

Water-use strategies of six co-existing Mediterranean woody species during a summer drought

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Abstract Drought stress is known to limit plant performance in Mediterranean-type ecosystems. We have investigated the dynamics of the hydraulics, gas exchange and morphology of six co-existing Mediterranean woody species growing under natural field conditions during a drought that continued during the entire summer. Based on the observed minimum leaf water potentials, our results suggest that the six co-existing species cover a range of plant hydraulic strategies, from isohydric to anisohydric. These differences are remarkable since the selected individuals grow within several meters of each other, sharing the same environment. Surprisingly, whatever the leaf

water potentials were at the end of the dry period, stomatal conductance, photosynthesis and transpiration rates were relatively similar and low across species. This result contradicts the classic view that anisohydric species are able to maintain gas exchange for longer periods of time during drought stress. None of the plants showed the expected structural acclimation response to the increasing drought (reduction of leaf-to-sapwood area ratio), thereby rejecting the functional equilibrium hypothesis for our study system. Instead, three of the six species increased photosynthetic area at the branch level. The observed dissimilar patterns of gas exchange, hydraulics and morphology across species seem to be equally successful given that photosynthesis at the leaf level was maintained at similar rates over the whole dry period.

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Abbreviations

A	Photosynthetic rate per area ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)
A_L	Branch leaf area (mm^2)
A_S	Cross-sectional stem area (mm^2)
GSF	Global site factor (unitless)
g_s	Stomatal conductance per area ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)
E	Transpiration rate per area ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)
K_h	Maximum conductivity ($\text{kg m MPa}^{-1} \text{ s}^{-1}$)
K_L	Leaf-specific hydraulic conductivity ($\text{kg m}^{-1} \text{ MPa}^{-1} \text{ s}^{-1}$)
K_S	Specific hydraulic conductivity ($\text{kg m}^{-1} \text{ MPa}^{-1} \text{ s}^{-1}$)
$P_{50\text{PLC}}$	Pressure causing 50% loss of K_h (MPa)
PPFD	Photosynthetic photon flux density ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)

Ψ_l	Leaf water potential (MPa)
Ψ_{pd}	Predawn leaf water potential (MPa)

Introduction

Plants of Mediterranean climates are exposed to a summer drought period that can last for several successive months (Archibold 1995). In contrast to many species from deserts (Levitt 1980; Smith and Nobel 1986) and tropical areas with strong rainfall seasonality (Lebrija-Trejos 2009), most Mediterranean plants retain their leaves during the summer and are therefore expected to be physiologically and morphologically adapted to maintain photosynthesis during dry periods (di Castri 1981; Joffre et al. 1999). Although plants experience a continuum of leaf water potentials, two contrasting strategies have been proposed (McDowell et al. 2008). At one extreme, plants rapidly close stomata when confronted with excessively dry soil conditions. Such plants limit transpiration, maintain relatively high (but negative) leaf water potentials and thus avoid hydraulic failure by cavitation of water columns in the xylem. At the other extreme, plants keep stomata open for longer periods of time, maintain relatively high transpiration rates and thus decrease leaf water potentials to more negative values during the summer. The declining water potential might cause hydraulic failure by inducing cavitation in the xylem (Tyree and Zimmermann 2002). Those strategies have been referred as isohydric and anisohydric strategies, respectively (Jones 1998; Tardieu and Simonneau 1998).

It is far from clear how the iso/anisohydric concept relates to the life history of plant species and associated plant traits in Mediterranean climates, where early successional shrubs and late-successional shrubs or trees may coexist for a long periods of time (Thompson 2005) because succession is very slow in these ecosystems. From a physiological point of view, early successional species are known for their high gas exchange rates (De Lillis and Federici 1993), and those high rates are accompanied by highly conductive xylem and leaves (Tyree and Zimmermann 2002 and references therein). Such high rates might be maintained during persisting droughts in the summer, although they may decline under extreme conditions. In contrast, late-successional species with lower gas exchange rates (Quero et al. 2006) are expected to remain physiologically active for much longer during the summer drought, since they consume water at a much slower rate. Actual proof for the existence of such different strategies is, however, limited, and it is particularly unclear how they relate to the isohydric/anisohydric concept (see Bonal and Guehl 2001, for tropical rainforest tree species).

From a morphological point of view, plants in drought-stressed environments are expected to invest proportionately more in structures involved in water acquisition and transport (functional equilibrium hypothesis, Brouwer 1962). It has been proposed that leaf-level homeostasis in water status is attained due to whole-plant changes in morphology and anatomy (Bhaskar and Ackerly 2006; Maseda and Fernandez 2006). For example, plants could adjust their hydraulic system to drought conditions through modification of the leaf-to-sapwood area ratio (Sterck et al. 2008; Martínez-Vilalta et al. 2009) to prevent the occurrence of low water potentials in plant tissues, reduce the risk of cavitation of water columns in the xylem (Zweifel and Zeugin 2008) and delay stomatal closure (Tyree and Zimmermann 2002).

To date, studies evaluating water-use strategies in plants have been based on the analysis of diverse variables, exploring either gas exchange between leaves and the atmosphere (Acherar and Rambal 1992; Sala and Tenhunen 1994; Medrano et al. 2009), hydraulic properties, such as vulnerability to xylem cavitation (Salleo et al. 1996; Iovi et al. 2009), or morphological adjustments (Sterck et al. 2008; Martínez-Vilalta et al. 2009). Although a number of studies on Mediterranean-type climates have combined gas exchange measures with hydraulic measures (i.e. Martínez-Vilalta et al. 2002; Bhaskar et al. 2007), we are not aware of any such study that included multiple plant species growing under natural field conditions and covering, with high temporal resolution, a whole-summer drought period. Moreover, it is far from clear how morphological adjustments are associated with the variation in physiological traits during ongoing summer drought for plants differing in life history. In this paper, we present a field study of six co-occurring evergreen woody species that are expected to include a variety of water-use strategies. The main objective of this study was to compare how these species varied in hydraulics, gas exchange and morphology over a period of 3 dry summer months, from the last precipitation in the first week of June until the first rains in September.

Based on this objective, we state three hypotheses. First, we expected to encounter both isohydric and anisohydric species in our study site, with the two species groups showing contrasting seasonal gas exchange. More specifically, isohydric species will achieve higher gas exchange rates early in the summer, but have lower rates later in the summer because they save water by stomatal closure. On the contrary, anisohydric species will be less productive under the more favourable conditions early in the summer, but will assimilate more (i.e. higher photosynthesis rates) under stressed conditions during late summer. Secondly, we expect coordination between the drought stress experienced (in terms of water potential) and plant attributes

that increase drought resistance (Pockman and Sperry 2000) during the Mediterranean summer. Finally, following the functional equilibrium model, we expect structural changes on leaf and sapwood area during the summer drought to mitigate water stress, resulting in a better water supply to leaves, expressed by a decreasing leaf-to-sapwood area ratio and a corresponding increase in leaf-specific conductivity.

Materials and methods

Study site

The study was conducted during the spring and summer of 2008 in Sierra de Cardena y Montoro Natural Park ('La Vegueta' area, 320–340 m a.s.l.; 38°14'N, 4°12'W; Cordoba, SE Spain) in a 5-ha fenced plot comprising a Mediterranean shrub community. This plot was selected because various approximately 1.5-m-tall plants of the six study species were all found in the same area, with the different individuals being scattered across the site, thus providing independent replicates [average distance 9 m, spatial pattern = random, 'average nearest neighbour' tool ArcGIS v9.2, (ESRI, Redlands, CA)]. Moreover, the area was fenced 2 years before the study year and was therefore protected against predation from large herbivores (*Cervus elaphus* L.), thus excluding a potentially confounding factor from this study. The climate is typical Mediterranean, with a mean annual precipitation of 752 mm (1989–2005), cold and rainy winters, lower rainfall in the spring and autumn and almost no rainfall in the summer. The average number of consecutive days without precipitation in the summer is 65 days (2001–2009), with maximum values of 93 and 89 days in 2008 and 2009, respectively. Average annual temperature is 15.3°C and the means of the coldest and hottest months (January and August) are 7.3 and 25.3°C, respectively. The bedrock is granodiorite and the soil is a regosol (Quero 2007).

Field sampling

Field data were collected from 12 May to 5 September 2008. The environmental variables included precipitation, radiation, vapour pressure deficit (VPD), temperature and physical soil properties. Precipitation was measured using an on-site pluviometer, and daily values of the remaining of variables were taken from the closest meteorological station (approx. 15 km apart, 38°03'N, 04°08'W, 208 m a.s.l.; Marmolejo, IFAPA, Junta de Andalucía, Spain).

Within the plot, we selected six species of different successional status that are typically found in the study site (Quero 2007) (Table 1): early successional (*Cistus*

Table 1 Summary of descriptive variables among studied species (n = 6)

Descriptive variables	<i>Pistacia lentiscus</i>	<i>Daphne gnidium</i>	<i>Quercus ilex</i>	<i>Myrtus communis</i>	<i>Cistus ladanifer</i>	<i>Olea europaea</i>
GSF (global site factor)	0.812 ± 0.065 a	0.706 ± 0.062 a	0.875 ± 0.025 a	0.781 ± 0.038 a	0.712 ± 0.059 a	0.735 ± 0.044 a
PPFD (μmol photon m ⁻² s ⁻¹)	1,320 ± 79 a	1,382 ± 5 a	1,374 ± 11 a	1,402 ± 5 a	1,385 ± 20 a	1,313 ± 37 a
Crown width (m)	1.96 ± 0.33 a	1.44 ± 0.23 ab	0.79 ± 0.07 b	1.74 ± 0.22 ab	1.53 ± 0.14 ab	1.74 ± 0.31 ab
Crown depth (m)	1.38 ± 0.19 ab	1.05 ± 0.21 b	0.97 ± 0.12 b	1.15 ± 0.15 ab	1.54 ± 0.24 ab	1.94 ± 0.21 a
Plant height (m)	1.38 ± 0.19 ab	1.43 ± 0.12 ab	1.04 ± 0.12 bc	1.15 ± 0.15 b	1.83 ± 0.21 ab	1.94 ± 0.21 a
Diameter main stem (mm)	28.93 ± 7.29 a	22.15 ± 5.24 a	25.29 ± 6.68 a	20.86 ± 5.03 a	24.3 ± 2.9 a	24.09 ± 4.92 a
Branch length (cm)	20.0 ± 1.0 a	18.2 ± 1.3 ab	14.4 ± 1.6 b	19.9 ± 0.5 a	20.2 ± 0.9 a	20.3 ± 1 a
P _{50PLC} (MPa) ^a	3.09 ± 0.57 b	3.34 ± 0.17 b	5.63	4.06 ± 0.49 ab	6.2 ± 0.8 a	8.45
K _S (kg m ⁻¹ MPa ⁻¹ s ⁻¹)	0.694 ± 0.053 a	0.455 ± 0.012 ab	0.108 ± 0.044 c	0.784 ± 0.023 a	0.652 ± 0.068 a	0.140 ± 0.041 bc
K _L (kg m ⁻¹ MPa ⁻¹ s ⁻¹)	1.98 × 10 ⁻⁴ ± 3.5 × 10 ⁻⁵ ab	3.51 × 10 ⁻⁴ ± 9.05 × 10 ⁻⁵ a	4.91 × 10 ⁻⁵ ± 1.64 × 10 ⁻⁵ b	3.22 × 10 ⁻⁴ ± 9.98 × 10 ⁻⁵ a	4.51 × 10 ⁻⁴ ± 6.78 × 10 ⁻⁵ a	8.29 × 10 ⁻⁵ ± 2.42 × 10 ⁻⁵ b

Values are given as the mean ± standard error (SE). Values followed by different lowercase letters are significantly different among species at p < 0.05 (Tukey's Honestly Significant Difference Post-hoc test)

See Abbreviations and text for definitions and descriptions of the variables

^a P_{50PLC} [pressure causing 50% loss of maximum conductivity (K_b)] values for *Quercus* and *Olea* were taken from Corcuera et al. (2004) and Ennajeh et al. (2008) (see text)

Fig. 1 Radiation (thick line), mean temperature (thin line), vapour pressure deficit (VPD; dashed line) and precipitation (white columns at bottom of graph) during the study period. Precipitation was measured on site; all other data are from the Marmolejo meteorological station (38°03'26"N, 04°07'46"W, 208 m a.s.l.), IFAPA, Junta de Andalucía, Spain

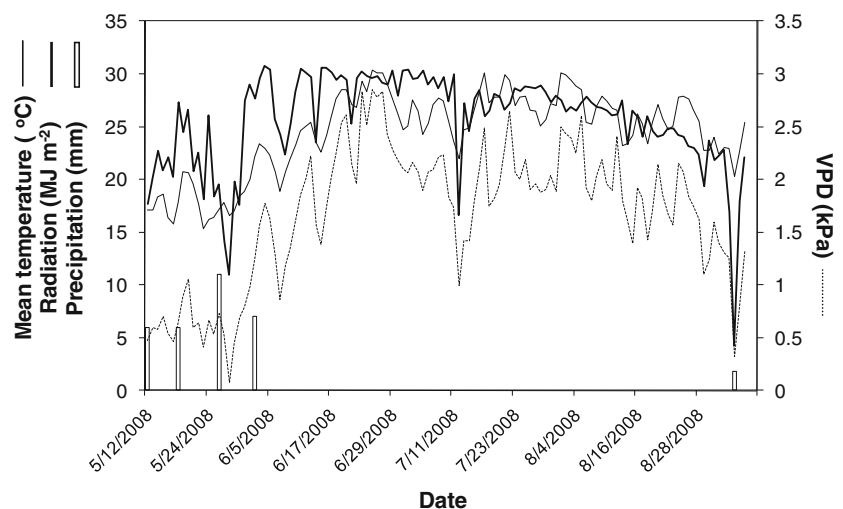


Table 2 Physical soil properties on the study site

	Mean \pm standard error
Bulk density (g cm^{-3})	1.45 ± 0.04 (11)
Clay (%)	3.9 ± 0.3 (4)
Loam (%)	9.4 ± 2.5 (4)
Sand (%)	86.8 ± 2.8 (4)
Field capacity (%)	13.2 ± 0.7 (4)
Soil compaction (MPa)	3.26 ± 0.3 (15)
Soil depth (cm)	50.5 ± 4.6 (25)

Numbers in parenthesis indicate sample size

Soil samples were randomly taken under the studied plants

ladanifer L., *Daphne gnidium* L.) and late successional shrubs (*Pistacia lentiscus* L., *Myrtus communis* L.) and trees [*Quercus ilex* ssp. *ballota* (Desf.) Samp., *Olea europaea* var. *silvestris* Brot.]; these will be referred to by their genus name hereafter. Six sun-exposed individuals per species were labelled for monitoring. To characterize the light environment, a global site factor (GSF) was quantified per individual with hemispherical photography, according to Quero et al. (2008a), and PPFD measurements were taken above each plant at midday on 30 July using an EMS7 canopy transmission meter (PP-systems, Hitchin, UK) (Table 1). All 36 individuals had a similar size of 1.34 ± 0.54 m [mean height \pm standard deviation (SD)]. Gas exchange and water potential measurements were performed weekly from 22 May to 28 July, and an extra measurement was performed on 4 September, just before the first autumn rains. As no precipitation events were registered from 4 June to 6 September, we monitored the entire 2008 summer drought period (Fig. 1). During this year, the cumulative summer precipitation (7 mm; June–August) was slightly lower than the averaged 2000–2008 value (10.7 mm; June–August), suggesting that the study

period represented a typical summer season. In addition, random samples of soil were taken under the studied plants to measure physical soil properties (Table 2). Bulk density was measured following standard soil methods (dry soil to soil volume ratio). The sieved <2-mm-diameter fraction (fine earth) was used to determine particle size distribution with the pipette method (Gee and Bauder 1986). Field capacity was calculated following to Saxton et al. (1986), and soil compaction was measured with a penetrometer (Penetrologger; Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) using a cylindrical probe with 3.3-cm² and 30°-angle cone. Soil depth was determined by making a hole until the bedrock was reached.

Predawn leaf water potentials (Ψ_{pd}) were measured on each sampling date between 3 and 4 a.m. in vertically oriented leader twigs (Scholander et al. 1965) at breast height, using a pressure bomb (range 0–15 MPa; Manofrígido, Lisbon, Portugal). Photosynthesis was measured on fully expanded leaves at breast height during the morning (8–10 a.m., solar time), when photosynthetic rates are at their maximum (Haase et al. 1999; Munné-Bosch et al. 2003). Although we did not measure diurnal courses in photosynthesis, the very similar trends observed over the morning across species were remarkable (see Results section) and suggest that among-species differences on diurnal courses were limited. A gas-exchange portable analyser (Ciras-2; PP-System), adjusted to have constant conditions of CO₂ concentration (390 ppm), flow (200 cm³ min⁻¹) and PPFD [800 $\mu\text{mol m}^{-2} \text{s}^{-1}$; this value provided enough to light saturate all species based on per-species light response curves (data not shown)], was used to measure photosynthesis. Each leaf was kept inside the chamber until measured values stabilized (approx. 2 min). Net assimilation rate (A), transpiration rate (E), stomatal conductance (g_s) and temperature inside the cuvette were recorded three times, and the average value was used as the data point in

the analysis. When a leaf did not completely fill the cuvette area (2.5 cm^2), a photograph was taken using a digital camera (Cyber-shot DSC-S75; Sony, Tokyo, Japan). In order to correct measurements by actual leaf area, the images were analysed using Image Pro-Plus image analysis software v4.5 (Media Cybernetic, Bethesda, MD). In the same branch, additional water potential measurements (Ψ_1) were synchronized with the measurement of gas exchange. Immediately after every photosynthetic measurement, an adjacent leafy twig was collected and measured with the pressure bomb.

At the beginning of sampling, one branch per individual was labelled in order to characterize morphological changes in leaf area and stem section throughout the drought period at branch level. At each of the sampling dates, stem diameter was measured to the nearest 0.01 mm at the same position of the stem using a digital calibre, and the number of fully expanded leaves was counted. Cross-sectional stem area (A_S) was calculated from measured diameters, taking the area of a circle into consideration. In order to avoid monitoring effects for the remaining drought period and to prevent potential branch death at the end of the drought period (September), only a subsample of fully expanded leaves per branch (range 5–20, depending on species) was harvested on 1 August, and laminas were scanned and processed with Image Pro-Plus image analysis software v4.5. We estimated branch leaf area (A_L) at every census by multiplying the number of leaves by the average leaf area of fully expanded leaves.

Vulnerability to xylem embolism

Vulnerability to xylem embolism was measured on four of the six studied species (see below for details). We harvested 24 stem elements, one per studied individual, at the end of the experiment (5 September 2008). Stem segments were collected of each branch that had been also selected for water potential and gas exchange measurements. In the laboratory, leaves were removed from the stems, and approximately 25-cm-long wood segments were re-cut underwater to be used for hydraulic measurements. Segments were inserted inside a pressure chamber, with both ends protruding. Proximal ends were connected to the measuring circuit, and hydraulic conductivity (K_h , $\text{kg m MPa}^{-1} \text{ s}^{-1}$) and vulnerability curves were measured according to Sperry and Saliendra (1994; but see also Martínez-Vilalta et al. 2002). To obtain the maximum conductivity, we first flushed the segments at high pressure (approx. 100 kPa) with the measure solution of KCl (degassed and filtered at $0.22 \mu\text{m}$) for 60 min to remove all native embolisms and then, for 10 min, lowered the pressure difference driving the flow to approximately 6 kPa and raised the pressure inside the chamber to 0.1 MPa for

10 min. Next, the pressure was lowered to a basal value of 10 kPa, and conductivity was measured again after the system equilibrated, which took approximately 15 min. The process was repeated at progressively higher injection pressures until the loss of conductivity was complete or a pressure of 7 MPa was reached. The percentage loss of hydraulic conductivity (PLC) after each applied pressure (P) was calculated by referring the conductivity after the treatment to the conductivity at 0.1 MPa: $\text{PLC} = 100 \times [1 - (K_{h,p}/K_{h,0.1})]$ (Martínez-Vilalta et al. 2002). PLC was plotted against P using the model of Pammenter and Vander Willigen (1998),

$$\text{PLC} = \frac{100}{1 + e^{a \times (P - P_{50\text{PLC}})}} \quad (1)$$

where $P_{50\text{PLC}}$ is the pressure causing a 50% loss of K_h , and a is related to the slope of the curve. A curve was adjusted for each segment using the nonlinear estimation module in STATISTICA ver. 7.0; Statsoft, Tulsa, OK), always resulting in good fits to the measured data points ($R^2 \geq 0.75$ in all cases, $p < 0.05$). $\text{PLC}_{50\text{PLC}}$ data for *Quercus* and *Olea* were taken from published vulnerability curves using the branch dehydration technique (Corcuera et al. 2004; Ennajeh et al. 2008, respectively), since the air-injection technique has methodological problems related to the very long vessels found in both species (Martínez-Vilalta et al. 2002 for *Quercus*; Hervé Cochard, personal communication, for *Olea*).

Specific hydraulic conductivity, K_S ($\text{kg m}^{-1} \text{ MPa}^{-1} \text{ s}^{-1}$) was calculated as the ratio between maximum K_h and mean cross-sectional area of the segment (without bark), and leaf-specific hydraulic conductivity, K_L ($\text{kg m}^{-1} \text{ MPa}^{-1} \text{ s}^{-1}$), was calculated as the quotient between maximum K_h and distal leaf area, where the leaf area was measured with a leaf area meter (Li-Cor-3100; Li-Cor, Lincoln, NE). An estimate of percent loss of hydraulic conductivity (i.e., native embolism) over the whole summer was calculated using vulnerability curves obtained in the lab and Ψ_1 measured in the field.

Data analyses

Water potentials, percent loss in hydraulic conductivity in the field, stomatal conductance and photosynthetic measurements were analysed using repeated measures analysis of variance (ANOVA; Proc GLM, STATISTICA, ver. 7.0; Statsoft) where species was introduced as a between-subject factor and date as a within-subject factor. When the differences were significant, a multiple comparison of means (post hoc Tukey's Honestly Significant Difference test) was carried out. We examined bi-variation relationships between the studied variables through least-squares fitting. Significance was fixed at the 0.05 alpha level throughout the

study. Variables were normalized and log-transformed to avoid heteroscedasticity when required (Zar 1996).

Results

Characterization of the dry environment

The experiment started on 22 May 2008 and lasted until 5 September 2008. The last showers of the summer 2008 fell on 4 June (7 mm), following which time the plants did not receive any rain until the end of the experiment. Over this dry period of 92 days, we expected that plants would be gradually exposed to drier soil conditions. Atmospheric conditions, however, also changed. The daily radiation, mean temperature and VPD increased in June, stabilized until the beginning of August and then declined slowly during August (Fig. 1). Analysis of the physical soil properties revealed a high sand content (approx. 87%, Table 2), which is due to the granodiorite nature of the bedrock (Quero 2007). This high sand content could explain the high soil penetration resistance and bulk density as well as the low water holding capacity, which may have some effects on the root capacity to penetrate the soil and to reach the water. Thus, plants had to face severe soil conditions, which were aggravated during the dry summer (see results below).

Plant's perception of environmental drought at the leaf level

The dynamics of the physiological (Fig. 2) and structural traits (Fig. 3) suggest that the plants studied strongly responded to the water dynamics in the soil and/or atmospheric conditions (see also Table 3). A repeated ANOVA with species and time as explanatory variables for the physiological and structural traits (Table 3) showed that the predawn and midday water potentials differed between species and time, with a significant time \times species interaction. Based on leaf water potentials at the end of the study period, some species tended to maintain the leaf water potential at the same level and closer to zero across time (the isohydric *Pistacia*, *Daphne* and *Quercus*), and others reduced the water potentials considerably over time (the anisohydric *Cistus*, *Myrtus* and *Olea*) (Fig. 2a, b). After the last rain showers on 4 June, both stomatal conductance and photosynthesis declined in all species until the end of the study period (Fig. 2c, d). Transpiration showed a similar pattern over time, with the exception of an increase over the first three to five sampling dates (Fig. 2e), probably because the transpirational demand increased over that period (Fig. 1). The species with the more negative water potentials (*Cistus*, *Myrtus* and *Olea*)

lost a higher percentage of their conductivity at the end of the drought period (Fig. 2f).

Plant's response to environmental drought at the branch level

Interactive effects of species \times time were observed for both morphological variables, A_S and A_L , as demonstrated by repeated measures ANOVAs (Table 3, Fig. 3). A_S increased over time for *Pistacia* and *Quercus*, but did not change significantly for the other species (Fig. 3a). A_L increased in *Pistacia*, *Quercus*, *Myrtus* and *Cistus*, but not in the other species (Fig. 3b). Accordingly, the resulting variable ($A_L:A_S$) also showed a significant interaction between time and species: the $A_L:A_S$ ratio increased with time for *Pistacia*, *Myrtus* and *Cistus*, but not for the other species (Fig. 3c). Native K_L decreased with time in anisohydric species (Fig. 3d), which can be attributed to the loss of conductivity over time (Fig. 2f). For isohydric species, the pattern in K_L did not differ with time, because these plants barely faced any loss in conductivity (native PLC <20%, Fig. 2f).

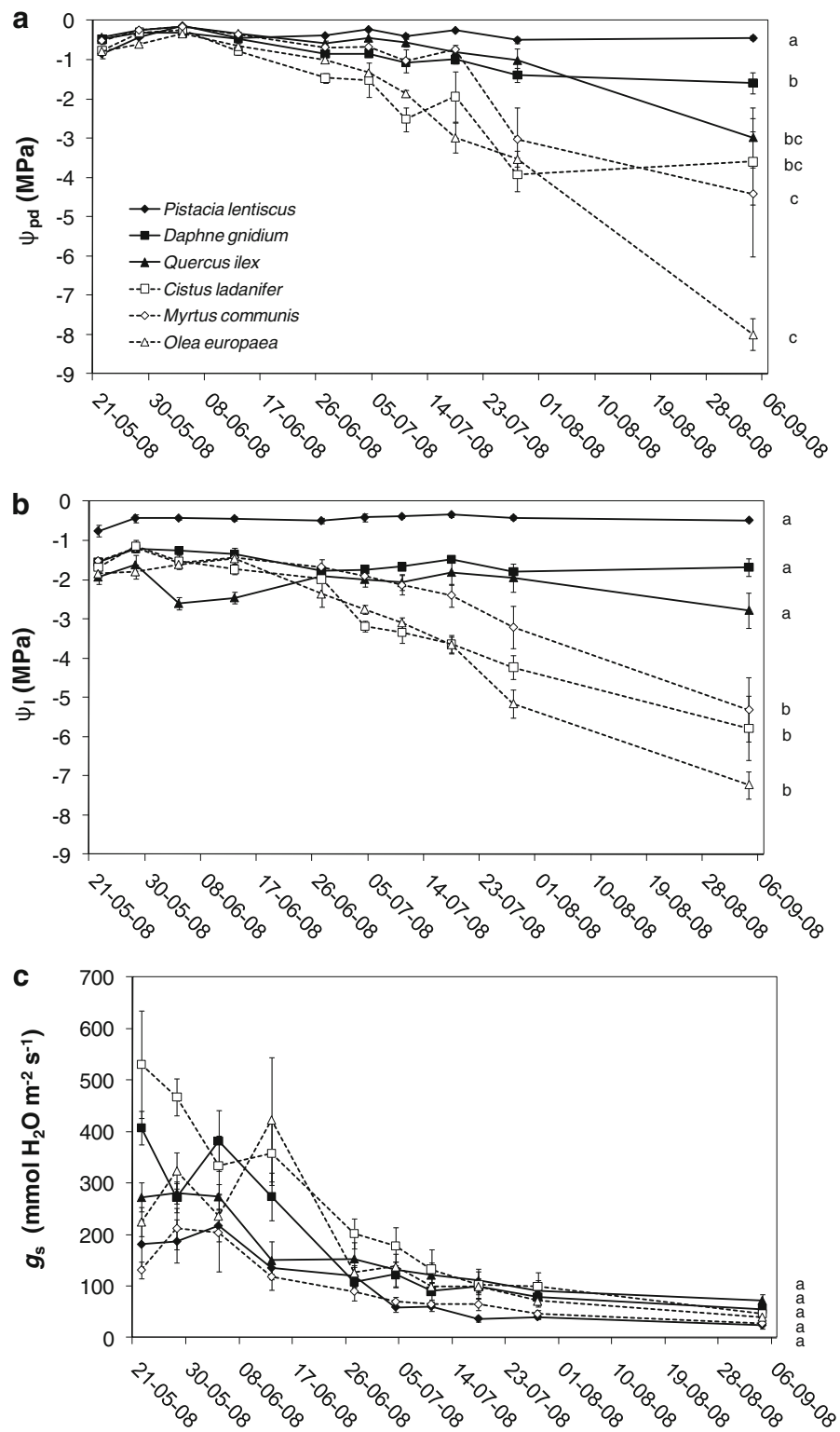
Maximum hydraulic conductivities and vulnerability to embolism

Specific hydraulic conductivity (K_S) varied among species, with both tree species (*Quercus* and *Olea*) having the lowest K_S values (Table 1). There were no consistent differences in K_S between isohydric and anisohydric species. Leaf-specific conductivity (K_L) followed similar trends to K_S , although a trend of increasing K_L was observed from tree to pioneer species. Vulnerability to xylem embolism varied markedly among species. The parameters of the fitted vulnerability curves, particularly P_{50PLC} , reflected differences between isohydric and anisohydric groups, with P_{50PLC} always being higher for the anisohydric species within a successional group. Among species, *Olea*, *Cistus* and *Quercus* were the most resistant (higher P_{50PLC}), followed by *Myrtus*, *Daphne* and *Pistacia* (Table 1).

Causal links among gas exchange, water relations and hydraulics

The trend towards anisohydric species being more resistant to xylem embolism (within a successional group at least) was not enough to compensate for their lower minimum leaf water potential, implying that hydraulic safety margins were narrower in the anisohydric species (Fig. 4). As a result, predicted levels of native embolism were lower in the isohydric species (PLC <20%, Fig. 2f) than in the anisohydric ones (PLC = 45–65%, Fig. 2f) at the end of the drought period.

Fig. 2 Water potentials (Ψ_{pd} predawn leaf water potential, Ψ_l leaf water potential), stomatal conductance (g_s), photosynthetic rate (A), transpiration rate (E), and percentage of loss of hydraulic conductivity in the field during the study period (spring and summer 2008). Ψ_{pd} was measured between 3 and 4 a.m., and Ψ_l between 8 and 10 a.m. (solar time), coordinated with gas exchange measurements. *Black and white dots* indicate isohydric and anisohydric behaviour, respectively, *squares, diamonds, triangles* pioneer shrubs, late-successional shrubs and trees, respectively. *Different letters* indicate significant differences among species ($p < 0.05$, post hoc Tukey's Honestly Significant Difference test) at the end of the drought period. *Error bars*: Standard errors (SE) ($n = 6$)

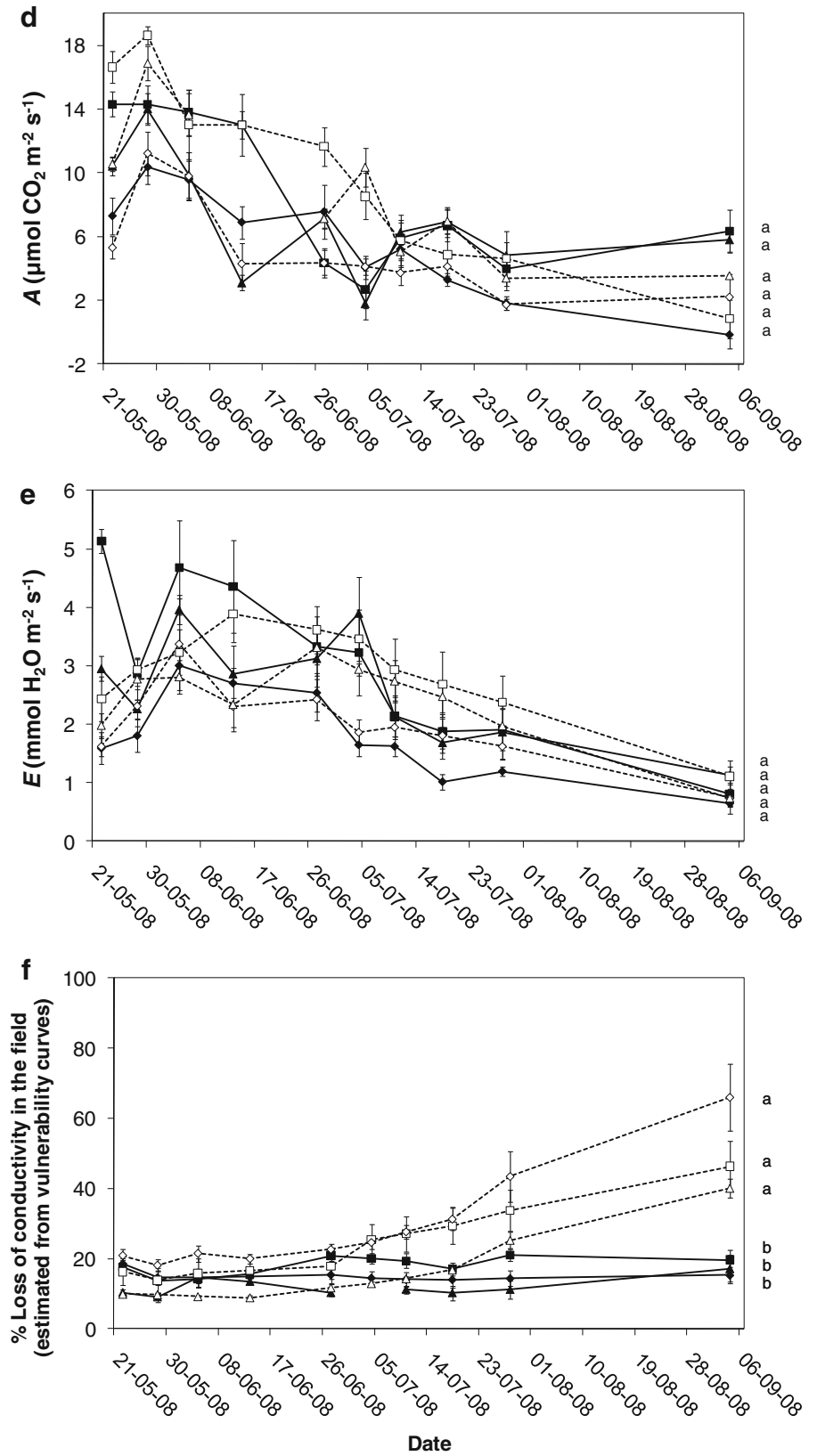


Discussion

Our results suggest that the six co-existing species cover a range of hydraulic plant strategies, from isohydric to

anisohydric. Based on the observed minimum leaf water potentials, these species can be ordered along an iso–anisohydric axis from *Pistacia*², *Daphne*¹, *Quercus*³, *Myrtus*², *Cistus*¹ to *Olea*³, where different superscript numbers

Fig. 2 continued



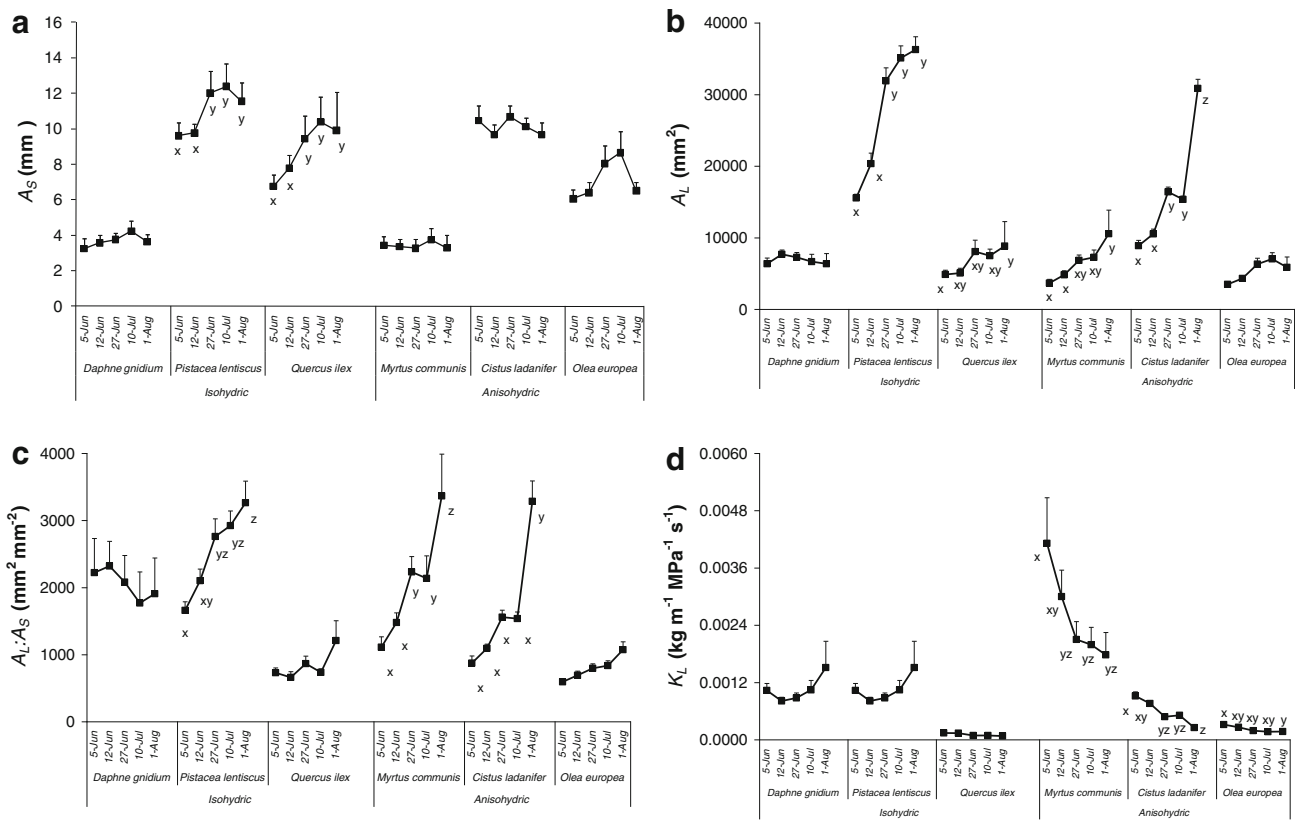


Fig. 3 Morphological changes at the branch level during the study period (spring and summer 2008): A_S Cross-sectional stem area, A_L branch leaf area, K_L native leaf-specific hydraulic conductivity. When

shown, *different letters* indicate significant differences within species ($p < 0.05$, post hoc Tukey's Honestly Significant Difference test) during the study period. *Error bars*: SE ($n = 6$)

refer to different successional groups, namely: 1, early successional shrubs; 2, late successional shrubs; 3, late successional tree species. Surprisingly, within each successional group always one species exhibited more anisohydric behaviour and the other exhibited a more isohydric one. Contrary to our expectations, isohydric and anisohydric species did not systematically differ in P_{50PLC} . However, within a successional group, the anisohydric species were always more resistant to embolism. Additionally, P_{50PLC} correlated with minimum leaf water potentials across species, which agrees with the theory that vulnerability to embolism limits the water potentials at which each species can operate. To the contrary, the species with more negative leaf water potentials had higher P_{50PLC} values, which allowed the plant to function close to the point of hydraulic failure by cavitation despite lower water potentials in the xylem. Indeed, these plants had a much higher loss of conductivity at the end of the dry season. Surprisingly, plants of all six species—and thus including the full range of hydraulic strategies—were able to maintain photosynthesis and invested in new leaf area at the branch level over the dry period. These results are remarkable and contrast with our initial hypotheses; as such, they may shed

new light on successful plant strategies in Mediterranean climates. In the following paragraphs, we discuss our hypotheses and then draw general conclusions.

Although the six species coexist in the same environment, leaf water potentials varied greatly among the species, particularly at the end of the dry period. Our first expectations was that more isohydric plants, i.e. those that close stomata at relatively high leaf water potentials, than anisohydric plants would show higher rates of net photosynthesis during and after the last showers in the early summer, but that the former ‘would pay’ for that activity with an earlier decrease of stomatal conductance and lower carbon gain later during the summer compared to the latter (Fig. 5a). This was clearly not the case: whatever the leaf water potential values, stomatal conductance, photosynthetic, and transpiration rates were relatively similar and low across species at the end of the dry period (Fig. 5b). Opposite results to those reported here, i.e. showing leaf water potential–gas exchange relationships, are usually found in the literature and have been found in different species and systems (Wullschleger et al. 1998). In our study, the more isohydric plants combined higher leaf water potentials with higher Ψ_{pd} (a measure of drought

Table 3 Results of the repeated measures analyses of variance (ANOVAs) for the study variables at the leaf and branch level, according to the factors species and time

Study variables	Factors		Interaction	R^2
	Species	Time		
Leaf level				
Ψ_{pd}	23.2***	57.3***	16.3***	96.8
Ψ_1	35.0***	26.9***	25.1***	87.1
g_s	12.7**	46.1***	13.4***	72.2
A	11.3**	46.6***	18.1***	75.9
Percent loss field conductivity	24.8***	23.2***	20.9***	68.9
Branch level				
A_S	70.7***	2.7***	2.5**	75.9
A_L	67.0***	12.1***	12.2***	91.7
$A_L:A_S$	37.2***	14.2***	13.4***	64.9
K_L	82.2***	5.4***	2.6***	90.7

The proportion of the explained variance [SS_x/SS_{total} (%)] and the level of significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) for each factor and the interaction are indicated

See Abbreviations and text for definitions and descriptions of the variables

R^2 Percentage of total variance explained by the model

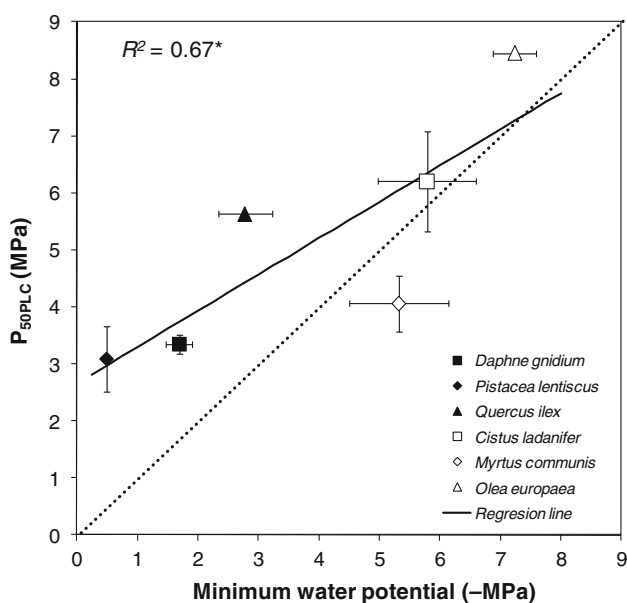


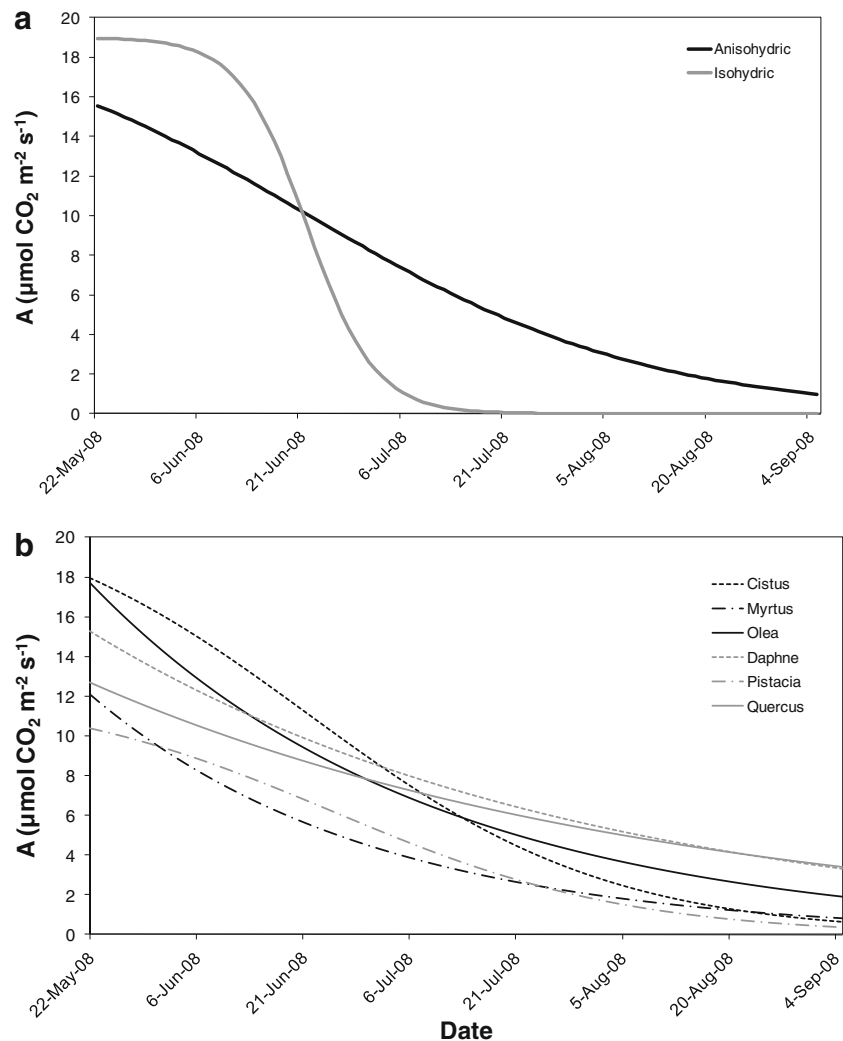
Fig. 4 Relationship between minimum water potential and pressure causing 50% loss of maximum conductivity (P_{50PLC}). Values of P_{50PLC} for *Quercus* and *Olea* were taken from Corcuera et al. (2004) and Ennajeh et al. (2008), respectively. Black and white dots indicate isohydric and anisohydric behavior, respectively, squares, diamonds and triangles pioneer shrubs, late-successional shrubs and trees, respectively. Dotted line is the 1:1 line. Asterisk indicates a significant relationship ($p < 0.05$). Error bars: SE ($n = 6$)

intensity; Resco et al. 2009), such that they were also able to maintain transpiration and carbon gain during the whole summer. Ψ_{pd} differed across species, and these differences became larger during the drought period between May and September (Fig. 2a, b). These differences are remarkable,

since the selected individuals grow within meters of each other, sharing the same environment. The Ψ_{pd} suggest that the soils around the roots of isohydric plants did not dry out as did the soils around the roots of the more anisohydric plants. The most obvious explanation for such differences in leaf water potentials is different rooting depth, as suggested by Bhaskar et al. (2007). Alternatively, it has been suggested that those differences in plant Ψ_{pd} do not necessarily reflect differences in access to water and rooting depth (Donovan et al. 2003), but might result from differences in night transpiration. Species that face ongoing night transpiration (Snyder et al. 2003; Howard et al. 2009; Rogiers et al. 2009) maintain the depletion zone around fine roots and create low Ψ_{pd} , while species without night transpiration might restore the water conditions around fine roots during the night. Concentrations of apoplastic solutes in leaf intercellular spaces have also been proposed as a mechanism contributing to differences in Ψ_{pd} (Donovan et al. 1999). Finally, we might also consider differences in fine root growth dynamics, with species able to grow rapidly to soil pockets with water ('water foraging') while others cannot; however, we are not aware of any studies reporting on such interspecific differences across co-existing plant species in Mediterranean areas.

Secondly, we expected coordination between the perceived drought stress and plant vulnerability to xylem cavitation, which was reflected in the Ψ_1-P_{50PLC} across the species' relationship (Fig. 4). It is reasonable that species experiencing lower leaf water potentials in the field show a higher resistance to xylem embolism. This relationship has been found in several studies on Mediterranean ecosystems

Fig. 5 Photosynthetic measurements (A photosynthetic rate per area) during the drought period: expected (a) and observed (b) trends. Observed trends are the result of fitting empirical data to a sigmoid equation ($R^2 > 0.9$ for all cases). *Black* and *grey lines* Anisohydric and isohydric species, respectively



(Martínez-Vilalta et al. 2002; Jacobsen et al. 2007), but not in all (Miranda et al. 2007). Whatever the case, it seems that a high resistance to xylem embolism (high $P_{50\text{PLC}}$ values) is not always translated into a better performance. Indeed, Vilagrosa et al. (2003) showed in a comparison of *Quercus coccifera* versus *Pistacia lentiscus* that the species more sensitive to embolism (i.e. *Pistacia*) had higher survival rate under field conditions. Our results suggest that in this community the capacity to cope with drought is related to hydraulic properties, such as resistance to xylem embolism. However, patterns of photosynthesis and transpiration are quite similar while the different species seem to undergo very different levels of drought intensity (i.e. Ψ_{pd}). This situation would appear to be a paradox: despite huge variation in predawn and midday water potentials over 3 months without rain, the gas exchange dynamics remained positive and similar across species during the morning hours. Because the species already differed markedly in their hydric status during the morning, we expect that such similar transpiration rates cannot be

explained from stored water sources in the sapwood or phloem (Waring and Running 1978; Phillips et al. 2002; Ryan 2002) because such storage largely depends on the hydraulic status itself (Zweifel et al. 2007).

Based on the functional equilibrium hypothesis (Brouwer 1962; Cannell and Dewar 1994), we expected that plants of all species would tune their hydraulic structure to improve the supply of water to the leaves by reducing the leaf-to-sapwood-area ratio during the summer drought (Sterck et al. 2008; Martínez-Vilalta et al. 2009). In contrast to this expectation, three of the six species showed the opposite response: these three species produced leaves more rapidly than sapwood area. The other three species also produced new leaf area and sapwood area but did not show a significant trend in the leaf-to-sapwood-area ratio (Fig. 3c). We thus concluded that none of the plants showed the expected structural acclimation response to the increasing drought condition (Maseda and Fernandez 2006) and, from this perspective, rejected the functional equilibrium hypothesis in our study system. In this regard, it should be

pointed out that since acute levels of water stress were only observed for a short period at the end of the summer, there might have been no time for structural responses (Quero et al. 2006, 2008b). Instead, half of the studied species increased photosynthetic area, which seems to have been a successful adaptation given that photosynthesis at the leaf level was maintained over the whole dry period in all species (Fig. 2d).

The results reported here show that the six Mediterranean species studied performed similarly in terms of gas exchange during a summer drought lasting for 3 months in which virtually no rain fell—despite increasing differences in perceived water stress (leaf water potential) and access to soil water. Therefore, the differences in leaf water potentials (i.e., the iso–aniso-hydric axis found) were likely to be more related to the need of the plant to avoid large losses of conductivity in the xylem due to embolism (Jacobsen et al. 2007) than to functional tradeoffs associated to the maintenance of gas exchange during the dry season. Moreover, these plants did not respond to the ongoing drought by reducing their leaf area. To the contrary, their structural responses were opposed to the functional equilibrium hypothesis and suggest that the transport efficiency in branches may not be a bottleneck for water transport and gas exchange during the summer drought in the studied species.

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