.342	0.846 (1)
Surface:volume ratio (SVR cm^{-1})				
Root system	88.5 ± 12.5	146.8 ± 47.6	0.003	0.044 (44)
Main axis	69.5 ± 13.9	117.0 ± 48.8	0.012	0.113 (18)
Laterals	140.9 ± 46.3	196.9 ± 68.0	0.075	0.175 (10)
External links	177.0 ± 53.9	294.5 ± 139.7	0.040	0.099 (22)
Specific tip density (STD g^{-1})	5.4 ± 4.1	20.4 ± 19.2	0.018	0.043 (18)
Internal links main:total ratio (ILR)	0.88 ± 0.12	0.66 ± 0.17	0.011	0.046 (35)
Branch frequency (%)				
Second order	80.1 ± 23.5	93.8 ± 6.2	0.129	0.175
Higher order	8.2 ± 8.3	50.9 ± 22.3	<0.001	0.003

For the phylogenetic comparisons, the proportions of analyses that were significant ($P < 0.05$) when using randomly generated data are shown in parentheses. For non-phylogenetic analyses, species was included in the model as a random factor nested within resprouting ability. SRL, RTD, SVR and STD were log-transformed while ILR were arcsine root-squared transformed

The number of tips diminished with depth ($P < 0.0001$), being higher for R− especially in the uppermost soil layers (significant depth-resprouting ability interaction; $P < 0.0001$; see dismissing *P* values with depth in the Fig. 5).

High SRL could be achieved by having either roots with small diameter or with lower tissue density (i.e. RTD). We did not find differences in RTD between resprouting abilities (Table 2); however, R+ showed thicker roots than R−, and this difference was observed for the whole root system, for the main axis and for the external links (Table 1, Fig. 4; Fig. S2 in the ESM). R− species showed higher root surface area per unit of root biomass, considering the whole root system, laterals and (marginally) the external links (Table 1), which is consistent with the longer and thinner roots found for R− and the shorter and thicker roots of R+. Due to such differences in root morphology, R− species had greater surface:volume ratio (SVR), not only considering the whole root system but also for the main axis and the external links (Table 2).

Regarding the branching pattern, R− species had higher STD (Table 2), indicating that R− had more branches than R+. In fact, whereas the frequency of second-order branches was equal for both resprouting abilities, the higher-order branches were more frequent in R− than in R+ (Table 2). However, the proportion of branches arising from the main axis was significantly higher in R+ (i.e. higher ILR; Table 2).

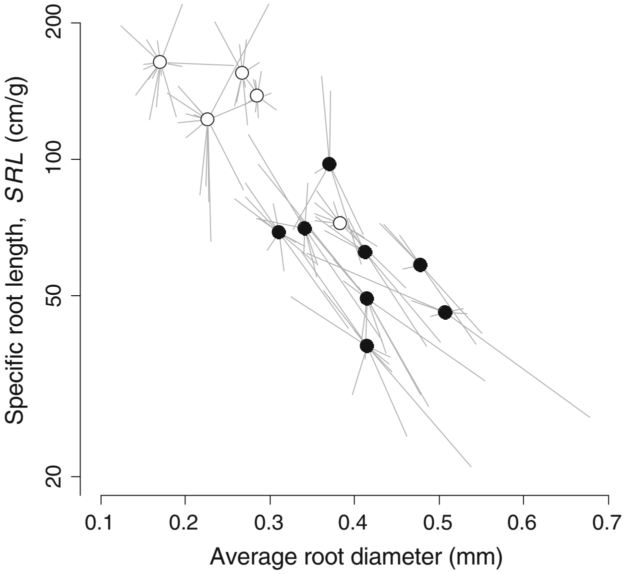

Fig. 4 Relationship between average root diameter and specific root length (SRL; log10 scale) for resprouting (R+ *closed symbols*) and non-resprouting (R− *open symbols*) species. Intra-specific variability is indicated by segments emerging from each symbol (mean species value). See Tables 1 and 2 for statistics

The results found for SRL, RTD and branch frequency were fully supported by phylogenetic-informed analyses, whereas those found for the SVR, STD and ILR were marginally supported (i.e. only for analyses conducted with the mean values per species; Table 2). For the remaining root traits (length, surface area and diameter), R+ and R− did not differ when considering the phylogenetic relatedness ($P \geq 0.05$ for more than 80% of the phylogenetic-informed analyses comparing resprouting abilities; Table 1). The results for total root length (including root biomass as covariate) and SRL differ because of the lower variability in SRL (which considers individual variability in both length and biomass; Fig. 4 and Fig. S2 in the ESM).

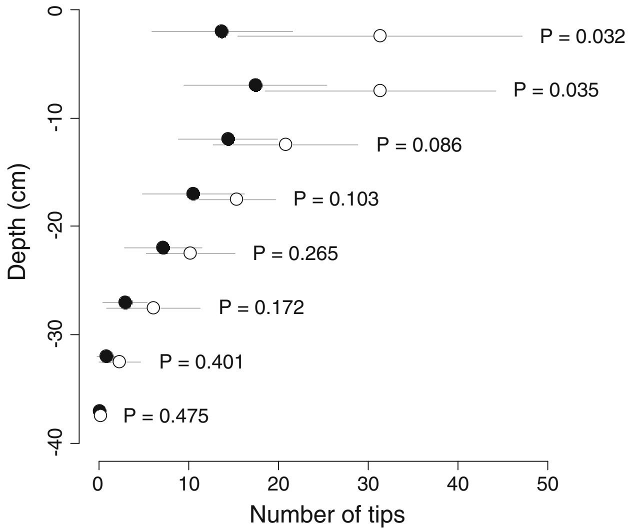

Fig. 5 Mean (±SD) number of tips at each 5-cm depth interval for resprouting (R+ *closed symbols*) and non-resprouting (R− *open symbols*) species. *P* values for cross-species analysis at each depth are shown. For phylogenetically-informed analysis, differences between resprouting abilities were only marginally significant for the upper interval ($P = 0.041$; $P \geq 0.05$ for 41% of the phylogenetic-informed analyses)

Discussion

As predicted, the root traits most strongly related to soil resource acquisition (i.e. SRL, SVR and branching pattern) differed between resprouting life histories, with R− having a potentially more efficient root system. These results, together with the higher drought resistance and water-use efficiency of R− stems and leaves (e.g. Paula and Pausas 2006; Pratt et al. 2008), suggest that R− counteract their lower allocation to roots with a suite of traits at both above- and below-ground level. Moreover, the higher efficiency in root allocation of R− is not driven by the species phylogenetic affiliation, reinforcing the idea that this pattern is the product of ecological trade-offs. On the other hand, we found significant morphological differences between R+ and R− (root length, surface area and average diameter), which were mainly explained by phylogenetic relatedness (i.e. they differed only in cross-species comparisons). That is, the present-day relationship between these root morphological traits and their resprouting ability is not the result of a trade-off but of historical effects that allowed the coexistence of functionally dissimilar species.

Non-resprouters had a higher maximum lateral spread (per unit of biomass) and thus explore larger soil areas. Such differences were qualitatively described in field studies for both juveniles from Australian Mediterranean-type ecosystems (Pate et al. 1990) and adults of the Epacridaceae family (Bell et al. 1996); in the latter case, the differences were related to climatic conditions. Our quantitative approach conducted in a common garden rules out environmental heterogeneity as the driver for the differences in lateral spread between resprouting life histories. Differences in lateral roots are also physiological, since in very dry conditions, the hydraulic conductivity in lateral roots of R+ is negligible, this being considered a mechanism to avoid total root dysfunction (Pratt et al. 2007a).

The highest lateral root length of R− is attained by increasing their branching degree, as indicated by the higher frequency of high-order branches. As a result, R− allocate more to the most absorptive region (i.e. external links). Furthermore, the root–soil interface of external links is higher in R− (i.e. greater SVR). Therefore, R− not only had higher absorptive surface per carbon unit invested considering the whole root system (i.e. higher SRL and SVR; see also Silva et al. 2002; Guerrero-Campo et al. 2006) but they also had a more efficient allocation pattern when only the most absorptive root region is considered. Roots with high SRL or profusely branched roots had potentially higher transport efficiency (Fitter 1986; Huang and Eissenstat 2000; Pemán et al. 2006; Hernández et al. 2010; see details in the "Introduction"). Therefore, our results suggest that the greater hydraulic efficiency recently found for R− Rhamnaceae species of the Californian chaparral (Pratt et al. 2010) could be a widespread pattern in fire-prone ecosystems.

A general way to maximize resource uptake efficiency is to maximize the absorptive surface with the minimum carbon cost (Wright and Westoby 1999). Whereas R− had higher SRL and SVR (see above), R+ had higher specific leaf area (Ackerly 2003; Knox and Clarke 2005; Paula and Pausas 2006; Pratt et al. 2007a) and therefore higher carbon assimilation per carbon unit devoted to leaf construction. Plant organs related to the uptake of limiting resources tend to be larger and more efficient than those that acquire highly available resources (Chapin et al. 1987). Therefore, we propose that, whereas R+ tend to maximize the surface and the efficiency of the organs for carbon uptake to ensure carbohydrate storage for resprouting (Iwasa and Kubo 1997), R− maximize the root surface, since their survival and growth may be limited by soil resources. Indeed, the persistence of R− species relies on seedling recruitment and fast growth for reaching maturation (to refill the seed bank before the next fire), and thus are strongly dependent on soil water and nutrients.

The branching pattern found in R+ roots (i.e. most branches emerging from the main axis) is expected in plants developing roots that function as storage organs or to obtain deep resources (Fitter et al. 1991). Thick storage roots, like those of R+, tend to have more parenchymatic cells and consequently higher storage capacity, which is developed early in seedling ontogeny (Verdaguer and Ojeda 2002). However, thick roots have low SVR and offer higher resistance to the radial flow of water, both causing lower root absorption potential (Larcher 1995; Huang and Eissenstat 2000). Furthermore, the abundance of xylem parenchyma is negatively correlated with the resistance to cavitation (Pratt et al. 2007b). The existence of a trade-off between carbohydrate storage and drought tolerance in roots may contribute to explain the differences in below-ground structure between R+ and R− species.

In addition, thicker roots increase the ability to penetrate into dry tough soils (Goss 1977), and the thick main axis of resprouters could allow them to reach deeper soil layers (see examples for adults in Hellmers et al. 1955; Bell et al. 1996). However, our seedlings of R+ species showed a thicker main axis, but similar rooting depth compared with R− species (see also Frazer and Davis 1988; Pratt et al. 2008). Similarly, we did not find differences in the shoot:root ratio, either in this experiment or in seedlings grown in the field (Paula and Pausas, unpublished data), discarding common garden conditions (i.e. enriched soil) as responsible for the similarities in biomass allocation (Gleeson and Tilman 1994). The lack of such differences in our study is due to the absence of secondary growth in young tissues. Indeed the shoot:root ratio increases throughout development in R− but not in R+ (Silva and Rego 2004). That is, although seedlings lack differences in biomass allocation between life histories, they consistently show a contrasted rooting pattern, supporting the idea that below-ground structure is ontogenetically defined and contributes to characterize the resprouting syndrome.

Differences in lateral spread and tip distribution at depth suggest the existence of a differential foraging strategy from very early development stages. Indeed, there are two contrasted syndromes of soil resource uptake in dry ecosystems (Fitter 1987; Schenk and Jackson 2002; Wright and Westoby 1999): (1) shallow and ramifying root systems with high lateral spread, which permit the exploration of larger top soil areas (rich in nutrients) and a rapid profit from the seasonal rainfalls; and (2) deeper root systems that are poorly ramified and, frequently, with thick roots which feed from the ground water table. These syndromes which have been described for different ecosystems are also reflected in coexisting R− and R+ of Mediterranean-type ecosystems. Furthermore, the differences in the foraging pattern contribute to explain the differences between life histories in the ability to survive grass competition at the seedling stage (Clark and Knox 2009).

In conclusion, through a common garden study, we found a contrasting foraging pattern between resprouting life histories, suggesting that, at an early stage, R− explore the upper soil layer more intensively and acquire water more efficiently by means of longer, thinner and more branched lateral roots. Such differences may contribute to explain the contrasted hydraulic architectures and survival rates previously reported in seedlings from different life histories in Mediterranean-type ecosystems. We propose that the trade-off between water and carbon uptake for resprouting may be the underlying process explaining our results.

Acknowledgments We thank all the volunteers for their help in the common garden and laboratory tasks, especially H. Simões, J. Bandeira, C. Pérez-Cervelló, S. Ribeiro and B. Moreira. The Banc de Llavors Forestals of Generalitat Valenciana provided the installations for the common garden experiment. This work has been financed by the Spanish projects SINREG (REN2003-07198-C02-02/GLO), PERSIST (CGL2006-07126/BOS), and GRACCIE (CONSOLIDER–Ingenio 2010 program; CSD200-00067). CEAM is supported by the Generalitat Valencia and Bancaixa; CIDE is supported by the Generalitat Valencia and the University of Valencia.

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