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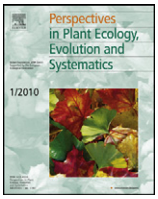

Research article

The role of hydraulic lift on seedling establishment under a nurse plant species in a semi-arid environment

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A B S T R A C T

Hydraulic lift (HL) has been shown to improve performance of species occurring next to a plant engaged in HL, but whether this process plays an important role in seedling survival and growth in arid environments remains largely unknown. Here, we tested the influence of HL on the interaction between *Retama sphaerocarpa*, a nurse lifter shrub from western Mediterranean basin, and the protégé shrub *Marrubium vulgare*. Seedlings of *Marrubium* were planted under the canopy of *Retama* in three tube types inserted into the soil. Tubes either did not affect (Mesh), reduced (Netting) or fully prevented root competition (PVC). At the same time, these tubes allowed (Mesh, Netting) or prevented (PVC) soil water flux to/from the tube. Additional seedlings were planted in Mesh tubes in gaps (open spaces between shrubs). Hydraulic lift was measured using soil psychrometers. We detected HL patterns only in mesh and Netting tubes but not in solid-walled PVC tubes. Seedling survival was comparable where root competition was reduced and HL allowed (Netting) and where they were fully prevented (PVC) while survival was 23% lower in Mesh tubes (full root competition plus HL) and no seedlings survived in gaps. Differences in seedlings biomass showed somehow similar patterns, although biomass was greatest in PVC tubes followed by Netting tubes. These results show that, although HL might have contributed to seedling performance under the canopy of *Retama*, it did not offset root competition from neighbours; when full root competition and HL occurred, competitive effects were stronger than facilitative effects of HL. Overall, intense root competition underneath nurse plants may outweigh the positive effects of the extra water meant by HL. Rather, microclimate amelioration and increased soil fertility under shrubs could account for the reported facilitation of *Marrubium* seedlings by *Retama*.

Introduction

Hydraulic lift (HL) is a process by which plants take up water from deep, moist soil layers and passively transport and release it into shallow, drier soil layers (Richards and Caldwell, 1987). This upwards movement of water can be beneficial for both the lifting plant and its neighbours (Caldwell et al., 1998; Ryel, 2004). For plants lifting water, HL can improve transpiration by providing an additional water source in the upper soil (Caldwell and Richards, 1989; Ryel et al., 2002) and can also sustain soil water availability during the onset of drought periods (Brooks et al., 2002; Meinzer et al., 2004). This additional water is effective in maintaining fine root survival and function (Caldwell et al., 1998; Bauerle et al., 2008) and in reducing xylem embolism in shallow roots (Domec et al., 2004), as well as preventing loss of mycorrhizal functioning in otherwise dry soils (Querejeta et al., 2003, 2007). For neighbours, HL can represent an additional water source (Caldwell, 1990; Dawson, 1993), as documented both in greenhouse (Hirota et al., 2004; Egerton-Warburton et al., 2007) and in field conditions (Caldwell, 1990; Warren et al., 2008; Hawkins et al., 2009).

The overall effect of a plant engaged in HL on its neighbours can be positive (Dawson, 1993; Zou et al., 2005), neutral (Fernández et al., 2008) or negative (Ludwig et al., 2003, 2004; Armas et al., 2010) depending on whether competition outweighs the benefits obtained from extra water provided through HL. This suggests that the net balance of the interaction between the lifter species and its neighbours may be context-dependent (i.e. availability of soil water). Such diverse outcomes highlight the need for further studies on the balance between competition and facilitation processes belowground, a topic that may be of paramount importance for understanding plant interactions in water-limited ecosystems where shallow-rooted species co-exist with deep-rooted species (Armas and Pugnaire, 2009). These latter species can tap groundwater (Haase et al., 1996; Jackson et al., 1999) and often perform HL (Hultine et al., 2004; Prieto et al., 2010a).

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Hydraulic lift is suggested as a mechanism that allows seedling recruitment under the canopy of lifter plants (Dawson, 1993; Caldwell et al., 1998; Callaway, 2007). Greater seedling survival has been found also in connection to mycorrhizal networks, which may suggest that HL played a role (Schoonmaker et al., 2007; Teste and Simard, 2008; Teste et al., 2009). However, the direct effects of HL on seedling survival have not been directly tested yet in semi-arid environments nor has it been quantified the extent to which HL contributes to facilitation by nurse plants (Ryel, 2004; Callaway, 2007). We expect that the balance between the positive effects of HL and the negative effects of belowground competition – consequence of overlapping root systems within the soil profile – be of special relevance for seedling establishment in arid systems where water is a main factor regulating such process (Moles and Westoby, 2004; Padilla and Pugnaire, 2007).

Here, we address the effect of HL on seedlings growing under a nurse species. We used a well-known system consisting of the large nurse shrub, *Retama sphaerocarpa* L. (Boiss), and the understorey protégée shrub, *Marrubium vulgare* L. (Pugnaire et al., 1996a). *Retama* facilitates the establishment and growth of many species in its understorey, including *Marrubium* (Pugnaire et al., 1996a,b; Pugnaire and Lázaro, 2000; Rodríguez-Echeverría and Pérez-Fernández, 2003). Amelioration of microclimatic conditions and increased resource availability under *Retama* are mechanisms that account for facilitative interactions (Moro et al., 1997a,b; Pugnaire et al., 2004), although the role of hydraulic lift by *Retama* remains little known (Prieto et al., 2010a). We conducted a field experiment in which we manipulated the interaction between *Marrubium* seedlings and *Retama* shrubs. By using tubes with different mesh size we altered belowground interactions preventing, reducing, or allowing interspecific root competition, while simultaneously preventing or allowing HL. Our main hypothesis was that HL under the canopy of *Retama* had a net positive effect on *Marrubium* survival and growth; i.e., the positive effect of HL would override the negative effects of root competition.

Methods

Study site and species

The field site was located in Rambla del Saltador, Almería province, SE Spain (37°08′N, 2°22′W, 630 m elevation). The site is a dry valley in the southern aspect of the Filabres Range, over micaschist bedrock, where slope erosion produced big alluvial fans and colluvial deposits. Soil in the valley bottom is therefore from alluvial origin and has a loamy sand texture (Puigdefábregas et al., 1996). Climate is semi-arid with a mean annual precipitation of 245 mm comprising a dry period from June to August (Haase et al., 1999). Mean annual temperature is 18.2 °C (Pugnaire and Lázaro, 2000).

The plant community in the riverbed and alluvial terraces is dominated by *R. sphaerocarpa* (Fig. 1), a large leguminous shrub found in the Iberian Peninsula and northwest Africa. It has an open canopy with photosynthetic stems and a dimorphic root system with a tap root that can reach 30 m deep (Haase et al., 1996) and shallow lateral roots (over 90%) which concentrate a large amount of fine roots in the uppermost 50 cm of soil (De Baets et al., 2007) where competition is intense (Pugnaire and Luque, 2001). The dimorphic root system allows *Retama* to perform HL (Prieto et al., 2010a,b). Gaps between shrubs are barely vegetated and most woody and herbaceous species are found below the *Retama* canopy. *Marrubium* is a drought-resistant, shallow-rooted species (root:shoot ratio = 0.46 ± 0.09, calculated from Fernández et al., 2010) able to stand low water potentials (pers. obs).

Fig. 1. View of the field site depicting *Retama sphaerocarpa* shrubs and the three different types of tubes used in this experiment. *Left*: PVC tube that did not allow root trespassing or water flow; *center*: Mesh tube that allowed neighbouring roots to get inside the tube and free movement of water; right: Netting tube that did not allow root trespassing into the tube but allowed free transfer of water between soil inside and outside. The field site is dominated by large *Retama sphaerocarpa* shrubs surrounded by barely vegetated areas. Note the facilitative effect of *Retama* shrubs on understorey plant community compared to gaps between nurse shrubs.

Experimental design

Three different types of tubes were inserted under *Retama*'s canopy (Fig. 1). One tube consisted of a cylindrical metal mesh, 10 mm mesh size, covered with a thin cloth 200 μm mesh size that restricted root access (Warren et al., 2008; Teste and Simard, 2008) into the tube but allowed transfer of water from/to the tube. This treatment (Netting, hereafter) reduced root interspecific competition and allowed the influence of HL on seedlings. The second type of tube consisted of the same cylindrical metal mesh but without cloth. This treatment (Mesh, hereafter) allowed roots to get inside the tube and movement of water, allowing for full root competition between seedlings and shrubs while simultaneously allowed the effects of HL on seedlings. The third treatment consisted of a solid-walled (5 mm thick) PVC tube that did not allow roots entering the tube or water movement. In this treatment (PVC, hereafter), no root interactions did occur due to the physical barrier. These three tube types were all 50 cm long and 12.5 cm internal diameter, open at the bottom to allow for water drainage.

According to our hypothesis, we expected (i) highest survival and growth rates under reduced belowground competition with HL allowed (i.e., Netting treatment) as seedlings would take advantage of lifted water along with reduced root interactions; (ii) higher survival and growth in the treatment where competition and HL were allowed (i.e., Mesh treatment) than when belowground competition and HL were prevented (PVC); (iii) a net facilitative effect of *Retama* on *Marrubium* seedlings, so that the lowest survival and growth would be found in gaps, far from *Retama* effects and with no microclimate amelioration.

A total of 34 *Retama* shrubs ($n = 30$ for seedling performance; $n = 4$ for microclimatic and soil water potential measurements) were randomly selected in a 1-ha, homogeneous alluvial flat in the Rambla del Saltador valley. Beneath each shrub, three 60 cm deep × 20 cm diameter holes were drilled with an automatic auger (Stihl, BT121, Andreas Stihl Ag & Co. Waiblingen, Germany) in three different aspects (N, E, W) in February 2008. Additionally, holes were dug in a similar way in open spaces between shrubs (Gaps, hereafter) at least 6 m away from the closest *Retama* individual, far from the influence of the shrub. Holes under *Retama* shrubs were made within 50 cm from the base of the shrub's main trunk. One type of experimental tube was then randomly assigned and inserted into each hole, refilled with the original soil, and compacted to previous bulk density. Each shrub had therefore the three treatments under its canopy (PVC, Netting and Mesh). In open spaces between shrubs (Gaps) we inserted Mesh tubes following the same procedure. Soil was allowed to stabilize for 8 months before plantation.

Marrubium seeds were germinated on vermiculite-filled trays (Verlite®, Vermiculita y Derivados SL, Gijón, Spain) in a greenhouse. In autumn 2008, seedlings were then taken to the field and three seedlings were planted inside each tube. Sixty millilitres of water were then supplied to each to reduce transplant shock. Upon plantation, seedlings were protected against herbivory with a mesh cage 18 cm width × 15 cm high, 2 cm mesh size. Fifteen days after planting, dead seedlings were replaced to ensure seedling establishment. One month after plantation and once overcome transplant shock, seedlings were thinned to one per treatment. Cages were kept throughout the experiment and no water was ever supplied other than the initial 60 ml.

Soil moisture and micrometeorology

Soil water potential (Ψ_s) was measured twice during winter and every month during spring and summer in the course of the experiment using twelve thermocouple psychrometers (PST-55-SF; Wescor, Logan, UT, USA) installed at 40 cm depth inside the three different types of tubes under 4 *Retama* shrubs (one psychrometer per tube, three psychrometers per plant). Psychrometers were inserted at this depth because patterns of hydraulic lift in *Retama* are strongest within this profile but not detected below 80 cm (Prieto et al., 2010b) and roots of *Marrubium* are able to reach such depth soon after plantation (*pers. obs.*). Each psychrometer was individually calibrated in the laboratory against salty solutions of known molality following Brown and Bartos (1982). Psychrometers were installed in the field in February 2008 and left to stabilize for 8 months until beginning of the experiment. All psychrometers were connected to a data logger (CR7, Campbell Scientific Inc., Logan, UT, USA) and soil water potential was recorded at hourly intervals for a period of 2–3 consecutive days in each measurement period. Offset values greater than ±5 were discarded. To minimize temperature effects on readings, cables were coiled and buried at 40 cm depth and the data logger was placed in a sealed metal box in the shade. The magnitude of the hydraulic lift (MPa) was calculated as the difference between the maximum and minimum water potential during nights. Hydraulic lift was considered to occur whenever there was an increase in soil water potential greater than 0.01 MPa, a pattern classically attributed to hydraulic lift (Millikin and Bledsoe, 2000).

At final harvest, gravimetric soil water content (%) was determined in soil samples from each treatment under *Retama* and in gaps between shrubs ($n = 4$). Soil was collected with a corer (AMS Inc., American Falls, ID, USA) at 10 cm depth and weighed immediately after collection. Samples were taken to the lab and oven-dried at 105 °C for 72 h and weighed again. Water content (%) was calculated as weight difference.

Air temperature and relative humidity were recorded under the canopy of shrubs and in gaps ($n = 3$) every 30 min with a data logger (Onset Computers Co., Pocasset, MA, USA) for two to three consecutive days during winter and summer. Photosynthetically active radiation (PAR) was recorded in the same microsites with appropriate sensors (SKP 215 PAR Quantum, Skye Instruments Ltd. Powys, UK). Measurements were taken every 30 s and a mean value was recorded every 10 min (AM16/32 and CR10X data logger, Campbell Scientific Inc., Logan, UT, USA).

Precipitation and evapotranspiration data were collected from the nearest station (Tabernas, Junta de Andalucía).

Seedling survival, growth and physiological measurements

Living and dead seedlings were recorded every 15 days from November 2008 to September 2009, thus encompassing autumn establishment, spring growth, and summer drought. Seedlings that died between two consecutive sampling dates were collected, oven-dried at 70 °C for 72 h and weighed to determine dry mass. Height of seedlings was measured from the soil surface to the most distal part of the stem bimonthly during autumn and spring, and on a monthly basis during summer. Living seedlings at the end of the experiment were harvested, oven-dried as above and weighed to determine final mass. Allometric relationships were obtained between height and mass of recently dead seedlings: $\mathit{Mass} = 0.0065 \times \mathit{Height}$ ($R^2 = 0.64$, $P < 0.01$). Here we assumed that no significant mass loss happened in dead seedlings between two sampling dates (i.e., 15 days). The former equation was used to estimate mass of living seedlings on each sampling date.

Statistical analysis

Differences in *Marrubium* seedling survival among the different treatments over time were analyzed using the Kaplan–Meier product limit method (Kaplan and Meier, 1958) with "*tube*" as treatment factor and "*day from seedling establishment*" (e.g., October 2008) as the continuous time variable. Treatments were randomly assigned to each aspect under *Retama* shrubs, and to be sure that the location of the treatments below *Retama* shrubs (aspect) did not have any effect on survival, we performed a Kaplan–Meier analysis and did confirm there was no effect of aspect on survival. Thus, this factor was not included in the analyses. Kaplan–Meier analyses were followed by pair-comparisons between treatments using the Log-rank test function (Schoenfeld, 1981). At harvest, differences in seedling survival among treatments were tested using binary logistic regression, where "*tube*" was the categorical factor and "*survival*" (dead or alive) was the response variable. To avoid type I error, pair-wise comparisons between treatments tested by binary logistic regression were followed by Bonferroni correction adjusted by Legendre and Legendre (1998).

Seedling growth over time was analyzed using ANCOVA, with "*tube*" as independent factor and "*sample date*" (day from seedling establishment) as covariate. Differences in seedling growth over the experiment were considered important when the "*tube* × *date*" interaction was significant (following García-Serrano, 2005). Nonetheless, seedling mass at final harvest was independently analyzed using a one-way ANOVA with "*tube*" as the independent factor. Seedling mass was log-transformed to ensure homoscedasticity. Differences in soil water potential among treatments over the course of the experiment were analyzed by using repeated-measures ANOVA with "*tube*" as the independent factor and "*time*" as the time factor. Tukey HSD post hoc tests were used to analyze differences between treatments for ANOVA or ANCOVA analyses.

PAR, temperature and relative humidity (RH) were analyzed using *t*-tests (under *Retama* canopy *vs.* gaps) on each sampling date, as repeated-measures ANOVA could not be performed due to low replication. Water content (WC) data from the three treatments under *Retama* were pooled because no differences were found between treatments inside *Retama*. Differences in WC of soils under *Retama* and in gaps were analyzed using *t*-tests. Data were analyzed with SPSS v.17.0 (SPSS Inc., Chicago, IL, USA). Results are presented as mean values ±1 SE throughout the text.

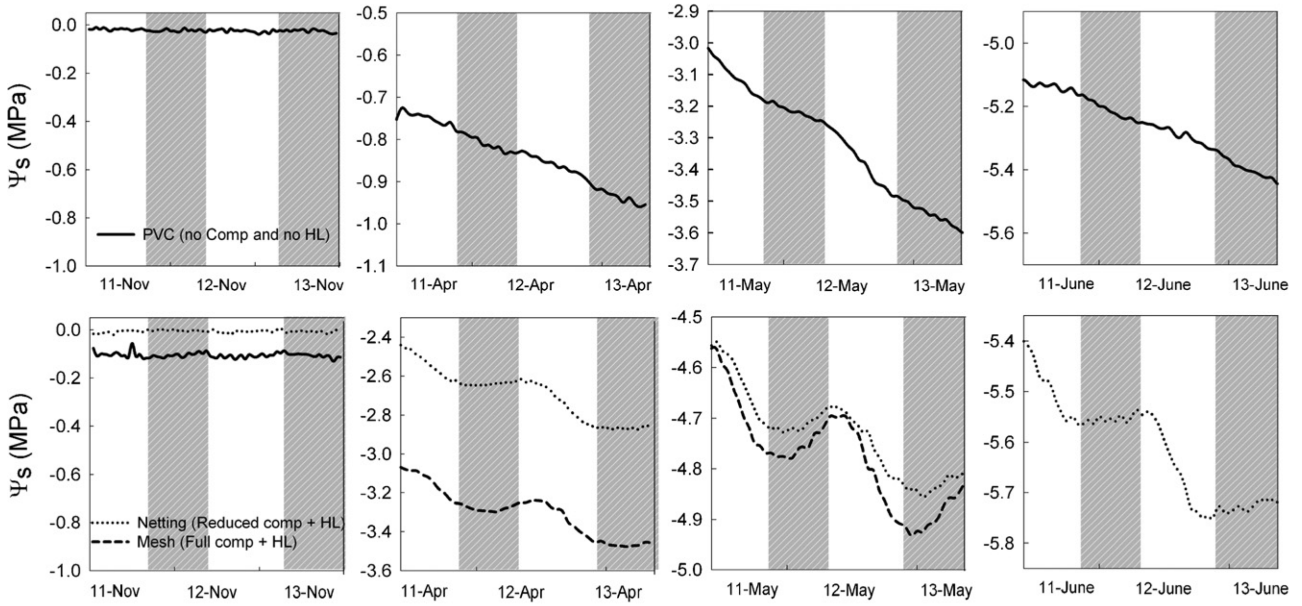

Fig. 2. Soil water potential in one representative psychrometer per treatment measured at hourly intervals at 40 cm depth inside treatment tubes. Hydraulic lift (HL) cycles (shown by increasing soil water potential at night) were only detected in Mesh and Netting tubes (lower panels). Dark areas indicate nighttime. Note the different scales on the *y*-axis.

Table 1
Mean changes in soil water potential ($\Delta\Psi_s$) during night calculated as the difference between soil water potentials at the beginning and the end of the night in the different tubes under *Retama* shrubs from April to June 2009 ($n = 4$ except in May where $n = 3$ for mesh and June where $n = 1$). Positive values indicate a net increase in soil water potential during night (indicative of HL) whereas negative values indicate a net decrease in soil water potential during the night. Data are mean ± 1SE. Different letters in a column indicate significant differences between treatments within each date (One-way ANOVA; $P < 0.05$). Data from November were not included, as HL cycles were not detected during this period.

Treatment	$\Delta\Psi_s$ (MPa)		
	April	May	June
PVC	-0.02 ± 0.00^a	-0.11 ± 0.02^a	−0.07
Netting	0.06 ± 0.02^b	0.09 ± 0.01^b	
Mesh	0.07 ± 0.02^b	0.06 ± 0.01^b	0.03

Results

Soil moisture and hydraulic lift

Hydraulic lift under *Retama* canopies was detected in Netting and Mesh tubes in April, May and June, but not in PVC tubes. In winter, HL was not detected in any tube (Fig. 2 and Table 1). In Mesh and Netting tubes, Ψ_s decreased during daytime and increased at night, with mean night increases being greater than 0.01 MPa (Table 1) indicating HL (Millikin and Bledsoe, 2000). A different trend was recorded in PVC tubes, as Ψ_s decreased during both day and night (Table 1). Summer was very dry and most psychrometers remained inoperative because Ψ_s dropped below −6 MPa, a value close to the lower limit for psychrometric measurements (Brown and Bartos, 1982). In winter, soils were likely too moist for HL to happen (Fig. 2 and Table 2).

Soil water potential was different among tubes (RM-ANOVA $F_{2,8} = 4.77$, $P < 0.05$) and changed in the course of the experiment (RM-ANOVA $F_{2,16} = 144.32$, $P < 0.001$). In addition, the pattern of soil drying with time differed among tubes, as indicated by the significant "*tube* × *time*" interaction (RM-ANOVA $F_{4,16} = 4.85$, $P < 0.01$, Table 1). Overall, soils remained moist (i.e., above −0.05 MPa) during winter in all tubes and got drier as seasonal drought progressed. Soils in PVC tubes tended to be moister than in Netting and Mesh tubes, and there were no significant differences between the two latter treatments on any sampled date (Table 2). Water content measured at harvest was significantly higher under the canopy than in gaps (Table 3). Depletion of soil moisture inside PVC tubes was lower than in treatments where competition for water was allowed (Mesh and Netting) despite HL. This led to greater soil water potentials inside PVC tubes in spring and summer (Table 2).

Microclimatic conditions

With a total of 293 mm of rain, year 2009 was slightly above average (Lázaro et al., 2001); however, 287 mm fell during winter and only 6 mm from May to September (Fig. 3).

Table 2
Mean daily soil water potential ± 1SE (Ψ_s, MPa) measured at 40 cm depth in treatments under *Retama* shrubs from November 2008 to June 2009. Upper-case letters indicate significant differences between dates within each treatment. Different lower-case letters in a column indicate significant differences between treatments (RM-ANOVA, M-ANOVA, Tukey post hoc test, $P < 0.05$, a cross next to the lower case letter for $P < 0.08$); $n = 4$ except in May where $n = 3$ for mesh and June where $n = 1$. Data from June 2009 was not included in the analysis due to the low number of replicates.

Treatment	Ψ_s (MPa)			
	November	April	May	June
PVC (No comp., no HL)	-0.01 ± 0.01^{Aa}	-1.82 ± 0.40^{Ba}	$-2.54 \pm 0.66^{Ba+}$	−5.27
Netting (reduced comp. + HL)	-0.04 ± 0.06^{Aa}	-2.59 ± 0.34^{Ba}	-4.72 ± 0.07^{Cb}	−5.61
Mesh (full comp. + HL)	-0.02 ± 0.03^{Aa}	-2.99 ± 0.26^{Ba}	$-4.13 \pm 0.41^{Bb+}$	–

Table 3
Microclimate conditions measured under *Retama* shrubs and in gaps in winter and summer. Variables are maximum photosynthetically active radiation (PAR), mean day temperature (*T*), mean relative humidity (RH), difference between day maximum and minimum temperature (Daily *T* diff) and gravimetric soil water content (WC). Data are mean ± 1SE ($n = 3$). Data for WC under shrubs were obtained by pooling data from the three treatment tubes ($n = 4$). Different letters in a row show significant differences between microsites each season (*t*-test, $P < 0.05$).

	Winter		Summer	
	Retama	Gaps	*Retama*	Gaps
PAR (μmol m^{-2} s^{-1})	985.50 ± 159.13^a	1523.95 ± 13.85^a	1048.11 ± 61.14^a	1795.88 ± 94.85^b
T (°C)	20.5 ± 0.16^a	21.4 ± 0.48^a	35.0 ± 0.32^a	38.65 ± 0.40^b
RH (%)	65.63 ± 7.54^a	64.19 ± 2.54^a	11.72 ± 0.39^a	9.10 ± 0.50^b
Daily *T* diff (%)	11.86 ± 0.30^a	11.49 ± 1.23^a	20.52 ± 0.68^a	26.05 ± 1.10^b
WC (%)	–	–	0.33 ± 0.03^a	0.16 ± 0.02^b

Microclimatic conditions were less severe under shrub canopies than in gaps. The *Retama* canopy blocked some radiation (PAR), which was on average 35% and 42% lower under the canopy than in gaps in winter and summer, respectively (Table 3). Mean day temperatures were also lower under the shrubs, and mean relative humidity was significantly higher under the canopy than in gaps. Differences in both variables were significant in summer but not in winter. Daily changes in temperature, measured as the difference between the daily maximum and minimum, were 5 °C greater in gaps than under the shrub canopy in summer. No significant differences were observed in winter.

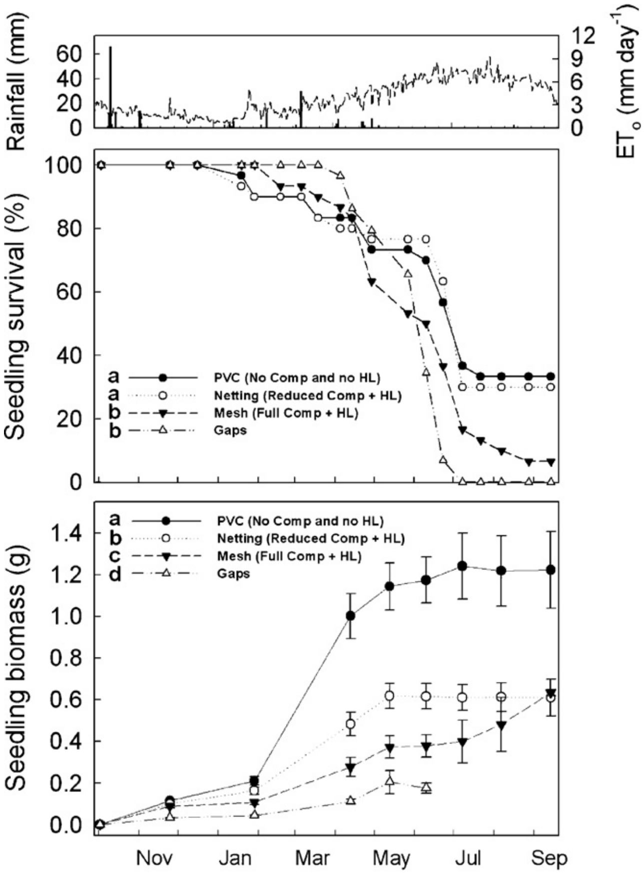

Fig. 3. Rainfall (upper panel, solid bars) and evapotranspiration (upper panel, dashed line), seedling survival (middle panel) and biomass (lower panel) of surviving *M. vulgare* seedlings planted in three different tube treatments under *Retama sphaerocarpa* shrubs (PVC, Netting and Mesh) and in Gaps. Data for biomass are mean ± 1SE. Different letters indicate significant differences among treatments (log-rank test for survival, $P < 0.001$; Tukey post hoc test for seedling biomass, $P < 0.001$). Rainfall and evapotranspiration data were collected from an adjacent meteorological station.

Seedling survival and growth

There were significant differences in seedling survival among treatments in the course of the experiment (Kaplan–Meier, $\chi^2 = 21.98$, $P < 0.001$; Fig. 3). In spring around 60–80% of seedlings were alive whereas at the beginning of the dry season seedling mortality increased, especially where full competition took place (Mesh tubes) or where abiotic conditions were harsher (Gaps); in both treatments survival by late June was just 37% and 7%, respectively, whereas survival in PVC and Netting tubes was 57% and 63% respectively. Survival differences were greatest among treatments at the end of the experiment in late summer, after >4 months of drought (logistic regression, $\chi^2 = 6.31$, $P < 0.01$). Overall, *Marrubium* seedlings had greatest survival rates in PVC and Netting tubes (33% and 30%, respectively), and lowest in Mesh tubes (7%) and in gaps (0%). It is worth noticing that no seedlings survived at the end of the experiment in gaps.

Seedling growth in winter was slow but a great increase in biomass did occur in spring (Fig. 3). Biomass increased five-fold in PVC tubes *vs.* three-fold in Netting tubes and less than two-fold in Mesh tubes and gaps. During summer, seedling growth remained almost arrested in all tubes, with the exception of an increase in seedling mass in Mesh tubes towards the end of the experiment (this effect was due to a greater mortality of smaller seedlings within this treatment rather than to true seedling mass gain in live seedlings; data not shown). Seedling growth patterns changed in the different experimental tubes as indicated by the significant "*tube* × *time*" interaction (ANCOVA $F_{3,613} = 4.207$, $P < 0.01$, Fig. 3). Seedlings without HL and root competition (PVC tubes) grew significantly more than seedlings where HL and root competition took place (Netting and Mesh tubes) and seedlings in Netting tubes gained significantly more biomass than seedlings in Mesh tubes where full root competition and HL occurred and in gaps. Seedling mass at harvest was significantly different among tubes (one-way ANOVA $F_{2,23} = 8.07$, $P < 0.01$) being greatest in PVC tubes while Netting and Mesh tubes did not differ (Tukey post hoc test, $P > 0.05$).

Discussion

Retama shrubs enhanced *Marrubium* survival and growth in its understorey compared to gaps, in agreement with previous reports showing that *Retama* is a nurse plant. But competition under *Retama* is strong (Pugnaire and Luque, 2001) and although we detected HL in Netting and Mesh tubes, it only slightly increased seedling survival during summer but had no effect on growth. As expected, PVC prevented the effects of HL inside tubes but also competition from *Retama*, producing the largest plants. Significant differences among all three treatments under the canopy point to belowground interactions between seedlings and shrubs as the cause, all other environmental variables being equal under the canopy. The shallow-rooted habit of *Marrubium* (Fernández et al.,

2010) along with the high density of *Retama* fine roots in the top soil (De Baets et al., 2007) suggest that, when growing together, root systems of the two species overlap and compete, with consequences on aboveground plant mass. Therefore although we did not measure root architecture or density, differences in growth and survival between treatments point to belowground competition (Casper and Jackson, 1997; Schenk, 2006).

Our main hypothesis did not hold, as the positive effect of HL on *Marrubium* seedling survival and growth did not override the negative effect of root competition with neighbours. In our study, hydraulic lift was detected in Netting and Mesh tubes with a magnitude similar in both tubes. Soil moisture was, however, lower than in PVC tubes. Seedling survival was higher in tubes with no (PVC) or reduced (Netting) competition, irrespective of whether HL was allowed (Netting) or not (PVC). Survival was lower under full root competition and HL (Mesh). Inconsistent with our hypothesis, our data suggests that the intensity of belowground competition outweighed the positive effects of HL on survival.

The similar survival in PVC and Netting tubes evidences that the effect of HL was weaker than the effect of competition. However, the fact that root competition outweighs HL does not preclude any positive effect of HL on seedlings. In fact, HL seems to have a positive effect on survival (i.e., comparable in PVC and Netting tubes) but not on seedling growth (i.e., different growth in PVC and Netting tubes). These data evidence that seedling responses to the interaction differ depending on the sensitivity of the variable measured (Goldberg et al., 1999; Burgess, 2011). In a similar experiment, Cahill and Casper (2000) used different kind of tubes to exclude neighbours' roots and observed a strong correlation between plant growth inside tubes and belowground competition intensity.

To our knowledge, this is one of the few reports analyzing the relative importance of HL on seedling survival under a nurse species engaged in HL and the first in semi-arid environments. A study in a mesic coniferous forest found that Douglas-fir (*Pseudotsuga meinziesii*) seedlings tended to have greater survival when seedlings were connected to donor trees through mycorrhizal fungi and hypothesized that HL might play a role in the interaction (Teste and Simard, 2008). They observed greater competition for soil resources next to the donor tree whereas facilitation was greater with increasing distance from the tree. Similar results were obtained by Ludwig et al. (2004) on adult grasses growing under *Acacia tortilis* trees when, similarly to our PVC tubes, belowground competition and HL were excluded. Their conclusions support our findings that competition for soil water between tree and understorey species was intense and outweighed the facilitative effects of HL. Hirota et al. (2004) also observed intense competition between rice plants (*Oryza sativa*) that had lower performance in the presence of the tree (*Markhamia lutea*), but in the long-term rice plants that had access to water lifted by the tree remained green whereas rice growing alone died.

Preventing HL while keeping all other root interactions in the understorey is difficult to achieve without disturbance, and separating HL effects from root competition effects in the field is not feasible. In our experiment, treatments that allowed flux of lifted water to seedlings (Netting and Mesh tubes) were effective since we detected HL inside these tubes. *Marrubium* seedlings most likely took up water lifted by *Retama* in treatments where hydraulic lift was allowed, as it is hard to believe that roots discriminate against lifted water once released in the soil. We also succeeded to create a less interactive treatment (Netting) where root competition for resources and interspecific root contact were reduced by preventing root growth inside Netting tubes. Our treatments thus allowed us to compare the effects of HL on a gradient from no root interactions (PVC) to reduced (Netting) and full (Mesh) root interactions, yet we still do not know what proportion of the effect found in the Netting tube is due to reduced competition or to HL.

Overall, our data show that moisture provided by *Retama* through HL did not fully compensate for belowground interactions in the understorey, as shown by seedling survival and biomass data. However, the overall net effect of this shrub species on *Marrubium* seedlings was positive, compared to seedlings performance in gaps. Hydraulic lift might contribute to this nurse effect by buffering soil moisture decline (Brooks et al., 2002; Meinzer et al., 2004), although belowground competition under *Retama* shrubs overrides the effect of HL. Most likely, the combined effects of microclimate amelioration and improved soil moisture and nutrients under the canopy of *Retama* outweighed negative root interactions.

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