Oecologia (2010) 162:11–21
DOI 10.1007/s00442-009-1447-1

PHYSIOLOGICAL ECOLOGY - ORIGINAL PAPER

Hydraulic lift and tolerance to salinity of semiarid species: consequences for species interactions

Cristina Armas · Francisco M. Padilla · Francisco I. Pugnaire · Robert B. Jackson

Received: 5 December 2008 / Accepted: 10 August 2009 / Published online: 4 September 2009

Abstract The different abilities of plant species to use ephemeral or permanent water sources strongly affect physiological performance and species coexistence in water-limited ecosystems. In addition to withstanding drought, plants in coastal habitats often have to withstand highly saline soils, an additional ecological stress. Here we tested whether observed competitive abilities and C–water relations of two interacting shrub species from an arid coastal system were more related to differences in root architecture or salinity tolerance. We explored water sources of interacting *Juniperus phoenicea* Guss. and *Pistacia lentiscus* L. plants by conducting physiology measurements, including water relations, CO_2 exchange, photochemical efficiency, sap osmolality, and water and C isotopes. We also conducted parallel soil analyses that included electrical conductivity, humidity, and water isotopes. During drought, *Pistacia* shrubs relied primarily on permanent salty groundwater, while isolated *Juniperus* plants took up the scarce and relatively fresh water stored in upper soil layers. As drought progressed further, the physiological activity of *Juniperus* plants nearly stopped while *Pistacia* plants were only slightly affected. *Juniperus* plants growing with *Pistacia* had stem-water isotopes that matched *Pistacia*, unlike values for isolated *Juniperus* plants. This result suggests that *Pistacia* shrubs supplied water to nearby *Juniperus* plants through hydraulic lift. This lifted water, however, did not appear to benefit *Juniperus* plants, as their physiological performance with co-occurring *Pistacia* plants was poor, including lower water potentials and rates of photosynthesis than isolated plants. *Juniperus* was more salt sensitive than *Pistacia*, which withstood salinity levels similar to that of groundwater. Overall, the different abilities of the two species to use salty water appear to drive the outcome of their interaction, resulting in asymmetric competition where *Juniperus* is negatively affected by *Pistacia*. Salt also seems to mediate the interaction between the two species, negating the potential positive effects of an additional water source via hydraulic lift.

Keywords *Juniperus phoenicea* · *Pistacia lentiscus* · Root system · Stable isotopes · Water sources

Communicated by Todd Dawson.

C. Armas and F. M. Padilla have contributed equally to this paper.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-009-1447-1) contains supplementary material, which is available to authorized users.

C. Armas · R. B. Jackson
Department of Biology, Duke University,
Box 90338, Durham, NC 27708, USA

Present Address:
C. Armas (✉) · F. M. Padilla (✉) · F. I. Pugnaire
Estación Experimental de Zonas Áridas,
Consejo Superior de Investigaciones Científicas,
General Segura 1, 04001 Almería, Spain
e-mail: cris@eeza.csic.es

F. M. Padilla
e-mail: fpadilla@eeza.csic.es

Introduction

Primary productivity in arid ecosystems is a function of water availability (Ehleringer et al. 1999; Huxman et al. 2004), which depends on both the temporal and spatial distribution of rainfall and on plant community composition. Rooting depth and access to soil moisture also influence plant C–water relations in such environments

(Noy-Meir 1973; Schwinning et al. 2005). Species with shallow root systems are unable to use deep soil water sources during drought and often experience water shortages as a result. By contrast, deep-rooted species may tap more deeply stored water (Haase et al. 1996; Jackson et al. 1999; Richards and Caldwell 1987) and can more successfully deal with water stress in dry periods (Ackerly 2004; Canadell et al. 1999; Padilla and Pugnaire 2007), extending their photosynthetic activity far into the dry season (Canadell and Zedler 1995; Haase et al. 1999). Species with dual root systems—shallow, horizontal roots and deep, tap roots—would thus be at an advantage in such systems, as they can use rainfall that infiltrate shallow soils as well as deeper soil water (Dawson and Pate 1996; Schwinning et al. 2002). Such dimorphic roots allow some plants to maximize water use throughout the year and to increase their competitive abilities (Ehleringer and Dawson 1992; Schulze et al. 1998; Williams and Ehleringer 2000).

Plant interactions are often mediated by access to water and the ability of plants to use ephemeral or permanent water sources, both of which have important consequences for ecosystem water and C balances and for plant responses to climate variability (Jackson et al. 1996; Schenk and Jackson 2002; Schlesinger et al. 1990; Schulze et al. 1996). There is clear evidence that neighboring plants in arid environments compete for water when root systems overlap, so differences in rooting depth and the ability to utilize different water sources may be mechanisms of species coexistence through niche differentiation and hydraulic lift-mediated facilitation (Dawson 1993; Filella and Peñuelas 2003b; Franco and Nobel 1990; Williams and Ehleringer 2000; Zou et al. 2005). However, such mechanisms of interaction remain poorly understood (Casper and Jackson 1997; Schwinning et al. 2004).

Rain scarcity in arid coastal areas is exacerbated by salinity and the very low water-holding capacity of sand. Soil salinity limits the ability of plants to take up water, reduces growth rate, and, like water stress, can lead to a decrease in water potential (Ψ) that further limits water uptake (Hasegawa et al. 2000). Overall, the physiological responses of plants to salt stress and water shortage are similar in many ways (Jakab et al. 2005; Munns 2002), but the mechanisms by which plants deal with salt stress differ among species (Ashraf and Harris 2004; Greenway and Munns 1980).

Research in an arid coastal sand dune system has shown contrasting physiological responses to precipitation of two dominant shrub species, *Juniperus phoenicea* subsp. *turbinata* and *Pistacia lentiscus* (Armas and Pugnaire 2009), whose interactions are mediated by water availability; the C/water relations of juniper plants depended strongly on the amount and temporal distribution of rainfall, while the co-occurring *Pistacia* (hereafter called "lentisc") displayed high and steady physiological activity throughout the year relatively independent of rainfall patterns. When both species grew nearby, strong asymmetric competition took place, and performance and survival of juniper was negatively affected by lentisc, particularly during dry summers. By contrast, lentisc was relatively unaffected by the presence of juniper. In addition, juniper seedlings were less able to withstand highly saline soils than lentisc seedlings were.

In this study we tested whether the physiological constraints of juniper in summer were caused by its lack of deep roots to access the salty groundwater under the dunes or if instead it had low tolerance to water salinity. We propose that the different ability of the two species to use saline water determines the competitive outcome of the interaction between them. Specifically, we hypothesized that: (1) the shallow-rooted juniper would use fresh water stored in the upper soil layers, (2) the deep-rooted lentisc would be able to use both salty groundwater and fresh water stored in the dunes, and (3) differential abilities and overlap in root systems would harm *Juniperus* performance more than *Pistacia* performance. We conducted physiological measurements, including water relations, CO_2 exchange, photochemical efficiency and sap osmolality, analyses of stable isotopes of C ($\delta^{13}C$), O ($\delta^{18}O$), and H ($\delta^{2}H$) of plants and soils, and measured soil electrical conductivity (EC) and moisture to identify the source of water used by plants. We also performed a greenhouse experiment to determine species' tolerances to salinity.

Materials and methods

Study site and species

The study was conducted in a coastal dune system in the Punta Entinas-Sabinar Nature Reserve, Almería, Spain (36°41′N, 2°42′W; 0–8 m elevation). The local climate is dry Mediterranean, with an annual rainfall of 220 mm. Mean monthly temperatures range between 12°C in winter and 30°C in summer, with high relative air humidity (mean around 70%). Sand dunes between 3 and 8 m in height formed over quaternary fossil beaches are stabilized by plants and are bisected by 1- to 1.5-m-deep valleys perpendicular to the coastline. The groundwater beneath the dune system is saline (25.3 ± 0.16 dS m^{-1}, soil EC; Pulido-Bosch et al. 1991), but soils from the top of the dune system (0- to 1-m depth) do not exceed 0.4 dS m^{-1} (Armas and Pugnaire 2009).

Vegetation covers 48% of the dune tops and is distributed in discrete patches dominated by co-occurring juniper and lentisc shrubs, with additional patches separated by bare or low-cover gaps where these species occur isolated

from each other. *Juniperus phoenicea* subsp. *turbinata* (Cupressaceae) is a monoecious evergreen shrub up to 6 m tall found in western Mediterranean coastal dune systems. Its root system is reported to be shallow, with preferential development in the first 50 cm of the soil profile (Castillo et al. 2002; Martínez García and Rodríguez 1988). *Pistacia lentiscus* (Anacardiaceae) is a dioecious evergreen shrub up to 3–4 m tall that is widespread in the Mediterranean basin. It has a dimorphic root system with deep roots that may reach well below 5-m depth (Martínez García and Rodríguez 1988; Specht 1988).

Sample collection for isotopic analysis

Plant water sources were determined in adult shrubs of each species growing alone and in clumps (one individual of each species) on top of the dunes ($n = 5$–6 per species and treatment). Plants were selected from different dunes and positions but overall were ~4–5 m above the water table. We determined the natural abundance of ^{18}O and deuterium (^{2}H or D) in xylem sap and compared these values to that of precipitation, dew, groundwater, and soil water isotopes at different depths. The relative abundance of ^{13}C in leaves was also measured.

In August 2006, three to four 15-cm leafless stems from the east side of each plant were sampled shortly after dawn and immediately enclosed in screw-cap polypropylene tubes (Corning) and sealed with Parafilm for xylem sap analysis. One of these stems was enclosed in a separate tube for sap osmolality measurements. At the same time we collected and wrapped in aluminum foil several sunlit leaf samples for C isotope analyses. Leaves were dried at 70°C for at least 48 h and then ground to a fine powder.

Soil cores of 5 cm diameter were extracted with a drilling rig in 50-cm increments from the surface to the water table on bare ground locations ($n = 6$ soil profiles). Soil samples were sorted into three parts; one part was sealed with a rubber stopper in a glass tube for water isotopic analysis; the second part was sealed in a container and analyzed for gravimetric water content (expressed as g water/g soil × 100); and the third sample was used to measure soil EC in a saturated paste extract (Conductimeter Basic 30; Crison Instruments, Alella, Spain). Due to the difficulty of mechanically drilling the soil under shrub canopies, we collected soil samples under shrubs only at 5-, 50- and 100-cm depth from the top of the dune with a manual soil corer ($n = 6$ per site, depth, species and treatment).

To compare water isotopes of xylem sap with those of different water sources, we collected rain water from winter to summer 2006 with standard rain gauges, and dew water during June and July of the same year. Rain was allowed to accumulate in a collector between monthly visits. A 5-mm layer of clear white mineral oil was added to the rain collector to prevent evaporation. Contact with mineral oil does not alter water isotopes (Williams and Ehleringer 2000). Dew was collected 50 cm above the soil surface with an 80 × 95-cm steel plate inclined 45° that condensed water vapor and channeled water to a funnel and a glass tube containing mineral oil. All water samples collected were filtered through 0.45-μm Teflon filters and kept refrigerated in tightly sealed glass vials. Soil and plant samples were kept frozen in the lab until water was extracted using cryogenic vacuum distillation (Ehleringer and Osmond 1989).

Water isotope content (δD, $\delta^{18}O$) and $\delta^{13}C$ of leaves were measured in a Finnigan MAT Delta Plus XL continuous flow mass spectrometer system (Finnigan, San Jose, Calif.) at the Duke University Environmental Stable Isotope Laboratory. Mass spectrometer measurements had a precision of 0.1‰ for both water ^{18}O and organic ^{13}C, and 0.3‰ for water ^{2}H. The isotopic abundance was expressed in delta notation (δ) in parts per thousand (‰) as

$$\delta = \left(R_{\text{sample}}/R_{\text{standard}} - 1\right) \times 1{,}000 \quad (1)$$

where R_{sample} and R_{standard} are the molar ratios of heavy to light isotope of the sample and the international standard (Vienna standard mean ocean water for $^{2}H/^{1}H$ and $^{18}O/^{16}O$; and Vienna Pee Dee belemnite for $^{13}C/^{12}C$).

To determine whether the aquifer contained seawater we analyzed anion concentrations with an ion chromatograph (ICS2000; Dionex, Sunnyvale, Calif.).

Sap osmolality in the field

We measured expressed sap osmolality of stems (mmol kg^{-1}, $n = 6$ per species and treatment) of plants of the two shrub species growing alone and in clumps. If stems of the same species living alone and in clumps have different sap osmolality, then they are probably taking up water with different salinity.

Stems were carefully peeled so they had no bark or phloem, cut into small pieces, and placed into 2-ml microtubes (Eppendorf, Hamburg). Expressed sap was obtained from thawed samples following the procedure of Callister et al. (2006); we made a fine hole at the base of each microtube, inserted the tube firmly into another empty 2-ml microtube and centrifuged for 15 min at 9,000 r.p.m. (Centronic; Selecta, Abrera, Barcelona). Each tube had two ball bearings of a combined weight of 1.8 g resting above the sample. Osmolality of expressed sap of stems was then determined using a vapor pressure osmometer (Vapro 5520; Wescor, Logan, Utah).

Physiological status of plants in the field

In August 2006 (summer) and April 2007 (spring) we measured pre-dawn (Ψ_{pd}) and midday (Ψ_{md}) Ψs, pre-dawn relative water content (RWC), early morning and midday photochemical efficiency of photosystem II (Fv/Fm), leaf water vapor conductance (g_s) and photosynthetic rate (A) on mature, attached leaves at the same height and on the east side of plants ($n = 7$ per species and treatment, i.e., alone or in clumps). Since juniper plants have scale leaves, all measurements were performed on green twigs. Ψ was determined on terminal shoots using a pressure chamber (SKPM 1400; Skye Instruments, Llandrindod Wells, UK). RWC was calculated following Barrs and Weatherley (1962). Fv/Fm was measured with a portable fluorimeter (PEA; Hansatech, Kings Lynn, UK) on leaves that were previously dark adapted for 30 min. g_s and A were measured under ambient CO_2 concentrations on green, mature, sunlit leaves using a portable infrared gas analyzer (LCi; Analytical Development Company, Hoddesdon, UK). Measurements were expressed on a projected leaf area basis, obtained from digitized images of leaves with an image area analyzer (Midebmp; Almería, Spain). For juniper we used Cregg's (1992) leaf area correction for cylindrical twigs.

Greenhouse salinity experiment

In January 2007, one-year-old saplings of both species grown from seeds and supplied by local nurseries were transplanted to 6 × 5-cm-wide, 19-cm-high plastic pots (Forest Pot 400®) filled with washed river sand. Only one sapling was planted in each pot. Plants were placed in a greenhouse and irrigated daily with distilled water for 5 weeks prior to being subjected to salinity. Any plant that died within this period was replaced. Saplings were then randomly assigned to one of four water salinity levels ($n = 10$ plants per treatment): 0, 4.5, 9, and 18 g l^{-1} of dissolved NaCl (Panreac, Barcelona), the greatest salt concentration being ~55% of seawater salinity. During the experiment, 10 ml of a standard nutrient solution (2 ml l^{-1} water of a 4–5–6 NPK fertilizer; KB, Lyon, France) was added weekly.

Salt treatments began in February 2007. To allow plants to acclimate to the salt, salinity was increased in increments of 1 g salt l^{-1} every 2 days until the target concentration was reached, taking 6 weeks to reach the highest concentration. Salt treatments ceased in June 2007. Plants were monitored weekly for survival, and dawn Ψ of twigs and early morning Fv/Fm, g_s and A on mature, attached leaves were measured prior to harvest. We also collected leaf samples for measuring relative abundance of $\delta^{13}C$ in a micro-Dumas combustion elemental analyzer (Fison NA1500 NC; Fison Instruments, Beverly, Mass.), at the EEZ Stable Isotope Laboratory (CSIC, Granada, Spain).

Statistical analyses

Differences in physiological variables under different treatments in the field were tested for each species independently using ANOVA at a significance level of 0.05. We used M-ANOVA or RM-ANOVA for time-repeated measurement analysis. Previously, the homogeneity of variances was checked using Levene's test. The Box M test was used to check the homogeneity of variances/covariances matrix. Differences in C and water isotopes were tested using one-way multivariate ANOVA. Post hoc differences were tested using Scheffé's test.

Differences among sapling responses of each species to each salinity concentration were tested using ANOVA. For each species, differences in sapling survival among salinity treatments were analyzed through simple binary logistic regression where survival at the end of the experiment was the dependent variable and salinity the predictor factor, followed by pair-comparisons after a conservative Bonferroni correction.

Analyses were performed with the SPSS 14.0 software (SPSS, Ill.). Data results throughout the text, tables and figures are presented as mean ± 1 SE.

Results

Soil profiles

In the upper meter of the dune surface (0- to 5-, 50- and 100-cm depths), soil EC in gaps was low and consistently around 0.31 ± 0.04 dS m^{-1} (Table 1). Conductivity and moisture increased with depth, particularly when approaching the water table (Fig. 1). Groundwater EC reached 25.3 dS m^{-1}, while at 1 m above the water table it was 3.6 dS m^{-1} (Fig. 1a). Similarly, gravimetric water content at the water table was 13.0 ± 1.3% but it was <2.9 ± 0.6% just 1 m above the water table (Fig. 1b).

Table 1 Soil electric conductivity (dS m^{-1}) at 0- to 5-, 50- and 100-cm depth from the dune surface in gaps without vegetation, and in the understory of juniper (J), lentisc (L) and clumps of both species ($J+L$) (mean ± SE, $n = 6$)

	Gaps	J	L	Co (J+L)
0–5	0.36 ± 0.04 a	2.22 ± 0.06 b	1.08 ± 0.03 c	1.57 ± 0.04 d
50	0.30 ± 0.02 a	0.11 ± 0.01 b	0.11 ± 0.02 b	0.09 ± 0.01 b
100	0.33 ± 0.06 a	0.13 ± 0.03 b	0.12 ± 0.02 b	0.10 ± 0.01 b

Different lower-case letters within a row show significant differences among sites

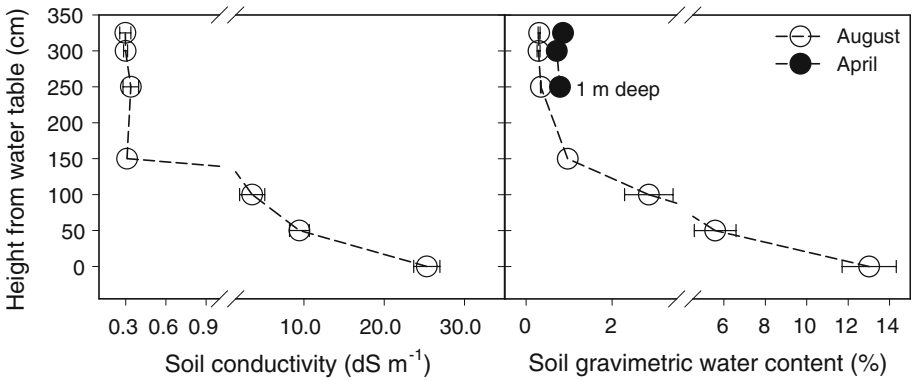

Fig. 1 Soil electrical conductivity (*left panel*) and gravimetric soil water content (*right panel*) at different heights from the water table in the dune profile (mean ± 1 SE; $n = 6$; *error bars* only visible if larger than the symbol)

Soil EC was significantly higher under shrubs than in gaps at the soil surface (Table 1), but soils at 50- and 100-cm depth in the understory had lower soil EC than gaps. Surface soils were slightly saltier under solitary junipers than under solitary lentiscs, whereas soil salinities in clumped vegetation patches were intermediate.

Water from the aquifer was a mix of fresh and sea water as suggested by the concentrations of Cl^-, SO_4^{2-} and Br^-, which had values approximately 70% of those of sea water. Salinity overall was ~48% of sea water (Fig 1; Table S1).

Isotope signature of xylem sap and plant water sources

Both lentisc and juniper plants growing with lentisc showed similar δD and δ^{18}O xylem sap values (Fig. 2) that differed from those of isolated junipers ($P < 0.05$). δD in lentisc sap and in juniper plants growing close to lentisc was intermediate between groundwater and water 1 m below the dune surface; δ^{18}O values of groundwater and soil water 1 m below the soil surface were very similar. In contrast, sap isotopic signatures of isolated juniper matched that of soil 1 m above the water table ($P = 1.00$, post hoc tests for both δD and δ^{18}O) and did not differ from values of soil 1 m below the dune surface ($P > 0.2$). The sap isotopic signature of junipers growing in clumps matched that of lentisc and groundwater, but differed from that of isolated junipers. Dew and water stored in the upper 25 cm of soil had no apparent influence on the isotopic composition of plants (Fig. 2; dew data not shown).

In spring (April), sap osmolality was significantly higher in lentisc stems than in juniper stems (Fig. 3; $P < 0.001$), but there were no significant differences between plants living alone or in clumps. Juniper sap osmolality increased from spring to summer, but it did not change in lentisc during the same time period ($P = 0.65$). In August, sap osmolality of junipers growing with lentisc was almost twice that of junipers growing alone, whereas sap

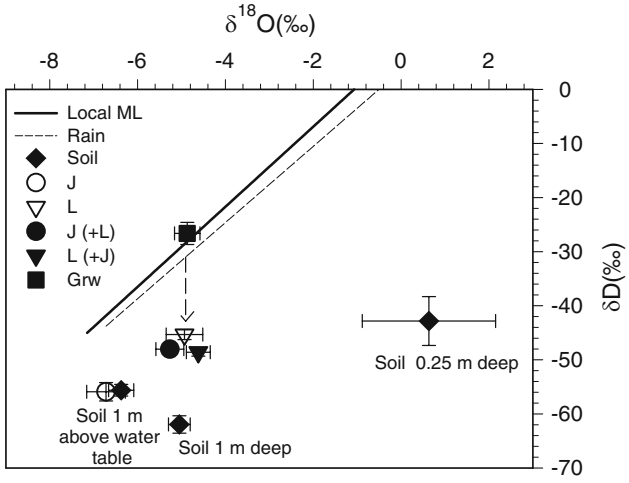

Fig. 2 Relation between natural abundance of D (δD) and O ($\delta^{18}O$) in: the local meteoric line for Almeria, Spain (*Local ML*; from GNIP); rain (*regression line*); water from soil at different depths in the dune profile; groundwater (*Grw*); and xylem water of lentisc (*L*) and juniper (*J*) plants growing alone or with the other species {for lentisc [*L (+J)*] and for juniper [*J (+L)*] plants}. Samples were collected in August (mean ± 1 SE; $n = 5$–6 for plants and aquifer; for soils $n = 4$). *Vertical arrow* suggests that plants taking up ground water fractionate water H isotopes leading to a D depletion of root xylem water relative to the salty water source

osmolality was similar for all lentisc plants regardless of juniper co-occurrence.

Plant physiological status in the field

Differences in plant Ψ were large between the two species ($F_{4,21} = 62.37$, $P < 0.001$; Table 2). Juniper plants were under apparent water shortage in summer, showing Ψs below −5 MPa, whereas lentisc plants showed little evidence of water deficit and displayed high, steady Ψ both in spring and summer (Tables 2, S2). Junipers associated with lentisc plants showed more negative Ψs than did individual junipers growing alone, especially in the dry season, when their Ψs reached below −7 MPa. By contrast, lentisc

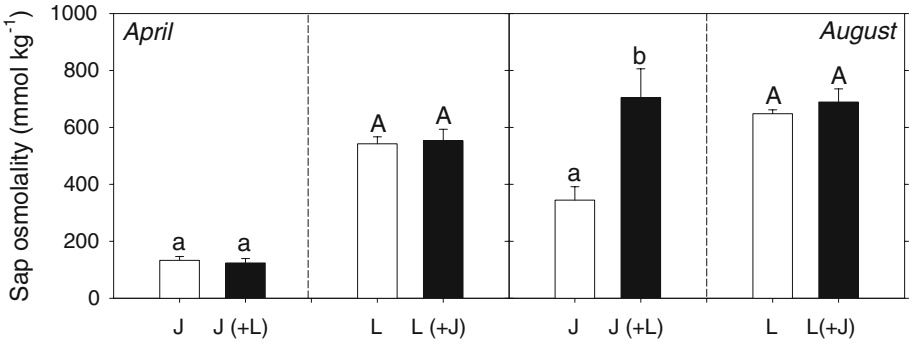

Fig. 3 Osmolality of expressed sap in L and J stems growing alone (*white bars*) or with the other species [*black bars*; L (+J) and J (+L)] in April (wet season) and August (dry season). Values are mean ± 1 SE; $n = 6$, $n = 4$ for juniper in August. *Different letters* within each species and month indicate significant differences between plants growing alone or with the other species at $P < 0.05$. For abbreviations, see Fig. 2

Table 2 Physiological status of juniper and lentisc shrubs growing alone or in clumps for the following variables measured in April and August: early morning relative water content (*RWC*), predawn (*pd*) or early morning (*a.m.*) and midday (*p.m.*) twig water potential (Ψ), photosynthetic rate (A) and conductance to water vapor (g_s) in leaves, predawn photochemical efficiency of PSII (Fv/Fm), and natural abundance of C ($\delta^{13}C$) in leaves in August (mean ± 1 SE; $n = 7$; $n = 6$ for isotope data)

Month	Juniper		Lentisc	
	Alone	With lentisc	Alone	With juniper
RWC (%)				
April	**81.56 ± 0.30** a	**77.60 ± 0.70** b	86.02 ± 1.03 a	88.53 ± 1.90 a
August	**68.76 ± 1.79** a	**58.49 ± 1.67** b	81.68 ± 2.27 a	82.95 ± 2.66 a
Ψ (MPa)				
April pd	**−1.42 ± 0.11** a	**−2.34 ± 0.10** b	−1.42 ± 0.32 a	−1.33 ± 0.28 a
April p.m.	−2.39 ± 0.11 a	−3.03 ± 0.53 a	−2.50 ± 0.26 a	−2.06 ± 0.38 a
August a.m.	**−4.83 ± 0.46** a	**−7.20 ± 0.23** b	−1.14 ± 0.19 a	−1.5 ± 0.18 a
August p.m.	**−5.46 ± 0.45** a	**−8.03 ± 0.17** b	−2.79 ± 0.35 a	−2.64 ± 0.44 a
A (μmol m^{-2} s^{-1})				
April a.m.	9.34 ± 1.31 a	6.81 ± 0.69 a	14.24 ± 0.82 a	13.12 ± 0.82 a
April p.m.	**4.07 ± 0.44** a	**1.23 ± 0.48** b	3.48 ± 0.87 a	4.45 ± 0.80 a
August a.m.	**3.07 ± 0.50** a	**1.33 ± 0.35** b	6.16 ± 0.82 a	8.09 ± 1.25 a
August p.m.	**1.77 ± 0.44** a	**0.41 ± 0.14** b	2.35 ± 0.75 a	2.18 ± 0.76 a
g_s (mol m^{-2} s^{-1})				
April a.m.	0.13 ± 0.03 a	0.09 ± 0.01 a	0.26 ± 0.07 a	0.20 ± 0.02 a
April p.m.	**0.04 ± 0.01** a	**0.01 ± 0.01** b	0.04 ± 0.02 a	0.06 ± 0.02 a
August a.m.	0.04 ± 0.01 a	0.03 ± 0.01 a	0.07 ± 0.01 a	0.09 ± 0.02 a
August p.m.	**0.02 ± 0.01** a	**0.00 ± 0.00** b	0.02 ± 0.01 a	0.02 ± 0.01 a
Fv/Fm				
April	0.780 ± 0.005 a	0.781 ± 0.007[a]	0.767 ± 0.007 a	0.759 ± 0.007 a
August	**0.722 ± 0.011** a	**0.629 ± 0.036** b	0.793 ± 0.006 a	0.783 ± 0.005 a
$\delta^{13}C$ (‰)				
August	−23.66 ± 0.13 a	−23.76 ± 0.17 a	−27.47 ± 0.48 a	−26.99 ± 0.42 a

Different letters in a variable within each species indicate significant differences between plants growing in the different treatments at $P < 0.05$ (*in bold*)

growing alone and associated with juniper showed no differences. The RWC of twigs revealed trends similar to those of plant Ψ (Table 2).

Overall, early morning gas exchange rates in lentisc were higher than in juniper, with relative differences being more pronounced during periods of water shortage. Both

species displayed a midday depression in A that was more pronounced in spring than in summer (Table 2). Significant differences in A between plants growing alone or in clumps were apparent only for juniper, with isolated plants having higher A than plants living in clumps with lentisc. Differences occurred mainly at midday in spring and in early morning in summer. g_s showed similar trends, but significant differences occurred only at midday (Tables 2, S2).

Juniper and lentisc showed contrasting Fv/Fm ($F_{2,23} = 18.31$, $P < 0.001$; Table 2). Differences in Fv/Fm between shrubs occurring alone or in clumps were significant only for juniper in summer.

The different response of juniper and lentisc to water shortage is reflected in differences in leaf $\delta^{13}C$. There were strong differences in $\delta^{13}C$ between species ($F_{1,24} = 106.51$, $P < 0.001$) but not between treatments (Tables 2, S2). Juniper had less negative $\delta^{13}C$ than lentisc (-23.71 ± 0.04 vs. -27.23 ± 0.17‰, respectively), suggesting that juniper had a higher water use efficiency reflecting increased water limitations (Dawson et al. 2002; Ehleringer 1993).

Greenhouse salinity experiment

Our two species showed strongly contrasting responses to salinity (Table 3). Survival of juniper was very low (28.6%) when watered with high salt concentration (18 g NaCl l^{-1}) but 100% of juniper saplings survived irrigation with distilled water. In contrast, survival of lentisc saplings grown at 18 g NaCl l^{-1} was much higher (87.5%) and comparable to plants receiving distilled water (100%). Juniper was very sensitive to salinity, and small increases in salt concentration (e.g., 0–4.5 g NaCl l^{-1}) reduced survival as much as 40%. In lentisc, however, the highest saline solution reduced survival by only 12.5% compared to control saplings.

Overall, lentisc plants displayed lower Ψ than juniper, and Ψ decreased in both species as salinity increased (Table 3). As plants apparently did not suffer water shortage (saplings were irrigated daily throughout the experiment and control plants of both species had Ψs around −1.2 MPa at harvest), these differences likely reflect species-specific responses to salinity. Salt stress also influenced leaf C isotopes (Table 3); increased salinity led to less negative $\delta^{13}C$ ($ANOVA_{salt}$, $P < 0.02$) in both species ($ANOVA_{species \times salt}$, $P = 0.16$), probably due to reduced stomatal conductance.

The effects of salinity on gas exchange and photochemical efficiency was quite different in the two species (Table 3). A and leaf g_s decreased in both species as salinity increased, but while lentisc continued to fix C at high rates even under a high salt concentration (6.45 ± 1.07 μmol m^{-2} s^{-1}), juniper nearly stopped

Table 3 Physiological status of juniper and lentisc saplings watered with different salt concentrations (g l^{-1}) for the following variables: twig Ψ and $\delta^{13}C$, Fv/Fm, A and g_s in leaves, and survival of saplings at the end of the greenhouse experiment (mean ± 1 SE; $n = 3$–7)

NaCl (g l^{-1})	Juniper				Lentisc			
	0	4.5	9	18	0	4.5	9	18
Soil EC (dS m^{-1})	0.096 ± 0.004 a	0.788 ± 0.031 b	15.02 ± 0.163 c	27.80 ± 0.242 d				
Survival (%)	100 a	60 b	60 b	28.6 b	100 a	100 a	100 a	87.5 a
Ψ (MPa)	−1.26 ± 0.11 a	−1.44 ± 0.04 a	−2.11 ± 0.25 ab	−2.37 ± 0.17 b	−1.26 ± 0.16 a	−2.03 ± 0.20 b	−2.14 ± 0.07 bc	−2.70 ± 0.13 c
$\delta^{13}C$ (‰)	−25.15 ± 0.29 a	−24.40 ± 0.50 ab	−25.17 ± 0.75 ab	−24.04 ± 0.40 b	−29.23 ± 0.68 a	−27.34 ± 0.45 ab	−27.78 ± 0.65 ab	−25.59 ± 0.61 b
Fv/Fm	0.778 ± 0.012 a	0.686 ± 0.060 ab	0.574 ± 0.080 ab	0.539 ± 0.091 b	0.813 ± 0.010 a	0.815 ± 0.007 a	0.749 ± 0.039 a	0.744 ± 0.030 a
			>9 g l^{-1}				>9 g l^{-1}	
A (μmol m^{-2} s^{-1})	3.71 ± 0.22 a	2.16 ± 1.03 ab	0.64 ± 0.27 b		20.16 ± 3.76 a	15.00 ± 2.25 a	6.45 ± 1.07 b	
g_s (10× mol m^{-2} s^{-1})	0.35 ± 0.07 a	0.16 ± 0.09 ab	0.02 ± 0.02 b		3.28 ± 0.92 a	2.24 ± 0.40a b	0.74 ± 0.14 b	

Different letters in a variable within each species indicate significant differences among plant responses to salinity treatments ($P < 0.05$). For abbreviations, see Table 2

photosynthesizing (0.64 ± 0.27 μmol m^{-2} s^{-1}). Similarly, g_s was higher in lentisc than in juniper plants. Lentisc showed only small decreases in chlorophyll fluorescence with salinity increases, reaching values around 0.75 at 18 g NaCl l^{-1}, whereas in juniper Fv/Fm decreased with salinity and reached as low of ≈0.54.

Discussion

We hypothesized that juniper water stress would be a consequence of its inability to tap permanent water sources and its greater sensitivity to salinity than lentisc. Indeed, we found that the two species differed substantially in salt tolerance and used different water sources, which in turn influenced their competitive interaction. Our data suggest that the salt-tolerant species could redistribute water to neighboring plants, with negative consequences for sensitive species.

Accessing water sources

In this dry system the physiology of lentisc seems to be relatively independent of rainfall. Our data support previous studies in which lentisc always displayed high leaf A and g_s rates and high Ψs throughout the year (Armas and Pugnaire 2009).

Lentisc $\delta^{18}O$ in xylem sap matched that of the salty aquifer, that was apparently its main water source. δD in the xylem sap was much lower than that of groundwater, probably because of fractionation by root membranes during saline water uptake, a process that leads to a decrease in D in xylem water relative to the surrounding soil (Ellsworth and Williams 2007; Lin and Sternberg 1993). Xylem sap water isotopes suggest that lentisc might also have used fresh water stored higher in the soil profile (up to 1 m below the dune surface). However, since moisture in the upper meter of the soil profile was quite low in summer, its contribution to lentisc's water source was likely small at that time. All of these data suggest that lentisc relied upon the salty groundwater during drought, allowing for a steady physiological performance and high Ψs. Salty water does not seem to have constrained lentisc performance, and it is known to be a relatively salt-tolerant species (Tattini et al. 2006; Valentini et al. 1992), as we observed in our greenhouse salinity experiment.

Xylem isotopic signatures of isolated junipers in summer matched that of soil 1 m above the water table, suggesting that these plants were taking up relatively fresh water (EC ca. 3.6 vs. 25.0 dS m^{-1} of the groundwater). Thus, our hypothesis that juniper lacked deep roots does not hold because our results suggest that juniper was able to tap water from the dune down to 1 m above the water table. The low Ψ experienced by juniper in summer may reflect the depletion of fresh water in the dune and its sensitivity to saline water, which prevents its access to the salty aquifer. These results also suggest that 1 m above the water table is the deepest layer from which *Juniperus* can take up water at our field site, as deeper soil layers become too salty for this species.

Importantly, the water source of isolated junipers seems to be different from the water source of junipers living in clumps. Sap water isotopes of isolated junipers differed from those of junipers living close to lentisc, which had similar δD and $\delta^{18}O$ values to lentisc, whose primarily water source was the aquifer. These data bear consistent evidence that lentisc may be providing nearby juniper with an additional source of water via hydraulic lift, the process whereby plants passively redistribute water through their root systems from deep, wetter soils to shallow, drier soils along Ψ gradients (Richards and Caldwell 1987).

Hydraulic lift and species interactions

Hydraulic lift has been shown to improve performance and water status of neighboring species in both mesic (Dawson 1993) and harsh environments (Filella and Peñuelas 2003a). Water lifted by lentisc might be expected to benefit neighboring junipers; instead, juniper shrubs associated with lentisc fared poorly in the dry season (e.g., showed more negative Ψ, less RWC, lower A and g_s, and lower photosynthetic efficiency than individuals growing alone). By contrast, lentisc always performed equally well either isolated or when associated with juniper. This is not the first report of asymmetric competition in this system, as Armas and Pugnaire (2009) also showed that the reproductive effort and long-term survival of juniper were negatively affected when it grew with lentisc. In other words, hydraulically lifted water supplied by lentisc seems not to have any net positive effect on juniper, as might be expected in an arid environment, and suggests that something else counterbalances the potential positive effects of hydraulic lift.

Ludwig et al. (2004) showed in a dry savanna that grasses growing in the understory of a tree lifting water performed worse than grasses near trees whose hydraulic lift had been suppressed. Trees extracted significant amounts of water from the top soil, so that belowground competition for lifted water overwhelmed the positive effects of hydraulic lift on neighbors. This could have happened in our system, and the poor performance of juniper in clumps might be due to competition with lentisc for lifted water. However, the performance of juniper growing with lentisc was unlikely to be caused only by competition for water, as juniper is a

notable drought-tolerant species, like most other *Juniperus* species from dry habitats (Willson et al. 2008).

One possibility is that lentisc plants were performing hydraulic lift of salty water. Salt-tolerant species are apparently able to redistribute soil water. For example, some *Eucalyptus* species perform hydraulic redistribution (Burgess et al. 1998, 2001), such as *Eucalyptus camaldulensis* that takes up water and tolerates aquifer salinities similar to those found in our field site (Nosetto et al. 2008). Shrubby species like *Prosopis vetulina* and *Prosopis glandulosa* also redistribute soil water (Scott et al. 2008; Zou et al. 2005), and both are salt-tolerant species that fractionate D during the uptake of salty water (Ellsworth and Williams 2007). Yet, to our knowledge, the salt content of lifted water has not been addressed to date. Uptake and exudation of some sort of salty water by roots may be possible. For instance, Salim (1988) showed a net loss of Na from roots and stems tissues to the rhizosphere of *Vigna radiata*. However, the release of salty water by lentisc roots via hydraulic lift remains uncertain.

An alternative explanation could be that salt accrued in soils under these plant canopies due to salt deposition in litter or leaf salt spray that washed off under plants. The negative effect of lentisc on juniper might thus be caused by mobilization of this salt in soils under shrubs, due to the hydraulically lifted water released by lentisc. Soil EC in the upper 5 cm of soil under shrubs was 5–7 times greater than in gaps; however, our data showed that deeper soil layers (i.e., 50- and 100-cm depth) had overall low and relatively equal ECs. We were unable to find salt accretion in soils 1 m below the dune surface, where isotope data suggest all monitored plants took up some water. With all these data in hand, this hypothesis seems improbable in our system.

Overall, the two proposed mechanisms: (1) the hydraulic lift of salty water, and (2) salt accumulation and solubilization beneath lentisc shrubs, may co-occur. Salt seems to be the mediating factor in the interaction between these two species counteracting the potential positive effects of an additional water source provided via hydraulic lift. This conclusion is supported by stem sap osmolality results. In August, sap osmolality of juniper plants growing with lentisc was twice that of isolated juniper plants (and also similar to that of lentisc); in contrast, in the wet season sap osmolality of juniper plants was low and similar in all junipers. Sap osmolality in lentisc xylem was high in spring and summer, probably as a result of salty groundwater uptake. This result is consistent with the work of Tattini et al. (2006), who showed that stem Na^+ concentrations of lentisc plants subjected to salty water irrigation were almost half values in the soil/water medium. Even more importantly, our findings are consistent with those of higher xylem sap osmolality as salinity increases, as in Pagter et al. (2009), who reported expressed sap osmolality of roots of *Phragmites australis* ranging from 150 to 600 mmol kg^{-1} as salinity rose, and in Sobrado (2001), with the mangrove *Avicennia germinans*.

In conclusion, the different abilities of our two species to use salty water appear to drive the outcome of an interaction in which juniper performance is negatively affected by salt when growing in clumps with lentisc. Our data show that a salt-tolerant species redistributes water from the aquifer via hydraulic lift, negatively affecting the performance of the neighboring salt-sensitive species. It remains unresolved, however, to what extent salt accumulation beneath shrubs, and hydraulic lift of salty water, may account for the reported negative effect over clumped junipers. These processes could have important consequences at both community and ecosystem scales.

Acknowledgments We thank the Junta de Andalucía Environmental Agency for permission to work in the Natural Reserve; Will Cook, Jonathan Karr and Antonio Delgado Huertas for help with isotope and ion analyses; Tracey Crocker and Maria José Jorquera for help with lab determinations and plant care, and Harriet Whitehead for comments on an earlier version. This work was partly funded by the Spanish Ministry of Education and Science (grants CGL2004-00090 and CGL2007-63718). C. A. was supported by a Spanish MEC-Fulbright fellowship and by an I3P-CSIC contract. F. M. P. acknowledges the isotope training received thanks to an exchange grant of the ESF-SIBAE programme.

References

Ackerly D (2004) Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. Ecol Monogr 74:25–44

Armas C, Pugnaire FI (2009) Ontogenetic shifts in interactions of two dominant shrub species in a semi-arid coastal sand dune system. J Veg Sci 20:535–546

Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Sci 166:3–16

Barrs HD, Weatherley PE (1962) A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust J Biol Sci 15:413–428

Burgess SSO, Adams MA, Turner NC, Ong CK (1998) The redistribution of soil water by tree root systems. Oecologia 115:306–311

Burgess SSO, Adams MA, Turner NC, White DA, Ong CK (2001) Tree roots: conduits for deep recharge of soil water. Oecologia 126:158–165

Callister AN, Arndt SK, Adams MA (2006) Comparison of four methods for measuring osmotic potential of tree leaves. Physiol Plant 127:383–392

Canadell J, Zedler PH (1995) Underground structures of woody plants in Mediterranean ecosystems of Australia, California, and Chile. In: Kalin-Arroyo MT, Zedler PH, Fox MD (eds) Ecology and biogeography of Mediterranean ecosystems in Chile, California and Australia. Springer, New York, pp 177–210

Canadell J et al (1999) Structure and dynamics of the root system. In: Rodá F, Retana J, Gracia CA, Bellot J et al (eds) Ecology of Mediterranean evergreen oak forests. Springer, Berlin, pp 47–59

Casper BB, Jackson RB (1997) Plant competition underground. Annu Rev Ecol Syst 28:545–570

Castillo JM, Casal AEB, Luque CJ, Luque T, Figueroa ME (2002) Comparative field summer stress of three tree species co-occurring in Mediterranean coastal dunes. Photosynthetica 40:49–56

Cregg BM (1992) Leaf area estimation of mature foliage of *Juniperus*. For Sci 38:61–67

Dawson TE (1993) Hydraulic lift and the water use by plants: implications for water balance, performance and plant–plant interactions. Oecologia 95:565–574

Dawson TE, Pate JS (1996) Seasonal water uptake and movement in root systems of Australian phreatophytic plants of dimorphic root morphology: a stable isotope investigation. Oecologia 107:13–20

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant ecology. Annu Rev Ecol Syst 33:507–559

Ehleringer JR (1993) Carbon and water relations in desert plants: an isotopic perspective. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant carbon/water relations. Academic Press, San Diego, pp 155–172

Ehleringer JR, Dawson TE (1992) Water uptake by plants: perspectives from stable isotope composition. Plant Cell Environ 15:1073–1082

Ehleringer JR, Osmond CB (1989) Stable isotopes. In: Pearcy RW, Ehleringer JR, Mooney HA, Rundel PW (eds) Plant physiological ecology field methods and instrumentation. Chapman & Hall, London, pp 281–300

Ehleringer JR, Schwinning S, Gebauer RE, Press MC, Scholes R, Barker MG (1999) Water use in arid land ecosystems. Advances in plant physiological ecology. British Ecological Society, Blackwell, London, pp 347-365

Ellsworth PZ, Williams DG (2007) Hydrogen isotope fractionation during water uptake by woody xerophytes. Plant Soil 291:93–107

Filella I, Peñuelas J (2003a) Indications of hydraulic lift by *Pinus halepensis* and its effects on the water relations of neighbour shrubs. Biol Plant 47:209–214

Filella I, Peñuelas J (2003b) Partitioning of water and nitrogen in co-occurring Mediterranean woody shrub species of different evolutionary history. Oecologia 137:51–61

Franco AC, Nobel PS (1990) Influences of root distribution and growth on predicted water uptake and interspecific competition. Oecologia 82:151–157

Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. Annu Rev Plant Physiol 31:149–190

Haase P, Pugnaire FI, Fernandez EM, Puigdef bregas J, Clark SC, Incoll LD (1996) An investigation of rooting depth of the semiarid shrub *Retama sphaerocarpa* (L.) Boiss. by labelling of ground water with a chemical tracer. J Hydrol 177:23–31

Haase P, Pugnaire FI, Clark SC, Incoll LD (1999) Diurnal and seasonal changes in cladode photosynthetic rate in relation to canopy age structure in the leguminous shrub *Retama sphaerocarpa*. Funct Ecol 13:640–649

Hasegawa PM, Bressan RA, Zhu J-K, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol 51:463–499

Huxman TE, Snyder KA, Tissue DT, Leffler AJ, Ogle K, Pockman WT, Sandquist DR, Potts DL, Schwinning S (2004) Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. Oecologia 141:254-268

Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED (1996) A global analysis of root distributions for terrestrial biomes. Oecologia 108:389–411

Jackson RB, Moore LA, Hoffmann WA, Pockman WT, Linder CR (1999) Ecosystem rooting depth determined with caves and DNA. Proc Natl Acad Sci USA 96:11387–11392

Jakab G, Ton J, Flors V, Zimmerli L, Metraux JP, Mauch-Mani B (2005) Enhancing *Arabidopsis* salt and drought stress tolerance by chemical priming for its abscisic acid responses. Plant Physiol 139:267–274

Lin G, Sternberg LSL (1993) Hydrogen isotopic fractionation by plant roots during water uptake in coastal wetland plants. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant carbon–water relations. Academic Press, New York, pp 497–510

Ludwig F, Dawson TE, Prins HHT, Berendse F, de Kroon H (2004) Below-ground competition between trees and grasses may overwhelm the facilitative effects of hydraulic lift. Ecol Lett 7:623–631

Martínez García F, Rodríguez JM (1988) Distribución vertical de las raíces del matorral de Doñana. Lagascalia 15:549–557

Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25:239–250

Nosetto MD, Jobbágy EG, Tóth T, Jackson RB (2008) Regional patterns and controls of ecosystem salinization with grassland afforestation along a rainfall gradient. Global Biogeochem Cycles 22:GB2015

Noy-Meir I (1973) Desert ecosystems: environment and producers. Annu Rev Ecol Syst 4:25–51

Padilla FM, Pugnaire FI (2007) Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. Funct Ecol 21:489–495

Pagter M, Bragato C, Malagoli M, Brix H (2009) Osmotic and ionic effects of NaCl and Na_2SO_4 salinity on *Phragmites australis*. Aquat Bot 90:43–51

Pulido-Bosch A, Navarrete F, Molina L, Martínez-Vidal JL (1991) Quantity and quality of groundwater in the Campo de Dalías (Almería, SE Spain). Water Sci Technol 24:87-96

Richards JH, Caldwell MM (1987) Hydraulic lift: substantial nocturnal water transport between soil layers by *Artemisia tridentata* roots. Oecologia 73:486–489

Salim M (1988) Loss of sodium from mung bean shoots to saline root media. J Agron Crop Sci 160:314–318

Schenk HJ, Jackson RB (2002) The global biogeography of roots. Ecol Monogr 72:311

Schlesinger WH et al (1990) Biological feedbacks in global desertification. Science 247:1043–1048

Schulze ED et al (1996) Rooting depth, water availability and vegetation cover along an aridity gradient in Patagonia. Oecologia 108:503–511

Schulze ED et al (1998) Downward flux of water through roots (i.e. inverse hydraulic lift) in dry Kalahari sands. Oecologia 115:460–462

Schwinning S, Davis K, Richardson L, Ehleringer JR (2002) Deuterium enriched irrigation indicates different forms of rain use in shrub/grass species of the Colorado Plateau. Oecologia 130:345–355

Schwinning S, Sala OE, Loik ME, Ehleringer JR (2004) Thresholds, memory, and seasonality: understanding pulse dynamics in arid/semi-arid ecosystems. Oecologia 141:191–193

Schwinning S, Starr BI, Ehleringer JR (2005) Summer and winter drought in a cold desert ecosystem (Colorado Plateau). Part I. Effects on soil water and plant water uptake. J Arid Environ 60:547–566

Scott RL, Cable WL, Hultine KR (2008) The ecohydrologic significance of hydraulic redistribution in a semiarid savanna. Water Resour Res 44:W02440

Sobrado MA (2001) Effect of high external NaCl concentration on the osmolality of xylem sap, leaf tissue and leaf glands secretion of the mangrove *Avicennia germinans* (L.) L. Flora 196:63–70

Specht RL (1988) Mediterranean-type ecosystems. A data source book. Kluwer, Dordrecht

Tattini M et al (2006) Morpho-anatomical, physiological and biochemical adjustments in response to root zone salinity stress and high solar radiation in two Mediterranean evergreen shrubs, *Myrtus communis* and *Pistacia lentiscus*. New Phytol 170:779–794

Valentini R, Scarascia Mugnozza E, Ehleringer JR (1992) Hydrogen and carbon isotope ratios of selected species of a Mediterranean macchia ecosystem. Funct Ecol 6:627–631

Williams DG, Ehleringer JR (2000) Intra- and interspecific variation for summer precipitation use in pinyon-juniper woodlands. Ecol Monogr 70:517–537

Willson CJ, Manos PS, Jackson RB (2008) Hydraulic traits are influenced by phylogenetic history in the drought-resistant, invasive genus *Juniperus* (Cupressaceae). Am J Bot 95:299–314

Zou CB, Barnes PW, Archer S, McMurtry CR (2005) Soil moisture redistribution as a mechanism of facilitation in savanna tree–shrub clusters. Oecologia 145:32–40