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Morphological traits and water use strategies in seedlings of Mediterranean coexisting species

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Abstract The distribution of plants is associated with their different patterns of response to their environment. Mediterranean plants have evolved a number of morphological and physiological adaptations that determine their ability to survive and grow, being an effective water uptake and use important factors for drought resistance. In this article, we evaluated interspecific differences in morphology, biomass allocation, and architectural traits and their relationship with water use strategies in seedlings of seven co-occurring Mediterranean species (*Anthyllis cytisoides* L., *Genista scorpius* L. DC., *Myrtus communis* L., *Pistacia lentiscus* L., *Rosmarinus officinalis* L., *Spartium junceum* L. and *Ulex parviflorus* Pourr.). The results showed that morphological root features vary among species and they are significantly correlated with root hydraulic conductance and leaf gas exchange variables. Species with high specific root length (SRL) showed a low hydraulic conductance per root length (K_{RRL}) but high specific hydraulic conductance (K_{As}). *M. communis* and *P. lentiscus* showed the most contrasting water use patterns with respect to the other species studied. The results are not affected when considering phylogenetic relatedness. Thus, the variability observed in root hydraulic properties and leaf gas exchange suggests important mechanisms for understanding species coexistence in water-limited ecosystems.

Keywords Hydraulic conductance · Leaf gas exchange · Mediterranean species · Root morphology

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Introduction

Summer droughts and wildfires are two main ecological factors shaping Mediterranean ecosystems (Specht 1987; Pausas 2004). Species composition of the mature vegetation is determined through the differential survival and growth patterns exhibited by seedlings (Levin and Schmidt 1985; Pratt et al. 2007). Seedling emergence and survival are crucial events in the life cycle of plants, being decisive for their performance and success in water-limited environments (Pausas et al. 2004; Ojeda et al. 2005).

Different species survive and coexist by means of a diversity of functional strategies (Vilagrosa et al. 2003; Paula and Pausas 2006; Galmés et al. 2007). However, the patterns and processes underlying this phenomenon are still not fully understood (Keeley et al. 2005; Pratt et al. 2007).

Different species ability to respond to changes in water availability has been associated with morphological and physiological traits and constraints (Keeley 1998; Mitchell et al. 2008). In this context, drought-resistant plant traits have been synthesized in several functional classifications, on the basis of leaf habit (Clemente et al. 2005; Jacobsen et al. 2007a), rooting structure (Kummerow 1981; Canadell and Zedler 1995; Jacobsen et al. 2007a), regeneration strategy (Paula and Pausas 2006) or hydraulic architecture and drought resistance strategy (Levitt 1980; Tyree et al. 1994; Valladares et al. 2008).

Morphological and physiological features and responses to water conditions are not independent but related (Ackerly 2004; Lambers et al. 2006). Species show different aboveground and belowground characteristics responsible for their survival, growth, and reproduction in different habitats. There is broad evidence supporting a correspondence between the various root system traits developed by species in drought-prone ecosystems and their performance (Davis et al. 1999; Bell 2001; Verdaguer and Ojeda 2002), with important implications on the extent of fluctuations in water status of species (Fleck et al. 1995, 1998). In addition, it has been reported that the first summer rainless period after seedling establishment is a major limiting factor when seedling roots are not fully developed (Vallejo et al. 1999; Pratt et al. 2007).

Roots impose the greatest resistance to liquid water flow in the soil–plant-atmosphere continuum (Sperry et al. 2002); thus, effective water uptake is an important factor in species performance (Rieger and Litvin 1999). The capacity for water transport from soil to leaves is highly dependent on root architecture (Addington et al. 2006; Chirino et al. 2008), which is important in both determining how plants respond to water availability and in setting maximum rates of gas exchange (Brodribb and Feild 2000; Hubbard et al. 2001).

On the other hand, the physiological basis for the regulation of water use efficiency at leaf level is not fully clear because it depends on complex arrangements and interactions of physiological mechanisms and plant architecture (Pou et al. 2008). In this regard, specific differences in water use strategies can determine the capacity to survive under situations of water deficit (Martínez-Vilalta et al. 2002; Vilagrosa et al. 2003). Species can display water-saving mechanisms in order to prevent water deficit damage, decreasing water loss by closing their stomata or regulating the water flow to leaves by low root hydraulic conductance. In contrast, water-spending species increase their root hydraulic conductance, thus increasing water absorption, which allows them to extract water from soil to support high leaf gas exchange rates (Levitt 1980; Lo Gullo and Salleo 1988) even if their stomatal behavior involves xylem cavitation and consequently loss of hydraulic conductance (Nardini and Salleo 2000). In addition, it has been reported that variations in water use efficiency at leaf-level are closely related to variations in hydraulic conductance showed by species (Tyree et al. 1998; Pratt et al. 2007; Medrano et al. 2009). Accordingly, stomatal response can be interpreted on the basis of plant hydraulic conductance (Cochard et al. 2000; Ewers et al. 2000), and it is highly dependent on growth form and species (Ackerly 2004; Galmés et al. 2007).

The aim of the present study was to better understand the diversity of morphological traits and water use strategies in coexisting Mediterranean species. We undertook a comparative nursery experiment with seven coexisting Mediterranean shrubs that encompass the range of dominant strategies in the study area (i.e., different leaf and stem habit, and different post-fire strategy). We hypothesized that coexisting species diverge in their set of traits related to water use strategies, and that root system traits determine different water use strategies (Huang and Eissenstat 2000; Pemán et al. 2006). We examined allocation to aboveground and belowground tissues, root system morphology and water-relation traits. The link between morphological and functional responses evaluated in these species was expected to reveal the diversity of adaptive mechanisms among coexisting species.

Materials and methods

Study species

The species were selected for their abundance and coexistence in Eastern Iberian Peninsula thermomediterranean ecosystems, with a mean annual rainfall of 250–600 mm. The species included were: *Anthyllis cytisoides* L., *Genista scorpius* L. DC., *Myrtus communis* L., *Pistacia lentiscus* L., *Rosmarinus officinalis* L, *Spartium junceu*m L., and *Ulex parviflorus* Pourr. (Table 1).

Seeds of these species were collected in the field from local provenance and placed in Petri dishes at 18°C in darkness in a germination chamber. After the germination and emergence of one true leaf, seedlings were individually transplanted into pots (20 l in volume, 40 cm in height) containing sandy loam soil (USDA soil texture classification) and grown in a nursery (39°28′N 0°31′W; *Banc de Llavors*, *Generalitat Valenciana*, Valencia, Spain). Seedlings of each species were randomly distributed under a light reduction mesh with an 80% transmittance of full sunlight. The experiment was performed on late spring-summer in 2005; the maximum average temperature during the experiment was registered in July (25.0°C) and the minimum in May (18.7°C). Mean irradiance during the experiment was 23.76 MJ m^{-2} day^{-1} and the mean monthly relative humidity of the air was 64.9% (Valencia weather station). The pots were equally well watered 3 days per week and plants were maintained at field capacity before the measurements. A sample of 4–6 seedlings from each species (a total of 38 seedlings) was selected to perform all subsequent measurements (gas exchange, hydraulic conductance, and morphological characterization). Plant age when measurements were taken depended on species phenology: *G. scorpius*, *S. junceum*, and *U. parviflorus* plants were 4 months old, while *A. cytisoides*, *P. lentiscus*, *M. communis*, and *R. officinalis* plants were 3 months old; however, all plants were phenologically similar and with similar sizes (basal diameter between 4 and 6 mm).

Morphological measurements

Stems, leaves, and roots of each seedling were separated. Root surface area (A_R, m^2), root length (RL, m) and photosynthetic area (A_P, m^2) were measured by scanning the material and analyzing the images with a specific software (WinRhizo, Régent Instruments Inc., Quebec, Canada). *G. scorpius*, *S. junceum*, and *U. parviflorus* have small leaves and green photosynthetic stems, and thus both leaf and stems areas were considered to determine A_P.

Sapwood area (A_S, cm^2) was calculated using the stems collected for measurements of hydraulic conductance. Stem cross-section was measured after the bark was removed at the root collar level and under the assumption that all xylem was sapwood. Cross-sectional area was calculated considering diameters in two perpendicular directions. The samples were weighed after drying in a forced-air oven at 65°C up to constant weight. The biomass allocation variables considered were: aboveground (AMF, g g^{-1}), root (RMF, g g^{-1}), stem (SMF, g g^{-1}) and leaf mass fraction (LMF, g g^{-1}), as well as the root to shoot ratio (g g^{-1}).

Specific root length (i.e., the root length per unit of root mass, SRL, m g^{-1}) and the ratio root length to total plant biomass (RL/B, m g^{-1}) were also measured. Leaf mass per area (LMA, g m^{-2}) was calculated in four expanded leaves from different individuals per species as the ratio of leaf dry mass to leaf area. Sapwood area per photosynthetic area

Table 1 List of the species included in this study, their family, code used, and brief description, including post-fire regeneration strategy

Species	Family	Species code	Description
Anthyllis cytisoides L.	Fabaceae	Acy	Semi-deciduous shrub, facultative
Genista scorpius L.	Fabaceae	Gsc	Green-stemmed shrub, facultative
Spartium junceum L.	Fabaceae	Sju	Green-stemmed shrub, facultative
Myrtus communis L.	Myrtaceae	Mco	Broad-leaved evergreen shrub, resprouter
Pistacia lentiscus L.	Anacardiaceae	Ple	Broad-leaved evergreen shrub, resprouter
Rosmarinus officinalis L.	Labiatae	Rof	Narrow-leaved evergreen shrub, seeder
Ulex parviflorus Pourr.	Fabaceae	Upa	Green-stemmed shrub, seeder

Species with green stems have very few and small leaves

(A_S:A_P, cm^2 m^{-2}) and root-to-photosynthetic area ratio (A_R:A_P, m^2 m^{-2}) were also calculated.

Gas exchange and root hydraulic conductance measurements

We measured gas exchange and hydraulic conductance parameters in each seedling prior to taking the above measurements on morphological traits. In each seedling we measured: instantaneous determinations of net CO_2 assimilation (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), and intrinsic water use efficiency (WUEi, μmol CO_2/mol H_2O) as the ratio of A/g_s at saturating light (1,500 μmol photon m^{-2} s^{-1}), 25°C, and 380 μmol -mol^{-1} CO_2. All these measurements were performed at mid-morning with a portable photosynthesis open-system (Model LI-6400, LI-COR Inc., Nebraska, USA), maintaining the relative humidity in the chamber at 55 ± 5%. Air temperature and humidity measurements were performed to ensure that the conditions inside the chamber were similar. The gas exchange data were recorded after equilibration to steady state when coefficient of variation was ≤3% (around 10 min). In *G. scorpius*, *S. junceum* and *U. parviflorus* species portions of stems with a varying number of leaves were placed in the chamber. Leaf and stem areas were then determined to scale gas exchange measurements per unit of total photosynthetic area.

Hydraulic conductance was measured on the whole root system of each seedling using a high pressure flow meter (HPFM, Dynamax, Houston, USA), as described in Tyree et al. (1995). Shoots were cut under water at the root collar level. Root systems were kept in their substrate and after connect to the HPFM were perfused with distilled and degassed ultra-pure water filtered through a 0.1 μm water filtration membrane. Root hydraulic conductance (K_R, mol MPa^{-1} s^{-1}) was calculated from transient measurements as: $K_R = F/\Delta P$, where F is the measured flow and ΔP was the applied pressure every few seconds. The slope of the relationship between F and ΔP was taken as K_R. K_R was corrected for water temperature and then scaled on a root length basis (K_{RRL}, mol m^{-1} s^{-1} MPa^{-1}) and stem cross-section area (K_{As}, mol m^{-2} s^{-1} MPa^{-1}). K_{As} is a measure of the potential of the root system to supply water to the aboveground part of the plant.

Statistical analysis

Differences among species were analyzed using ANOVA. Species pairwise comparisons were performed with Tukey post hoc tests at the $P < 0.05$ significance level. The relationships between the studied variables were analyzed using the Pearson correlations in cross-species analysis. In order to verify to what extend the results are due to phylogenetic relatedness among species, the correlation between variables was also performed using phylogenetic independent contrasts (PICs; Felsenstein 1985). A phylogenetic tree was assembled based on the supertree available in the "Phylocom" software (Webb et al. 2008) and considering the phylogeny of Fabaceae (Pardo et al. 2004). To better understand the multidimensional strategy of the coexisting species, a principal components analysis (PCA) was conducted with all measured morphological and functional variables, using mean values of the variables for each species.

Results

Morphological measurements

The species studied showed different patterns of biomass allocation (Table 2). *M. communis* and *P. lentiscus* were the species with the highest allocation to roots (RMF), while the green-stemmed species (*G. scorpius*, *S. junceum*, and *U. parviflorus*) showed the highest allocation to stems (SMF) and the lowest to leaves (LMF). Root to shoot ratio ranged from 0.17 in *U. parviflorus* to 0.51 g g^{-1} in *M. communis*. The structure of the root systems also differed among the species. *R. officinalis*, *P. lentiscus* and *S. junceum* showed the highest specific root length (SRL), and this result was corresponded with the highest values of root length to total plant biomass (RL/B). *M. communis* was the species that showed the lowest SRL and RL/B, while *P. lentiscus* and *R. officinalis* displayed the highest RL/B values (Table 2).

A highly significant positive correlation was found between RL/B and SRL through the seven species studied for both cross-species analysis and PICs (Fig. 1; Table 3). LMA and the root-to-photosynthetic area ratio (A_R:A_P) showed significant differences among species. *G. scorpius* and *R. officinalis*

Table 2 Mean (±SE) values of aboveground mass fraction (AMF), root mass fraction (RMF), stem mass fraction (SMF), leaf mass fraction (LMF), root:shoot ratio, specific root length (SRL), root length to total plant biomass ratio (RL/B), leaf mass per area (LMA), sapwood area per photosynthetic area (A_S:A_P) and root-to-photosynthetic area ratio (A_R:A_P) studied across the different species

Species	AMF (g g^{-1})	RMF (g g^{-1})	SMF (g g^{-1})	LMF (g g^{-1})	Root:shoot ratio (g g^{-1})	SRL (m g^{-1})	RL/B (m g^{-1})	LMA (g m^{-2})	A_S:A_P (cm^2 m^{-2})	A_R:A_P (m^2 m^{-2})
Acy	0.77 ± 0.10ab	0.23 ± 0.01ab	0.38 ± 0.03ac	0.39 ± 0.03abc	0.29 ± 0.02ab	57.7 ± 8.42ab	7.35 ± 1.35a	97.2 ± 8.66ab	0.57 ± 0.06a	3.67 ± 0.78ab
Gsc	0.80 ± 0.01ab	0.20 ± 0.01a	0.47 ± 0.01c	0.33 ± 0.02bcd	0.25 ± 0.02b	36.1 ± 2.07c	6.83 ± 0.70a	167.1 ± 52.7a	3.38 ± 0.36bc	4.96 ± 1.30b
Sju	0.77 ± 0.01ab	0.23 ± 0.02ab	0.52 ± 0.06c	0.25 ± 0.05cd	0.29 ± 0.01b	72.2 ± 6.57ab	8.37 ± 0.68a	28.1 ± 5.78b	1.62 ± 0.29ab	1.80 ± 0.22ab
Mco	0.66 ± 0.05a	0.34 ± 0.05b	0.24 ± 0.03ab	0.42 ± 0.02abc	0.51 ± 0.06a	8.10 ± 0.75d	1.38 ± 0.11b	88.7 ± 4.12ab	3.08 ± 0.08bc	1.02 ± 0.16a
Ple	0.71 ± 0.05ab	0.29 ± 0.04ab	0.19 ± 0.04ab	0.52 ± 0.06ab	0.40 ± 0.01ab	72.2 ± 4.66ab	15.0 ± 1.53c	116.3 ± 13.7ab	3.00 ± 0.66abc	2.93 ± 0.28ab
Rof	0.78 ± 0.01ab	0.22 ± 0.01ab	0.19 ± 0.01b	0.59 ± 0.01a	0.28 ± 0.01ab	81.8 ± 2.36a	15.4 ± 1.38c	143.0 ± 13.5a	1.93 ± 0.25ab	4.00 ± 0.45b
Upa	0.85 ± 0.02b	0.15 ± 0.02a	0.73 ± 0.09d	0.12 ± 0.03d	0.17 ± 0.02b	49.0 ± 2.62bc	5.76 ± 1.55ab	121.2 ± 15.5ab	5.04 ± 0.76c	4.52 ± 1.03b
P values	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001	<0.001

Species code as in Table 1. A total of 4–6 seedlings per species were considered for all the variables. Different letters indicate significant differences between species (ANOVA, Tukey test, $P < 0.05$)

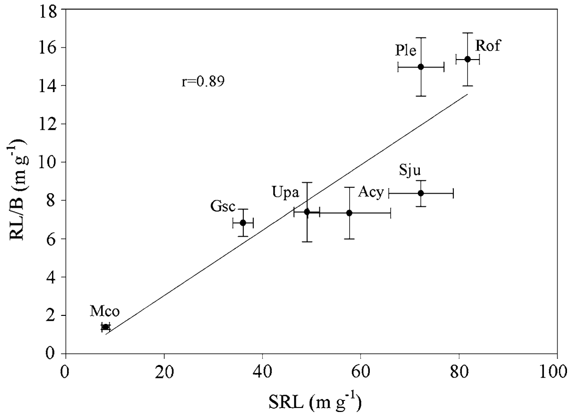

Fig. 1 Relationship between root length to total plant biomass ratio (RL/B, m g^{-1}) and specific root length (SRL, m g^{-1}) for the seven species studied. Each value represents the mean. *Bars* indicate ±SE (n = 4–6). Species code as in Table 1. Cross-species and PIC correlations are shown in Table 3

showed significantly highest LMA compared with *S. junceum*, while *G. scorpius*, *R. officinalis* and *U. parviflorus* showed the highest A_R:A_P, differing significantly from *M. communis*. *U. parviflorus* showed a high sapwood area per photosynthetic area (A_S:A_P) respect to the rest of the species (Table 2).

Gas exchange and root hydraulic conductance

Significant differences were found among the species with respect to the physiological variables studied (Table 4). The highest leaf photosynthesis (A) was found in *P. lentiscus*, followed by *A. cytisoides* and *R. officinalis*. *M. communis* was the species that presented both the lowest A and stomatal conductance (g_s). However, *M. communis* showed the highest intrinsic water use efficiency (WUEi). Root hydraulic conductance in a root length basis (K_{RRL}) and specific root hydraulic conductance (K_{As}) also showed significant differences among the species. *M. communis* was the species with the lowest K_{As}, differing significantly from *P. lentiscus* which showed the highest value. Nevertheless, *M. communis* and *G. scorpius* were the species that exhibited the highest K_{RRL} value (Table 4).

A was positively correlated with K_{As} for cross-species and a marginally significant correlation for PICs; however, a negative significant correlation was found between A and K_{RRL} for both cross-species and PICs across species (Fig. 2; Table 3). Physiological traits were found to be correlated with the

Table 3 Correlation coefficients (*r*) and the *P* values of the statistical comparison according to cross-species analysis and phylogenetic independent contrasts (PICs) between selected variables discussed in the text: net CO_2 assimilation (*A*), stomatal conductance (g_s), specific root hydraulic conductance (K_{As}), root hydraulic conductance per root length (K_{RRL}), specific root length (SRL) and root length to total plant biomass ratio (RL/B)

Traits	Cross-species correlations		PIC correlations	
	r	*P* values	*r*	*P* values
A				
K_{As}	*0.87*	*0.010*	0.73	0.060
K_{RRL}	−0.73	0.063	−0.44	0.326
SRL	*0.81*	*0.029*	0.64	0.120
RL/B	*0.86*	*0.013*	*0.82*	*0.023*
g_s				
K_{As}	*0.85*	*0.016*	*0.78*	*0.037*
K_{RRL}	−0.66	0.108	−0.48	0.272
SRL	0.63	0.131	0.57	0.178
RL/B	0.59	0.159	0.64	0.122
SRL				
K_{As}	0.68	0.094	0.65	0.111
K_{RRL}	*−0.90*	*0.006*	*−0.84*	*0.018*
RL/B	*0.89*	*0.010*	*0.82*	*0.024*

Italic text indicates a significant correlation ($P < 0.05$)

See Electronic Supplementary Material for a full correlation matrix

morphological characteristics of the root system measured. *A* was positively correlated with SRL for cross-species analysis and with RL/B for both cross-species analysis and PICs. In contrast, K_{RRL} showed a negative correlation with SRL and RL/B for both cross-species analysis and PICs (Table 3).

Multivariate analysis

Principal components analysis, computed from species' mean values of the morphological and physiological variables, resulted in two axes explaining ca. 72% of the total variance. The first axis (PC1, explaining 43.1% of the total variance) was related to physiological variables. It was positively linked to g_s (0.94), K_{As} (0.89), and *A* (0.83), and negatively to WUEi (−0.84). PC1 separated the species with a higher variation in gas exchange variables and specific hydraulic conductance from the species with a high WUEi. The second axis (PC2, explaining 28.7% of the variance) separated the species in relation to root and stem biomass allocation and root hydraulic architecture traits. PC2 was positively linked to RMF (0.96) and root to shoot ratio (0.90), and negatively linked to A_R:A_P (−0.90) and SMF (−0.60), with *M. communis* in the positive extreme and *U. parviflorus* in the negative one (Fig. 3).

Discussion

Morphological traits among species

Morphological traits differed widely among species. We found that the two obligate resprouters *M. communis* and *P. lentiscus* showed the highest

Table 4 Mean (±SE) values for net CO_2 assimilation (*A*), stomatal conductance (g_s), intrinsic water use efficiency (WUEi), root hydraulic conductance scaled on a root length basis (K_{RRL}) and specific root hydraulic conductance (K_{As}) studied across the different species

Species	*A* (μmol CO_2 m^{-2} s^{-1})	g_s (mol H_2O m^{-2} s^{-1})	WUEi (μmol CO_2 mol^{-1} H_2O)	K_{RRL} (mol m^{-1} s^{-1} MPa^{-1} × 10^{-5})	K_{As} (mol m^{-2} s^{-1} MPa^{-1} × 10^{2})
Acy	17.8 ± 1.48ab	0.44 ± 0.01ab	40.0 ± 2.69a	0.33 ± 0.11a	0.93 ± 0.18ab
Gsc	12.6 ± 1.19bc	0.24 ± 0.02cd	52.0 ± 3.73ab	1.32 ± 0.19bc	0.35 ± 0.04b
Sju	14.6 ± 1.52bc	0.34 ± 0.01bc	43.3 ± 8.84a	0.65 ± 0.04ab	0.48 ± 0.13b
Mco	4.92 ± 1.04d	0.06 ± 0.01e	86.4 ± 9.85b	1.88 ± 0.27c	0.10 ± 0.04b
Ple	22.3 ± 0.47a	0.54 ± 0.01a	41.4 ± 2.11a	0.43 ± 0.03a	1.65 ± 0.35a
Rof	17.2 ± 2.26ab	0.21 ± 0.03d	71.6 ± 9.13ab	0.39 ± 0.16a	0.82 ± 0.24b
Upa	8.03 ± 1.03cd	0.16 ± 0.05de	59.3 ± 16.7ab	0.55 ± 0.11ab	0.58 ± 0.21b
P values	<0.001	<0.001	<0.001	<0.001	<0.01

Species code as in Table 1. A total of 4–6 seedlings per species were considered for all the variables. Different letters indicate significant differences between species (ANOVA, Tukey test, $P < 0.05$)

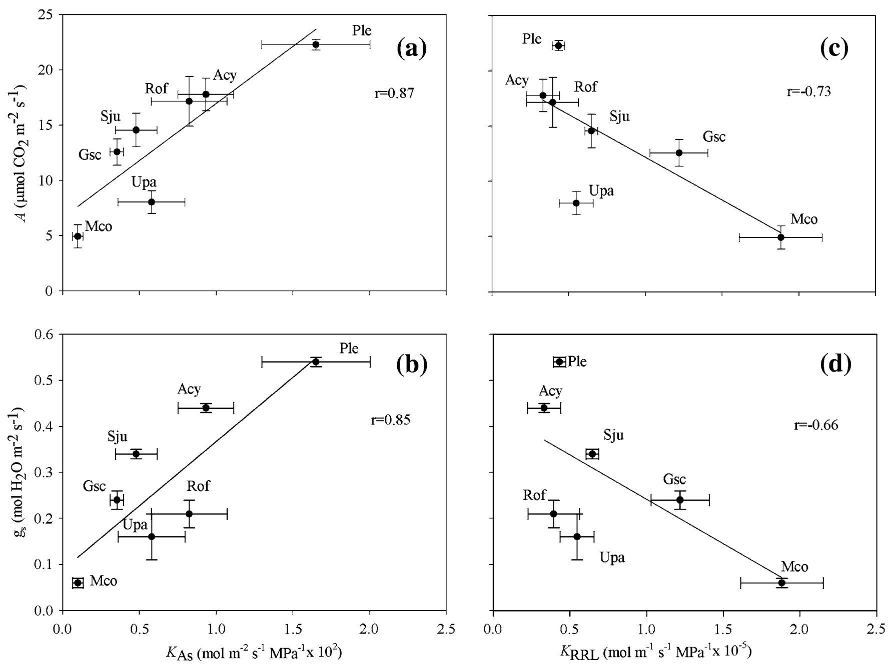

Fig. 2 Relationships between physiological characteristics for the seven considered species. **a** Relationship between leaf photosynthesis (A) and specific root hydraulic conductance (K_{As}). **b** Relationship between stomatal conductance (g_s) and K_{As}. **c** Relationship between A and root hydraulic conductance per root length (K_{RRL}). **d** Relationship between g_s and K_{RRL}. Each value represents the mean. *Bars* indicate ±SE ($n = 4$–6). Species code as in Table 1. Cross-species and PIC correlations are shown in Table 3

allocation to roots (RMF), since these species have to sustain regrowth after a disturbance (Ackerly 2004; Pausas et al. 2004; Schwilk and Ackerly 2005). *G. scorpius*, *S. junceum*, and *U. parviflorus* differed from the other species in stem and leaf parameters. These three species allocated a high proportion of biomass to stem and a low proportion to leaves. All three species have green stems, which have been considered as an advantageous trait in arid habitats with high irradiance regimes (Valladares et al. 2003).

The root morphology and distribution is related to the ability of plants to access water and nutrients (Steudle 2000; Green et al. 2005). In our study, morphological traits related to the structure of the root system showed a significant correlation with plant functional traits. This is in agreement with many studies evidencing the close relationship between root system development and aboveground physiological responses (Norby et al. 2001; Filella and Peñuelas 2003; Trubat et al. 2006; Chirino et al. 2008). Root structure, root soil colonization, and root:shoot ratio are important for evaluating hydraulic limits in the soil–leaf continuum (Davis et al. 1998; Sperry et al. 2002), and thus play an important role in the species' performance.

Specific root length (SRL) is a trait that characterizes the economical aspects of the root system construction costs and it has been related to root's efficiency to soil exploration and to water and nutrient acquisition, since it indicates the amount of root length achieved per unit root mass invested (Lambers et al. 2006; Ostonen et al. 2007). The positive correlation found between SRL and the root length to total plant biomass ratio (RL/B) (Fig. 1; Table 3) suggests that differences in SRL are associated with the root system extension. *M. communis* was the species with the lowest SRL and RL/B but with the highest root:shoot ratio in agreement with its high RMF, indicating high root construction costs by this species. High root:shoot ratios and low SRL values for *M. communis* have also been reported in field conditions (Silva et al. 2002).

G. scorpius, *R. officinalis*, and *U. parviflorus* showed significantly higher root-to-photosynthetic area ratio (A_R:A_P) values than *M. communis*. This parameter is related to plant hydraulic architecture, associated to the amount of water that can be extracted from the soil and transported to leaves (Addington et al. 2006). A high A_R:A_P favors the ability to extract water from soil by increasing the surface in contact with soil to better supply to the photosynthetic organs (Ewers et al. 2000; Hacke et al. 2000). This structural trait could be related to the higher drought tolerance reported in post-fire seeders

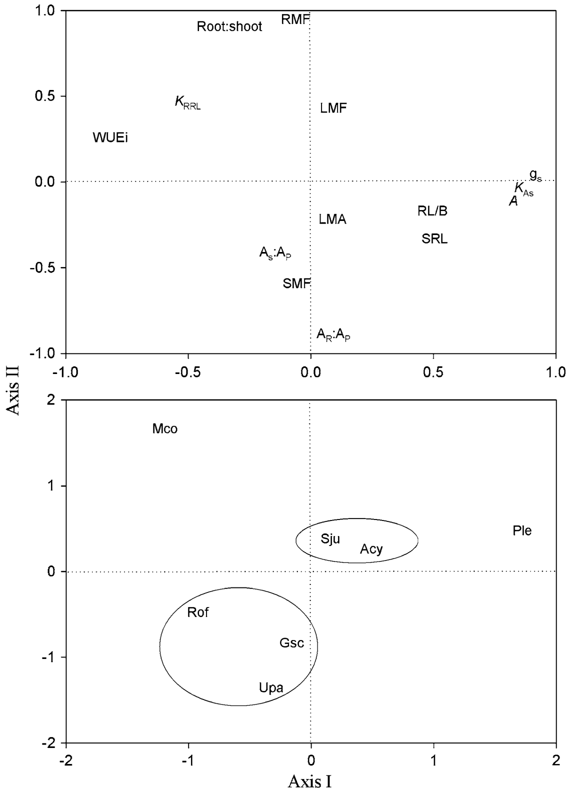

Fig. 3 Distribution of traits (*upper panel*) and the studied species (*lower panel*) as a function of principal components 1 and 2 resulting from multivariate analysis among the morphological (RMF, SMF, LMF, root:shoot ratio, SRL, RL/B, LMA, A_s:A_P, and A_R:A_P; variables code as in Table 2) and physiological traits included in the study (A, g_s, WUEi, K_{As}, K_{RRL}; variables code as in Table 4). Species code as in Table 1

species (Paula and Pausas 2006; Pratt et al. 2007). In contrast, *M. communis* showed the lowest A_R:A_P value, which could limit its water extraction potential under drought conditions.

Relationship between root morphology and plant physiology

Specific root length and RL/B were positively correlated with the net CO_2 assimilation rate (A) and negatively correlated with the root hydraulic conductance per root length (K_{RRL}) across all the species studied. The species with thinner roots (i.e., high SRL) showed a low K_{RRL} but a high capacity for soil exploration with high specific hydraulic conductance (K_{As}). These patterns suggest that both leaf gas exchange and plant hydraulic capacity are determined by the root system morphology. A higher SRL has been associated with a higher nitrogen absorption rate, which, in turn, is correlated with plant functional responses as increases in maximum relative growth rate (Osone and Tateno 2005). The positive correlation between SRL and K_{As} indicates that the root systems characterized by less massive roots per unit length have a higher hydraulic conductance. Previous significant correlations between root morphology parameters as SRL and hydraulic conductivity in herbaceous and woody species have also been reported (Rieger and Litvin 1999; Pemán et al. 2006), highlighting the role of root architecture in plant water status. Indeed, root structure has been related to the variability in physiological traits, including the vulnerability to cavitation in Mediterranean shrubs of California (Jacobsen et al. 2005, 2007a).

M. communis was the species that displayed the lowest SRL, K_{As}, and stomatal conductance (g_s) values but the highest K_{RRL}. Rieger and Litvin (1999) also reported low SRL associated to high K_{RRL} values, suggesting a strong role for the radial path length in the root system hydraulic conductivity. Leaf gas exchange variables (A and g_s) were positively correlated with K_{As} across the species (Fig. 2; Table 3). Species with greater K_{As} displayed greater leaf gas exchange values consistent with previously reported correlations (Bond and Kavanagh 1999; Sperry et al. 2002; Hernández et al. 2009). High photosynthetic rates and the correspondingly high stomatal conductance must be supported by high hydraulic conductance (Mencuccini and Comstock 1999; Hubbard et al. 2001; Sellin and Kupper 2007). Under optimal soil water supply, high K_{As} allows more efficient water transport from stems into photosynthetic organs to compensate leaf water loss and consequently maintain daily water potential high (Ackerly 2004; Jacobsen et al. 2007b). However, in this study there was no significant correlation among sapwood area: photosynthetic area ratio (A_S:A_P) and K_{As}. These results suggest that changes in xylem anatomy (i.e., vessel size and density) among species could be related to plant functional strategies. Previous studies have suggested that there may be a range of ecologically significant vessel strategies available to species (Ackerly 2004; Preston et al. 2006; Jacobsen et al. 2007b).

Among these species, there was variability in leaf WUEi, but probably the differences in leaf WUEi are more noticeable when species are under stronger water stress conditions (Medrano et al. 2009). In any case, *M. communis* and *P. lentiscus* were the species that showed the most contrasted water use strategies, with a significantly higher WUEi in *M. communis*. Leaf WUEi showed a negative trend respect to SRL, since plants with a high WUEi presented lower SRL values. In this regard, Jacobsen et al. (2008) suggested that different water-use strategies highlight the importance of root structure and function among Mediterranean species. The high WUEi at leaf level in *M. communis* under watered conditions could be due to its low capacity to transport water to leaves together with its low stomatal density (Medrano et al. 2009) and therefore, low stomatal conductance. In contrast, *P. lentiscus* showed low WUEi but high K_{As} and g_s values. Additionally, the high stomatal conductance measured in *P. lentiscus* could also be related to its high stomatal density compared with other species (Galmés et al. 2007).

Multivariate analysis: morphological, physiological traits and interspecific variability

The principal components analysis (PCA) including all the characters measured reflected the existence of specific differences in morphological and physiological traits among the seven species studied (Fig. 3). The main variation trend, marked by the PCA axis I, underlined the link between the physiological water use variables, evidenced by the association of K_{As} and gas exchange variables (A and g_s). Indeed, a trade-off was detected between A, g_s, K_{As}, and WUEi, reflecting the contrasted strategies of *P. lentiscus* and *M. communis*. The PCA evidenced a net separation between *P. lentiscus* and *M. communis*, in spite of having very similar life form (broad-leaved evergreen resprouting shrubs). The high WUEi associated with *M. communis* could offset the low gas-exchange rates. In contrast, *P. lentiscus* showed low WUEi respect to the rest of the species due to its high stomatal conductance rates under a favorable plant water status. However, high values of WUEi in *P. lentiscus* have been reported in field-grown under drought conditions (Flexas et al. 2001).

The second component was related to morphological traits, reflecting a strong negative relationship between root allocation (root:shoot, RMF) and A_R:A_P. The PCA displayed an association between species, underlining the high similarity on the one hand of *A. cytisoides* and *S. junceum*, and on the other hand of *G. scorpius* and *U. parviflorus* respect to morphological and physiological traits. *R. officinalis* was closer to *G. scorpius* and *U. parviflorus* than to the other species. These species showed high ratio A_R:A_P but low root allocation, which could be related with the high drought tolerance exhibited by these species in Mediterranean ecosystems (Valladares et al. 2003; Paula and Pausas 2006).

In summary, the different physiological behavior found in the seedlings of seven Mediterranean species appears to be related mainly to their architectural traits. These results highlight the role of root traits, such as SRL and plant hydraulic capacity, as important determinants of interspecific differences in leaf responsiveness in Mediterranean species. Most significant cross-species correlations were supported by significant phylogenetically independent contrasts, suggesting a lack of evolutionary associations between the traits measured across species. Therefore, the different species strategies observed in this study with regard to morphological and physiological traits are indicative of the great diversity of mechanisms that allow Mediterranean plants with similar and different life forms to persist and coexist in water-limited environments.

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