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Hydraulic lift promotes selective root foraging in nutrient-rich soil patches

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Abstract. Hydraulic lift (HL) – the passive movement of water through plant roots from deep wet to shallow drier soil layers – can improve root survival in dry soils by providing a source of moisture to shallow roots. It may also enhance plant nutrient capture, though empirical evidence for this is scarce and whether HL promotes the selective placement of roots in nutrient-rich soil enhancing nutrient capture in dry soils remains unknown. We tested this with a split-pot design in which we separated the root system of *Retama sphaerocarpa* (L.) Boiss shrubs into two pot compartments: a lower, well-watered one; and an upper, drier one. Half the shrubs grew under natural light conditions hence allowed to perform HL, whereas the other half had impaired HL by maintaining continuous illumination at night. Resource-rich (organic matter enriched in ^{15}N and P) and resource-poor soil patches were inserted in the upper compartment after a drought treatment was imposed. Artificial illumination did impair HL at night. Soil moisture in both the whole upper compartment and in soil patches was lower in plants illuminated at night and reduced the allocation of roots to nutrient-rich soil patches at the expense of root growth in nutrient-poor patches (i.e. root foraging precision). Plant nitrogen capture was also lower in shrubs with impaired HL. Overall, these results demonstrate that HL favoured the selective placement of roots in nutrient-rich patches as well as nutrient capture under drought, a process that may secure nutrient capture and maintain plant performance during drought periods.

Additional keywords: drought, hydraulic redistribution, isotopes, nutrient capture, root growth, soil nutrient heterogeneity.

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Introduction

Plants in arid ecosystems tend to have deep roots (Schenk and Jackson 2002) that span and explore large volumes of soil connecting layers with very different levels of moisture. Under these conditions and in periods of low transpiration demand (i.e. night-time) water can move passively through the root system driven by water potential gradients. Some of this water is passively released into shallow soil, moistening dry layers in a phenomenon termed 'hydraulic lift' (HL, Richards and Caldwell 1987).

The process of HL has been extensively addressed, mostly focusing on its potential benefits for both the lifting plant and its neighbours (see Prieto *et al*. 2012 for a recent review). Most of these benefits come from water supplied overnight by HL, which moistens shallow soil layers (Meinzer *et al*. 2004), increasing plant transpiration rates and carbon gains (Dawson 1997; Caldwell *et al*. 1998; Ryel 2004). HL also benefits belowground plant parts and the rhizosphere because (i) water redistribution within roots (from parts experiencing high water potentials to drier root parts) minimises the hazardous effect of soil drying and increases root survival (Bauerle *et al*. 2008*a*); (ii) internal redistribution of water fills up xylem vessels reducing root embolism, thus, maintaining greater hydraulic conductivity (Domec *et al*. 2004); (iii) it maintains roots hydrated at low soil water potentials (Valenzuela-Estrada *et al*. 2009); and (iv) some of the water transported to shallow roots can be passively transferred to root symbionts (e.g. arbuscular mycorrhizal fungi), thereby increasing their survival during drought periods (Querejeta *et al*. 2003; Warren *et al*. 2008). All the above processes should help maintain fine root growth and function and increase root growth into dry soil (Caldwell *et al*. 1998).

Nutrients in soil are not homogenously distributed so resource-rich soil patches can co-occur with nutrient and water-depleted patches (Jackson and Caldwell 1993). Typically, plants show a plastic response to soil nutrient heterogeneity: roots proliferate in patches where nutrients and water are most available and avoid those where resources are in short supply (Hutchings and de Kroon 1994; Bauerle *et al*. 2008*b*). Most studies that analysed root responses to nutrient heterogeneity or root foraging behaviour were generally conducted under abundant water conditions (see Hodge 2010 for a review), so data from heterogeneously distributed nutrients in dry soils are scarce and restricted to few publications (de Kroon *et al*. 1998; Suriyagoda *et al*. 2010). In such scenarios (i.e. arid and

semiarid ecosystems) HL could promote root growth into nutrient-rich soil patches by hydrating fine roots, increasing soil moisture and enhancing plant nutrient capture.

Plants take up nutrients from the soil solution via mass flow or diffusion; both of these processes are dependent on soil moisture conditions (Comerford 2005). Low soil moisture prevents mass flow and nutrient diffusion in the soil solution (Pregitzer and King 2005), thus, limiting nutrient capture. Plant roots, through the rewetting of soil at night via HL, may indirectly facilitate nutrient capture from shallow soil layers where nutrients mostly accumulate (Caldwell *et al*. 1998; Liste and White 2008). However, conclusive evidence in this field is still scarce (Armas *et al*. 2012). Most studies linking HL with nutrient capture involved grass species (see review by Armas *et al*. 2012), whereas the few that used tree or shrub species either lacked true controls with HL suppressed, used liquid nutrient solutions that can easily be absorbed by plants irrespective of soil humidity conditions (Armas *et al*. 2012) or did not directly measure nutrient capture (Dawson 1997). However, Aanderud and Richards (2009) showed that the daily soil drying–rewetting cycles due to HL could enhance organic matter decomposition in dry soils. These authors hypothesised that greater decomposition rates could promote greater nutrient availability, although they did not directly measure it. However, Armas *et al*. (2012) showed that HL not only promoted organic nitrogen (N) mineralisation under dry conditions, but also that HL makes soil N readily available for plants and that plants take up this N. This emphasises the importance of HL for nutrient capture but the role of HL on root growth still remains unknown.

In this study we hypothesised that HL supports root growth and foraging in resource-rich soil patches under dry soil conditions. This hypothesis was tested in a greenhouse experiment using a split-pot design with discrete nutrient-rich (organic matter enriched in ^{15}N and P) and control soil patches. The system included a lower-wet pot and higher-dry pot kept hydraulically apart by an air barrier that prevented water flow between soil layers (Querejeta *et al*. 2012) and the use of night-time illumination as a method to impair HL. This method has been used to curtail the process of HL (Dawson 1997; Bauerle *et al*. 2008*a*; Armas *et al*. 2012) based on the fact that water within the plant moves passively following a complex source–sink system of water potentials from lower to higher water potentials (see Prieto *et al*. 2012 for a detailed discussion). When stomata are open the negative water potential around leaves moves water from the soil into the roots and out to the atmosphere, but during stomatal closure the water potential gradient between the soil and the atmosphere is reduced and the greater water potential gradient is established between the lower and upper soil compartments. Hence, water is transported into roots in the lower, wetter compartment and flows out of the roots and into the soil in the upper, drier compartment. By preventing stomatal closure by night-time illumination, the water potential gradient is continuously forced in the direction of the leaves and atmosphere, thereby preventing water transfer between soil compartments.

The target species was *Retama sphaerocarpa* (L.) Boiss, a phreatophytic, leguminous shrub with the ability to be engaged in hydraulic lift (Prieto *et al*. 2010). After 10 weeks of imposed drought in upper compartments, we analysed root growth in the discrete soil patches and plant N capture from the nutrient rich patches and determined the occurrence of hydraulic lift.

The hypotheses tested were: (i) that greater water availability in upper compartments in plants engaged in HL would lead to greater root growth in response to nutrient addition (NR patches) than in those individuals where HL occurrence was impaired by illumination at night; and (ii) that greater root growth in NR patches would lead to increased plant nutrient capture.

Materials and methods

Soils

Soil was collected between 10 and 30 cm depth from a dry riverbed in Almería province, SE Spain (37°08′N, 2°22′W, 630 m altitude). It was an eutric fluvisol with 0.77% of organic matter, 1.4 mg g^{-1} total N, 0.064 mol kg^{-1} Ca, 0.0061 mol kg^{-1} Mg, 0.0017 mol kg^{-1} Na and 0.0006 mol kg^{-1} K. Cation exchange capacity was low (0.0232 meq) (Puigdefábregas *et al*. 1996). Soil was air-dried and sieved (2 mm mesh size) to eliminate the coarser fraction and thoroughly mixed with type III vermiculite at 2 : 1 v/v (Verlite, Vermiculita y Derivados SL, Gijón, Spain). The mix was used as soil substrate for the mesocosms split-pots.

Plant material and mesocosm establishment

Seeds of *Retama sphaerocarpa* (L.) Boiss (see Fig. S1, available as Supplementary Material to this paper) were sown in potting soil in February 2006. Four weeks after emergence, seedlings (one per pot) were transplanted to experimental mesocosms (split-pots) and grown in a greenhouse for three years until the beginning of the experiment in May 2009. Mesocosm set up was similar to that of Querejeta *et al*. (2012). Mesocosms consisted of two opaque PVC pots 23 cm diameter × 21 cm height placed one on top of the other. Both pots were connected through a 2 cm diameter bottom opening in the upper pot ensuring root penetration and growth into the lower compartment. One week before the treatments started we made sure that all shrubs had roots colonising the lower compartment. Upper pots were then fixed using scaffolding and the upper three centimetres of the lower pot were cut. Soil between pots was washed off creating an air barrier that prevented water flow between compartments. Exposed woody roots were protected with a fine layer of Vaseline (Unilever, Rotterdam, The Netherlands) to avoid root damage (Fig. S1).

When treatments started in May 2009, watering was withheld in all upper compartments, whereas bottom compartments were maintained at or near saturation by frequent irrigation (twice a day) throughout the experiment. Bottom compartments had small holes in the bottom to allow drainage. Four randomly assigned shrubs were subjected to 22 h light +2 h dark period cycles to prevent hydraulic lift (impaired hydraulic lift treatment, I-HL hereafter), whereas the remaining five shrubs grew under natural conditions, with ~12 h light +12 h dark cycles (hydraulic lift allowed treatment, HL hereafter). Each treatment had a soil control consisting of three pots with the same soil mixture but without shrubs and subjected to 12 h light and 12 h dark period (S1; $n = 3$) and 22 h light and 2 h dark period (S22; $n = 3$). Four 18W Grolux fluorescent tubes (Osram Sylvania, Danvers, MA, USA) and two 150 W CFL Phillips Agrolite light bulbs (Phillips International B. V., Amsterdam, The Netherlands) supplemented

a total of 400 μmol m^{-2} s^{-1} of PAR light. Lights were switched on each day at dusk and turned off 2 h before dawn to achieve the 22-h light cycle. The beginning and end of the light cycle was adjusted as the experiment progressed to keep the lighting period constant throughout the experiment.

Labelled organic matter preparation and soil cores

Faba bean (*Vicia faba* L.) seeds were sown (three seeds per pot) in April 2008 in 14 cm diameter × 11 cm height pots filled with riverbed sand. After emergence of seedlings, each pot was watered daily with tap water for 10 weeks except twice a week when pots were watered with 100 mL of a Hoagland solution supplemented with $^{15}NH_4{}^{15}NO_3$ (800 mg L^{-1}) and KH_2PO_4 (340 mg L^{-1}l). Plants were harvested 10 weeks later and leaves separated from stems. Leaves were then oven-dried at 70°C for 72 h and finely ground and homogenised with a grinder. The resulting dry organic matter (^{15}N-P-OM) had a $\delta^{15}N$ value of ~9000 (3.5 atom %) and a concentration of 0.23% of total P. This organic matter was then used to create discrete nutrient rich cores (NR patches, hereafter) of 18 cm^3 in volume by thoroughly mixing 1 g of the enriched OM with 19 g of original soil (sieved through 2 mm mesh) and placing the resulting mixture within a plastic cylinder 60 mm height × 20 mm diameter. Each patch represented ~3.5% of the total upper-pot soil volume. The cylinder was open in both ends and the wall was meshed with 112 rectangular 5 × 3 mm holes that allowed root growth into the patches. Control cores were created using the same procedure but the cylinder was filled with 20 g of sieved soil (thus, they were nutrient-poor soils, C patches hereafter). Each soil core received 5 mL of water (25% gravimetric water content) before they were inserted in the pots to homogenise the initial conditions between treatments and core types.

One week after water was withheld in the upper compartments and artificial light was supplemented, NR and C patches were inserted into the upper soil compartment at 10 cm depth; within each pot, two NR and two C patches were inserted in opposite directions with a cross-like design and at a middle distance between the shrub stem and the pot edge.

Irrigation with deuterium-labelled water

Nine weeks after treatments started 500 mL of deuterium-labelled water was added to each of the lower compartments. The lower pots were placed inside a small bucket that contained the labelled water until all water was taken up by plants (~4 days). Buckets were covered with an aluminium foil to prevent over-heating and evaporation of labelled water that would lead to isotopic fractionation before root water uptake and redistribution. Deuterium-labelled water (δD ~5000‰) contained 0.78 mL of pure D_2O (99.8% deuterium enrichment, Sigma Chemical Co., St Louis, MO, USA) per L of water.

Physiological measurements

Ten weeks after treatments started and 1 day before harvest, we assessed the physiological status of shrubs. We measured net photosynthetic rate (A) and stomatal conductance to water vapour (g_s) on mature, well developed green cladodes or photosynthetic stems that act as leaves in *R. sphaerocarpa*. Measurements were taken at 0600–0800 hours solar time to avoid potential photoinhibition with an infrared gas analyser (LI-6400, Li-Cor Inc., Lincoln, NE, USA) under ambient CO_2 concentration (380 μmol mol^{-1}) and artificial light and expressed on a leaf area basis. Maximum photochemical efficiency of PSII (F_v/F_m) was measured at dawn with a portable fluorimeter (PEA, Hansatech, Kings Lynn, UK) in cladodes previously dark-adapted for 30 min.

Harvest, sampling and laboratory procedures

At the end of the experiment, the NR and C soil patches were retrieved and plants harvested. Aboveground tissues, fine (<2 mm in diameter) and coarse (>2 mm) roots from upper and lower compartments were separated into different samples, oven-dried at 70°C for 72 h and weighed. Root mass ratio was calculated as the ratio between total root mass and total plant mass to estimate mass allocation patterns. In addition, the ratio between absorbing organs above- (green cladodes) and belowground (fine roots) was also calculated to estimate how much of the green mass is supported by absorbing fine roots.

On each plant, 6–8 cladodes (~2–3 g) and 2–3 g of fine roots (<2 mm diameter) from the lower compartments were selected, finely ground and then analysed for ^{15}N. Cladodes were also analysed for total N, P and C. Before the beginning of the experiment (t_0), cladode samples were also randomly collected from five shrubs to determine ^{15}N content.

Immediately after the dark period, NR and C patches, as well as soil samples from upper pots were carefully excavated, placed in plastic vials and immediately weighed for gravimetric water content determination (WC) and deuterium analyses. Then, samples were oven-dried to constant weight at 70°C for 72 h and weighed again. Soil gravimetric water content (%) was obtained for upper pots (bulk samples) and NR and C soil patches (soil from curlers); WC was calculated as the weight difference (g) between wet and dry soil per unit dry soil (g). Subsamples (1 g each) of soil from patches were finely ground and homogenised using a mortar and the $\delta^{15}N$ composition was then determined. Previously, roots had been cleaned, oven-dried and weighed to obtain root density per patch (mg_{roots} $g_{soil}{}^{-1}$).

Root foraging was determined with the relative interaction index (RII, see Armas *et al*. 2004). The index is calculated as $((RB_{NR} - RB_C)/(RB_{NR} + RB_C)) \times 100$ where RB_{NR} is the root mass in NR patches and RB_C is the root mass in C patches. The index ranges from –100 to 100 with positive values indicating greater placement of roots into NR patches and hence increased precision of root foraging, whereas negative values indicate the opposite.

Water for hydrogen isotope analysis was extracted from bulk soil samples collected at a depth of 10–15 cm in the upper compartments using a cryogenic vacuum distillation line (Ehleringer and Osmond 1989). Both deuterium (δD) and $\delta^{15}N$ analyses were conducted at the Stable Isotope Facility, University of California, Davis, CA, USA. Deuterium content was determined using a laser-absorption spectroscopy technique (LGR DLT-100 water analyser, Los Gatos Research, Inc., Mountain View, CA, USA). Analytical precision was <0.8‰. Values are expressed in delta notation and referred to the Vienna Standard Mean Ocean Water (V-SMOW). The proportion of the irrigation labelled water that was lifted and thus present, in upper

compartments (f_s) was estimated using a two-end member model (Dawson *et al*. 2002):

$$f_s(\%) = \frac{(\delta_s - \delta_b)}{(\delta_w - \delta_b)} \times 100, \quad (1)$$

where δ_s is the delta value of the water in the soil sample collected in upper compartments, δ_b is the background delta value and δ_w is the delta value of the labelled water (5000‰). To be conservative in the calculations, we used the lowest value from soil water in all the upper compartments (–33.9‰) as a background value (δ_b) assuming that this value corresponded to the irrigation water value before application of labelled water.

$\delta^{15}N$ content was determined with a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK). Analytical precision was <0.8‰. Values are expressed in delta notation and referred to the atmospheric air standard value for ^{15}N. We calculated the percentage recovery of the ^{15}N applied ($^{15}N_{capture}$) at the end of the experiment following Armas *et al*. (2012), as:

$$^{15}N_{capture}(\%) = \frac{^{15}N_{cladodes} - ^{15}N_0}{^{15}N_{patches}} \times 100, \quad (2)$$

where $^{15}N_{cladodes}$ and $^{15}N_{patches}$ are mg ^{15}N in cladode tissue and mg ^{15}N of the NR-patches at the end of the experiment, and $^{15}N_o$ is mg ^{15}N in cladode tissue before the experiment (t_0).

Total N, P and C analyses were conducted at the Servicio de Ionómica, Centro de Edafología Aplicada del Segura in Murcia, Spain. Total C and N content were determined using a Flash EA 1112 CHN analyser (Thermo Finningan, Rodano, Italy) and total P content was determined using an Iris Intrepid II XDL analyser (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Statistical analyses

Differences between treatments in cladode, root and soil ^{15}N content, cladode nutrient content (N, P, C), above- and belowground mass, root foraging (RII), soil deuterium content and plant physiological responses were tested using one-way ANOVA (from the initial nine plants we ended up with seven for analyses, $n = 3$ for HL and $n = 4$ for I-HL. Two plants from the HL treatment did not perform HL since main roots that connected both lower and upper compartments were damaged (see 'Results'). For soil water content in upper compartments we performed one-way ANOVA with 'treatment' as the independent factor ($n = 3$ for HL, $n = 4$ for I-HL and $n = 3$ for S12 and S22 respectively). Differences between treatments were analysed using Fisher's LSD *post hoc* test. Differences in soil patch root density and soil patch water content were analysed with a split-plot ANOVA considering 'treatment' as the main plot factor and 'patch' as the subplot factor. For each plant (mesocosms) we calculated average values for each variable measured in the patches (curlers); one from the two NR patches and one from the two C patches and these values were used as single replicates ($n = 3$ for HL and $n = 4$ for I-HL). Data were log-transformed to ensure homostedasticity when necessary. Differences between patches were analysed using Fisher's LSD *post hoc* test. Pearson's correlation analyses were performed across soil and plant variables ($n = 9$). All statistical analyses were performed using SPSS ver.17.0 (SPSS Inc., Chicago, IL, USA). Results are presented as mean ± s.e.

Results

Plant growth and physiological response

Total aboveground mass was not affected by the application of light at night (Table 1, $P = 0.76$). When divided into green (photosynthetic) and senesced cladode mass, differences in mass between treatments were still not significant ($P = 0.86$ and $P = 0.76$ for green and senesced mass respectively). Total belowground mass was slightly greater in shrubs growing in the HL treatment than in the I-HL (Table 1, $P = 0.03$). These differences were due to higher amount of fine roots in lower pots in the HL treatment ($P < 0.01$). Coarse (>2 mm) roots in lower pots ($P = 0.69$) or coarse and fine (<2 mm) root mass in upper compartments were not different ($P > 0.30$ in both cases). Differences in fine root mass did not affect the root weight ratio ($P = 0.10$) or the fine root : green mass ratio ($P = 0.30$).

Photochemical efficiency of PSII (F_v/F_m) was around the optimum of 0.8 (Maxwell and Johnson 2000) in both treatments but shrubs in the HL treatment displayed significantly greater F_v/F_m values ($F_{1,5} = 6.75$, $P < 0.05$). Treatment plants did not differ in A or g_s ($P = 0.73$ and $P = 0.18$ respectively; also see Table S1, available as Supplementary Material to this paper).

Soil moisture and isotope analysis

At the end of the experiment, after 10 weeks of imposed drought, water content (WC) in the upper compartments was one-third greater in the HL than in the I-HL treatment (Table 2, one-way ANOVA $F_{3,9} = 12.719$, $P = 0.001$). Irrespective of the treatment, WC was low (less than 1% w/w). In the HL treatment, WC was significantly greater than that of its no-plant control (S12) and

Table 1. Dry mass of above- and belowground plant parts, green and senesced cladodes, suberised stem and fine (<2 mm diameter) and coarse (>2 mm diameter) roots from upper and lower pot compartments of *Retama sphaerocarpa* shrubs engaged in hydraulic lift (HL) and with HL impaired (I-HL)

Data are means ± s.e. ($n = 3$ for HL and $n = 4$ for I-HL). Different letters in the same row show significant differences between treatments (one-way ANOVA, $P < 0.05$)

Mass (g)	Treatment	
	HL	I-HL
Total aboveground	10.41 ± 2.41a	11.09 ± 1.34a
Green	6.81 ± 1.98a	7.46 ± 2.20a
Dry	1.89 ± 0.73a	2.32 ± 0.80a
Stem	1.71 ± 0.69a	1.31 ± 0.21a
Total belowground	20.52 ± 1.88a	13.04 ± 1.11b
Upper compartments		
Fine roots (<2 mm)	3.09 ± 0.94a	2.00 ± 0.46a
Coarse roots (>2 mm)	6.54 ± 0.73a	5.53 ± 0.36a
Lower compartments		
Fine roots (<2 mm)	8.58 ± 1.03a	3.53 ± 0.30b
Coarse roots (>2 mm)	2.31 ± 0.61a	1.98 ± 0.34a
Root weight ratio	2.35 ± 0.43a	1.23 ± 0.18a
Fine root : green mass ratio	2.22 ± 0.52a	1.40 ± 0.73a

Table 2. Gravimetric water content (WC), deuterium content (δD), percentage water in upper compartments derived from labelled water (% water) in soil samples from upper compartments and ^{15}N content in nutrient rich patches (Soil $\delta^{15}N$)

All samples were collected at the end of the experiment. Data are means ± s.e. ($n = 3$ for HL and $n = 4$ for I-HL). Different letters in a row show significant differences between treatments (Fisher's LSD, $P < 0.05$). HL, mesocosm with plants engaged in hydraulic lift; I-HL, mesocosm with plants with impaired hydraulic lift; S12, bare soil under 12 h of light; S22, bare soil under 22 h of light. The proportion of lifted water derived from deuterium-enriched water (% water) was significantly different from zero in the HL treatment (*t*-test $t_2 = 11.00$, $P < 0.01$) but not in the I-HL treatment (*t*-test $t_3 = 2.85$, $P = 0.07$)

	HL	I-HL	S12	S22
WC (%)	0.89 ± 0.05a	0.60 ± 0.05b	0.57 ± 0.04b	0.48 ± 0.03b
δD (‰)	44.9 ± 7.16a	–11.8 ± 7.8b	–	–
Water (%)	1.57 ± 0.14a	0.44 ± 0.15b	–	–
Soil δ $^{15}N \times 10^3$ (‰)	6.18 ± 0.21a	6.13 ± 0.04a	–	–

Table 3. Pearson's correlation (*r*) coefficients for variables measuring the magnitude of HL in upper root compartments: deuterium in soil water, (δD) and gravimetric soil water content, WC; soil water content in nutrient rich soil patches, NR-WC) and variables related to plant nutrient capture (root foraging precision, RII; and ^{15}N content ($\delta^{15}N$), total N; and total P content in shrub cladodes)

All samples were collected at the end of the experiment ($n = 9$; included those plants excluded from analyses of differences between treatments). Significant differences are indicated: *, $P < 0.05$; **, $P < 0.01$

	δD	WC	NR-WC	RII	Cladode $\delta^{15}N$	Cladode N
δD (‰)	–					
Soil water content (WC, %)	0.61*	–				
NR-WC (%)	0.43	0.77**	–			
Root precision (RII)	0.76**	0.56*	0.48*	–		
Cladode $\delta^{15}N$ (‰)	0.60*	0.41	0.18	0.38	–	
Cladode N (%)	0.01	0.02	0.00	0.01	0.02	–
Cladode P (%)	0.01	0.05	0.11	0.00	0.04	0.71**

that in pots where hydraulic lift was impaired (I-HL). Soil moisture in the I-HL treatment was marginally different ($P < 0.06$) from its no-plant control (S22) and did not differ from the no-plant control under 12 h light cycle (S12) (Fisher's LSD test, $P > 0.30$).

Deuterium content (δD) in soils was consistent with WC data since soil water collected from upper compartments in the HL treatment was significantly richer in deuterium than the upper compartments in the I-HL treatment indicating a transfer of water from lower to upper compartments in the HL treatment (Table 2). Moreover, at the end of the experiment and across treatments, δD in soils from upper compartments was linearly and positively correlated with WC (Table 3), showing that deuterium concentration was linked to the amount of water hydraulically lifted to upper compartments. Deuterium concentration differed by ~30‰ between treatments. The estimated fraction of lifted water derived from deuterium-enriched water was low (less than 2%) but significantly higher in the HL treatment compared with I-HL (Table 2). Although there was a significant proportion of D-enriched water uplifted in the HL treatment (values were different from zero), this proportion was not significantly different from zero in the I-HL treatment (Table 2). The two split-pots with plants from the HL treatment with damaged roots had lower soil water contents that were similar to those in the I-HL treatment. These individuals also showed deuterium signatures similar to those in the I-HL treatment (–13.35‰ and –24.65‰ respectively) indicating that they did not perform HL likely due to root damage when they were manipulated to create the air barrier between compartments. Thus, they were excluded from ANOVA analyses, but served us as a proxy for comparisons across treatments (in such case $n = 9$).

At the end of the experiment, WC in discrete soil patches placed on the HL treatment was significantly greater than those in the I-HL treatment, irrespective of OM addition (Fig. 1*a*, treatment effect, $P = 0.042$, see Table S2 for ANOVA results). Nutrient-rich soil patches (NR) had significantly greater water content than soil-only patches (C patches, Fig. 1*a*, patch effect $P < 0.001$). The interaction between the two factors was not significant (treatment × patch effect, $P = 0.23$), thus, indicating that differences between treatments followed a similar trend in NR and C patches. Across treatments, δD in soils from the upper compartments was marginally correlated with WC in NR patches (Table 3, $P = 0.056$).

Root growth into the NR and C soil patches and plant nutrient capture

There was an effect of treatments on root growth into soil patches (Fig. 1*b*). Root growth into soil patches was dependent on the presence or absence of OM (treatment × patch effect, $P = 0.046$, see Table S3 for ANOVA results). Root foraging precision (RII) was significantly greater in shrubs that were engaged in HL, which allocated 92% of new root mass grown in discrete soil patches into the nutrient-rich patches whereas shrubs in the I-HL allocated ~67% (Fig. 1*c*). RII was positively correlated with δD and WC in soils from NR patches and WC in soils from the upper compartments (Table 3).

Cladode $\delta^{15}N$ before treatment application (background conditions, t_0) was 4.58 ± 0.39‰, significantly lower than at the end of the experiment, when on average $\delta^{15}N$ in cladodes was 12.95 ± 2.17 and 7.10 ± 0.31‰ in HL and I-HL treatments, respectively (Table 4), suggesting a significant capture of ^{15}N from NR patches in both treatments. The proportion of ^{15}N capture (%) from soil patches differed significantly between treatments. After 10 weeks of drought, ^{15}N capture by shrubs in the HL treatment was 3-fold that of shrubs in the I-HL treatment (Table 4).

Across treatments, $\delta^{15}N$ in cladodes was positively correlated with δD and hence, to the amount of water released in upper compartments via HL (Fig. 2), indicating a positive influence of hydraulically lifted water shed into the soil on ^{15}N capture. Cladode $\delta^{15}N$ was also marginally correlated with root foraging precision (Table 3, $P < 0.08$). The amount of total N, P and C in cladodes followed a trend similar to that observed for $\delta^{15}N$; there was, however, no significant differences between treatments (Table 4, $P > 0.30$).

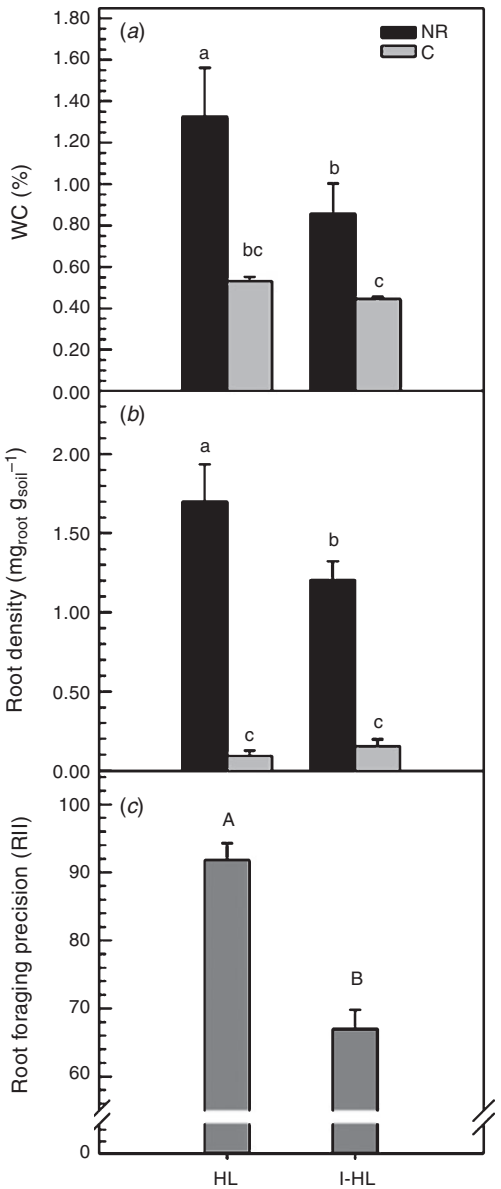

Fig. 1. Gravimetric soil water content (*a*), root density (*b*) and root foraging precision of *Retama sphaerocarpa* shrubs (*c*) in nutrient rich (NR, closed circles) and control (C, open circles) soil patches in upper compartments HL, mesocosm with plants performing hydraulic lift; I-HL, mesocosm with plants with impaired HL. Data are means ± s.e. ($n = 4$ for I-HL and $n = 3$ for HL). Error bars are shown only when larger than symbol. Different lowercase letters indicate significant differences between NR and C curlers within and between HL treatments (HL or I-HL) and uppercase letters indicate significant differences between HL treatments ($P < 0.05$).

Discussion

Our data show that HL occurs in *R. sphaerocarpa* shrubs and that continuous illumination successfully impaired this process. Root foraging precision was 25% greater in shrubs engaged in HL; i.e. showed greater root growth into nutrient-rich patches than shrubs with reduced HL ability. Shrubs engaged in HL captured three times as much ^{15}N as those that did not perform

Table 4. Cladode and root $\delta^{15}N$ (‰) composition, proportion of ^{15}N taken up from NR patches in plant cladodes (%) and total nitrogen (N), carbon (C) and phosphorus (P) content (%) in mature *Retama sphaerocarpa* cladodes collected one week before treatment application (background conditions, t_0) and at the end of the experiment after 10 weeks of drought in shrubs engaged in hydraulic lift (HL) and with impaired HL (I-HL)

Total N content (%) in roots is also shown. Data are means ± s.e. ($n = 3$ for HL and $n = 4$ for I-HL). Different letters in a row show significant differences between treatments (one-way ANOVA, $P < 0.05$; and Fisher's LSD *post hoc* differences when t_0 was included in the analysis, $P < 0.05$); n.a., not available

	t_0	HL	I-HL
Cladode $\delta^{15}N$ (‰)	4.58 ± 0.44a	12.95 ± 2.17b	7.10 ± 0.31c
^{15}N capture (%)	–	0.30 ± 0.09a	0.10 ± 0.03b
Cladode N (%)	2.24 ± 0.15a	2.62 ± 0.18a	2.48 ± 0.10a
Cladode P (%)	0.08 ± 0.01a	0.09 ± 0.01a	0.09 ± 0.01a
Cladode C (%)	44.8 ± 0.21a	45.8 ± 0.40a	45.6 ± 0.95a
Root $\delta^{15}N$ (‰)	n.a.	22.2 ± 3.63a	19.2 ± 2.89a
Root N (%)	n.a.	1.67 ± 0.08a	1.65 ± 0.03a

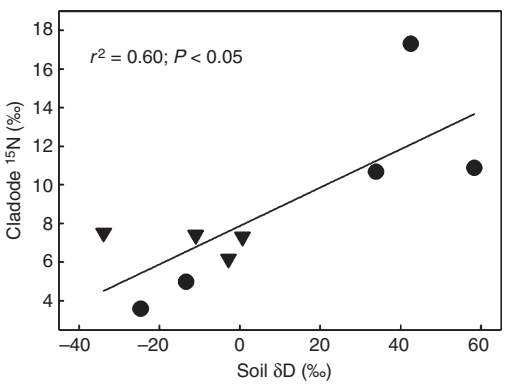

Fig. 2. Correlation between cladode $\delta^{15}N$ content in *Retama* cladodes and soil water deuterium content (δD) in soils from un-watered upper compartments ($n = 9$; all plants, included those plant excluded for between treatment analyses). Circles, HL; triangles, I-HL.

HL. The strong linear relationship found between the magnitude of HL (measured as soil δD content in upper compartments), soil water content, root foraging precision (RII) and $\delta^{15}N$ indicated that HL played a significant role in promoting root growth into nutrient-rich patches and helped nutrient capture from them. Overall, our results show that enhanced water availability in the upper compartments in the HL treatment favoured root growth in nutrient rich patches (^{15}N enriched soil organic matter patches) and led to greater ^{15}N capture by shrubs engaged in HL compared with those where HL was impaired.

Water content was significantly greater in the HL treatment, both in upper compartments and in discrete soil patches and was highly correlated with δD in upper compartments even after a relatively long drought period. Moreover, when comparing soil moisture in the HL treatment with its control without shrubs (S12), the latter was significantly drier. Lower compartments were continuously wet and a steep water potential gradient was created between lower and upper compartments that favoured the conditions for HL. Overall, these data support the idea that HL

took place in pots with shrubs under 12 h of light (HL). In contrast, the I-HL treatment showed significantly lower WC values in upper compartments than the HL ones and similar values to its control without shrub (S22), suggesting these plants were not engaged in HL. This also suggests that water evaporation was similar in S22 and I-HL pots and that I-HL plants could not take up water from the dry, upper compartments, at least towards the end of the experiment. In agreement with soil moisture data, we measured greater positive δD values in soil water in the upper compartment of pots where HL was allowed, providing clear evidence of HL in shrubs growing under 12 h of light (HL treatment). Applying labelled water to deep soil layers and its detection in upper soil layers, when the only pathway for water movement is through plant roots, has been used before to evidence HL (Dawson 1993; Leffler *et al*. 2005). In our case, upper and lower pots were hydraulically isolated and connected only through plant roots and isotopic data show that soil in upper pots in the HL treatment was wetted by water lifted from wet, lower compartments (a mix of tap water and enriched deuterium water). The tight relationship between δD and WC supports this point, an indication that only plants performing HL were able to maintain higher soil moisture in upper compartments and that water transported through HL from lower compartments was the source of this extra soil moisture. We conclude that *R. sphaerocarpa* shrubs were engaged in HL under 12 h of light (HL treatment) whereas continuous illumination at night (I-HL treatment) stopped, or greatly reduced, the process. Nonetheless, the proportion of lifted water was low compared with those found in other species under greenhouse conditions (de Kroon *et al*. 1998; Hawkins *et al*. 2009), but these low values are not surprising. Deuterium enriched water was applied after 10 weeks of drought with very dry soil conditions in the upper compartments (Table 2) and HL magnitude has been shown to decrease under very dry soil conditions in *Retama* shrubs (Prieto *et al*. 2010, 2011). The small but positive percentage of labelled water observed in upper compartments in the I-HL treatment could come from the conservative choice of using the lowest deuterium value in the upper pots, or might also represent small amounts of water lifted during the two-hour dark period, but it did not differ from zero anyway. Overall, differences in the HL capacity between treatments led to one-third as much greater soil water availability in the upper compartments of the HL treatment compared with I-HL treatment.

Plants actively forage for resources in heterogeneous environments (Bauerle *et al*. 2008*b*; Hodge 2010) and roots proliferate in resource-rich soil patches at the expense of resource-poor patches (Hutchings and de Kroon 1994). In our study, we found clear evidence confirming the hypothesis that HL would enhance root foraging under drought conditions. In the present study, *R. sphaerocarpa* shrubs displayed an active foraging behaviour in upper pots where NR rich patches were placed as noted by the positive root foraging precision (RII) and greater root growth into NR patches compared with soil-only C patches. Root foraging precision of shrubs was significantly greater in individuals that lifted water (HL) than in individuals that did not (I-HL) and there was a tight correlation between δD in soil water in upper compartments and root foraging precision (RII) indicating that greater water availability in upper pots through HL extended root growth into NR soil patches. The most likely pathway through which HL enhances root foraging precision is related to enhanced OM decomposition and nutrient cycling in OM-rich soil patches when water is available. In a similar study with buffalo grass (*Bouteloua dactyloides*), OM decomposition and N mineralisation was dependent on soil moisture conditions and was greater in plants engaged in HL than in plants where HL was impaired (Armas *et al*. 2012). This could have happened in our experiment, where greater HL maintained greater soil moisture in NR patches, which could have enhanced OM decomposition and mineralisation (Aanderud and Richards 2009; Armas *et al*. 2012) leading to greater nutrient availability. Moreover, increased water availability at night in upper compartments through HL may have increased nutrient diffusion and mobility in the soil (Dawson 1997), increasing nutrients available to plants and thus triggering a preferential root growth response in such nutrient-rich patches. This is consistent with the fact that soil patches were colonised by fine new roots that were not suberised where water exchange and thus, HL takes place preferentially (Caldwell *et al*. 1998). Although it may be difficult to differentiate between the direct effect of enhanced soil moisture conditions for root growth and the indirect effect concerning OM decomposition and nutrient availability in wetter soils, it is clear from this study that HL favours root growth and the placement of roots in nutrient rich soils. Nonetheless, root growth in NR patches in shrubs with hydraulic lift impaired also suggests that HL was not the only mechanism affecting root foraging behaviour. Roots are able to identify moisture and nutrient rich microsites and grow preferentially into these microsites (Bauerle *et al*. 2008*b*). At the beginning of the light treatments soil was moist in all soil patches, which could have favoured root foraging in NR patches in both treatments at least for a short period of time before the upper soils dried.

In addition to the root foraging response, we also found greater $\delta^{15}N$ in cladodes from plants engaged in HL than in plants with impaired HL, supporting our hypothesis that HL would increase plant nutrient capture from dry soil. Not surprisingly, we observed a positive correlation between the amount of water released into upper soil dry layers through HL (measured as δD) and plant $\delta^{15}N$. These results are consistent with those obtained for a grass species by Armas *et al*. (2012). Although in our study shrubs from both treatments assimilated relatively small amounts of ^{15}N (<0.5%) during the several weeks of the study, it is worth noting that plants engaged in HL took up three times as much ^{15}N than plants from the I-HL treatment – evidence that HL enhanced nutrient availability and plant nutrient capture from NR patches.

The method of applying continuous artificial light to plants during night-time periods has been used previously in HL experiments with successful results (Caldwell and Richards 1989; Bauerle *et al*. 2008*a*). However, this artificial manipulation might affect plant physiology and growth. Continuous illumination (CI) can have positive effects on seedling mass, photosynthetic rates and concentration of chlorophyll pigments in plants subjected to 24 h of light with a low PPF (150–500 $\mu mol\, m^{-2}\, s^{-1}$), similar to conditions used in our study (Lefsrud *et al*. 2006; Xiao *et al*. 2007). However,

we found no significant differences between treatments in net photosynthetic rates (A) or stomatal conductance (g_s); not in green or dead plant mass or in carbon content in cladodes. Continuous illumination could also affect plant nutrient status, although responses seem to be highly species specific and contradictory (Velez-Ramírez *et al*. 2011). We found no differences between treatments in the overall total N content in cladodes thus suggesting that CI *per se* did not affect plant N status. Overall, these data indicate that the differences between treatments that we observed in root growth, root foraging and ^{15}N capture from NR patches were most likely due to differences in water availability and in soil N availability rather than differences in the light regime applied.

Overall, our data show that greater nutrient availability for the HL-shrubs in NR patches is a consequence of greater HL that triggered greater root foraging precision in such shrubs. Although these data have to be interpreted carefully, this mechanism can be important to species in dry areas with access to deep-water sources and able to perform HL. These species could benefit from a selective placement of roots in nutrient-rich patches that may be also wetter due to water shed via HL, favouring nutrient capture that would be otherwise limited during drought periods. Although N capture in plants with HL may represent a small fraction of total plant nitrogen capture it could be an important source in arid regions where water availability in shallow layers is scarce all year round and thus governs nutrient availability to plants (Noy-Meir 1973; Armas *et al*. 2012).

In summary, this study provides evidence that *R. sphaerocarpa* actively forages for nutrients and that, under drought conditions, water provided through HL enhanced root proliferation in nutrient-rich patches in heterogeneous soils. This increased root proliferation and better moisture conditions lead to greater plant ^{15}N capture. To our knowledge, this is the first direct evidence linking the ability of plants to perform HL with root growth and foraging processes.

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