REGULAR ARTICLE

Species-specific roles of ectomycorrhizal fungi in facilitating interplant transfer of hydraulically redistributed water between *Pinus halepensis* saplings and seedlings

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Abstract

Background and aims Interplant transfer of hydraulically redistributed water (HRW) can take place via mycorrhizal hyphal networks linking the roots of neighboring plants. We conducted a mesocosm experiment to evaluate the influence of reduced extraradical hyphal lengths on interplant HRW transfer.

Methods Ectomycorrhizal *Pinus halepensis* saplings and seedlings were grown together in two-compartment mesocosms (fungicide-treated or control), and deuterium-labeled water was supplied to the taproot compartment (accessible to sapling taproots) during a 9-day soil drying cycle.

Results Upper soil water contents and seedling water potentials at the end of the drying cycle were lower in fungicide-treated than in control mesocosms. The stem water δD values of seedlings increased (marginally) with increasing soil hyphal length in both treatments separately, suggesting that interplant HRW transfer was at least partly mediated by fungal hyphae. In fungicide-treated mesocosms, the difference in δD values between the stem water of seedlings and upper soil water decreased sharply with increasing soil hyphal length, supporting a key role of ectomycorrhizal fungi (EMF) in interplant HRW transfer at low soil hyphal densities. However, two dominant EMF morphotypes differing in their water repellence properties and hyphal exploration types (*Thelephora terrestris* and *Suillus granulatus*) had contrasting impacts on hydraulic redistribution patterns, as only the EMF producing hydrophilic hyphae (*T. terrestris*) enhanced HRW transfer between pine saplings and seedlings.

Conclusions Changes in the abundance and/or composition of EMF communities in response to anthropogenic disturbance or climate change could affect facilitative plant interactions through alterations of interplant HRW transfer.

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Introduction

Hydraulic redistribution is a widespread process by which plant roots passively transfer water from wetter to drier layers of the soil profile along a water potential gradient (Caldwell et al. 1998; Prieto et al. 2012). Hydraulic redistribution is beneficial to the plants conducting the water movement, as it leads to prolonged root longevity and functionality and enhanced nutrient uptake in drying upper soil layers (Bauerle et al. 2008; Armas et al. 2012; Cardon et al. 2013; Prieto and Ryel 2014), as well as increased transpiration and carbon assimilation during dry periods (Dawson 1996; Emmerman and Dawson 1996; Domec et al. 2010). Furthermore, hydraulic redistribution by deep-rooted plants also provides an important water source for neighboring adult plants and seedlings during rainless periods. Caldwell and Richards (1989) reported that deuterium-labeled water fed to deep roots of *Artemisia tridentata* shrubs was redistributed overnight to upper soil layers and taken up by the neighboring, shallow-rooted grass *Agropyron desertorum* in a dryland ecosystem. In a mesic temperate forest, water redistributed upwards by sugar maple (*Acer saccharum*) supplied up to 60 % of the water used by coexisting shallow-rooted species, and the leaf water potentials and stomatal conductance values of these neighboring plants decreased with increasing distance from the redistributing tree during dry periods (Dawson 1993).

Querejeta et al. (2003) first described the direct transfer of hydraulically redistributed water (HRW) from host plants to ectomycorrhizal fungi (EMF), which allowed the persistence of functional extraradical EMF hyphae in dry upper soil layers during protracted drought. In a follow-up study, Egerton-Warburton et al. (2007, 2008) showed that common mycorrhizal networks linking the roots of neighboring plants can provide preferential pathways for interplant transfer of HRW. The findings of these and subsequent studies (Plamboeck et al. 2007; Schoonmaker et al. 2007; Warren et al. 2008; Booth and Hoeksema 2010; Teste and Simard 2008; Bingham and Simard 2012) suggest that preferential movement of HRW through EMF and arbuscular mycorrhizal fungal (AMF) hyphal networks could enhance seedling survival during drought. However, there are some indications that the functional ecophysiological traits of the mycorrhizal fungi involved in this process can influence the magnitude of interplant transfers of HRW (Plamboeck et al. 2007).

For example, EMF taxa differ in their water repellence properties (hydrophilic or hydrophobic; Unestam 1991; Unestam and Sun 1995) as well as in their hyphal diameters and exploration types (Agerer 2001), both of which might determine their ability to facilitate interplant transfers of HRW. Hydrophilic EMF form spreading mycelia with small-diameter hyphal conduits that transport water symplastically and also apoplastically along their hydrophilic hyphal walls (Unestam and Sun 1995). In contrast, hydrophobic EMF form coarse mycelial cords, strands and rhizomorphs with large-diameter empty vessels in their center (Unestam and Sun 1995). Hydrophobic EMF transport water symplastically and via their central vessels, but not apoplastically due to the hydrophobic nature of their hyphal walls (as only the hyphal tips retain hydrophilic properties in hydrophobic EMF; Unestam and Sun 1995). The thick mycelial cords and rhizomorphs with empty vessel-like hyphae typical of hydrophobic EMF likely have greater water transport capacity than the small-diameter hyphae of hydrophilic EMF when soil water is plentiful (Duddridge et al. 1980; Brownlee et al. 1983; Unestam 1991; Lehto and Zwiazek 2011). However, hydrophilic EMF might be more efficient at translocating the small amounts of water involved in hydraulic redistribution thanks to the capillary properties of their hyphal walls, which can wick water and might thus enhance interplant transfer of HRW along apoplastic pathways in drying soil. In addition, hydrophilic and hydrophobic EMF also differ in their tolerance to drought, which is generally higher in hydrophobic EMF (Unestam and Sun 1995; Agerer 2001). The differential sensitivity to drought stress between hydrophilic and hydrophobic EMF might also affect their role in hydraulic redistribution during progressive soil drying. Overall, very little is known about how EMF functional traits might modulate interplant transfer of HRW via common mycorrhizal networks.

Pinus halepensis Mill. (Aleppo pine) is the most widespread and abundant tree species in semiarid areas of the Western Mediterranean Basin, covering a total surface of 25,000 km^2 (Maestre and Cortina 2004). *Pinus halepensis* is the only native pine species present in the lowlands of semiarid Southeastern Spain, where it often forms monodominant forests (both natural and planted; Maestre and Cortina 2004). *Pinus halepensis* is an ectomycorrhizal species (Brundrett 2009) that conducts hydraulic redistribution (Filella and Peñuelas 2003), which makes it a suitable species to evaluate

the influence of changes in EMF hyphal abundance and EMF community composition on hydraulic redistribution patterns and interplant HRW transfer. The transfer of HRW between trees and conspecific seedlings through shared EMF hyphal networks could be a potentially important ecological process, as it could enhance seedling survival and performance in monodominant semiarid forests where seedling regeneration is strongly limited by drought stress.

The majority of field and mesocosm-based studies investigating the role of mycorrhizal fungi in the transfer of HRW among plants have used mesh screens that separate the roots of donor and receiver plants by excluding ingrowth or outgrowth of roots, but which allow unimpeded passage of mycorrhizal hyphae and rhizomorphs linking the roots of neighboring plants (e.g. Brownlee et al. 1983; Egerton-Warburton et al. 2007; Plamboeck et al. 2007; Warren et al. 2008). This experimental approach, while allowing passage of mycorrhizal hyphae, prevents spatial root overlap and contact between donor and receiver plants, which might hamper interplant HRW transfer to some extent. An alternative experimental approach is fungicide application to decrease mycorrhizal fungal abundance in roots and soil, which allows unimpeded intermingling and contact between the roots of donor (redistributing) and receiver plants (Querejeta et al. 2012). In the mesocosm experiment reported here, fungicide application allowed us to evaluate the differences in hydraulic redistribution patterns and magnitudes between pines with contrasting levels of EMF colonization and extraradical hyphal abundance during a soil-drying period. This approach is also more realistic and informative than comparing hydraulic redistribution patterns between mycorrhizal and non-mycorrhizal (non-inoculated) plants, as all pine species are obligately mycorrhizal and heavily colonized by EMF in nature (Brundrett 2009).

We hypothesized that fungicide application would reduce EMF frequencies in both roots and soil, and would thus reduce the contribution of the EMF hyphal pathway to the transfer of HRW between neighboring plants. We predicted that smaller amounts of deuterium-labeled HRW would be transferred from donor pine saplings to receiver seedlings in fungicide-treated mesocosms than in control mesocosms, due to the reduction in the functionality of the EMF hyphal pathway caused by fungicide addition. Furthermore, we predicted that this reduction of interplant HRW transfer through EMF pathways would negatively affect the water status

of receiver seedlings, thus leading to increased seedling drought stress at the end of the drying cycle in fungicide treated mesocosms, relative to control mesocosms with intact EMF networks. Finally, we predicted that interplant transfer of HRW in drying soil would increase with increasing frequency of hydrophilic EMF roots and hyphae with capillary properties that should enhance water translocation along apoplastic hyphal pathways (Unestam and Sun 1995).

Materials and methods

Mesocosms establishment

Pinus halepensis Mill. (Aleppo pine) saplings were grown in two-compartment mesocosms consisting of two opaque PVC pots of 21 cm height and 15 cm upper and 12 cm lower diameters, respectively (3 l) placed on top of each other on a scaffolding structure (Fig. 1). The lower (taproot compartment hereafter) and the upper compartments of each mesocosms were separated from each other by a 10 cm air gap to prevent capillary and mass flow of water between them. Both compartments were filled with a 2:1 mixture of riverbed sand and forest soil collected at 0–30 cm depth from a nearby monospecific Aleppo pine forest located in the Sierra de El Valle (Murcia, Southeastern Spain). The forest soil was collected next to 10 adult *P. halepensis* trees and we ensured visually that it contained plenty of ectomycorrhizal roots and rhizomorphs. In December 2005, seeds of *P. halepensis* were planted in the upper compartments of the mesocosms (one pine per mesocosm), with a total of eleven replicate mesocosms established. In addition, one extra mesocosm was used as a background control for deuterium enrichment in soil water as a result of evaporative isotopic fractionation during a soil drying cycle (Allison et al. 1983). Mesocosms were kept outdoors, irrigated to field capacity once a week, and fertilized with Hoagland's solution (Hoagland and Arnon 1950) once a month for 5 and half years. The air gap between upper and taproot compartments was initially filled with the soil mixture to allow root growth and extension into the taproot compartment. Within 6 months after mesocosm establishment, woody taproots (1-3 per mesocosm) were observed bridging the gap between upper and taproot compartments, and the soil filling the gap was then washed and removed. The pine sapling taproots bridging the gap between upper

Fig. 1 Experimental mesocosms containing donor pine saplings (*Pinus halepensis*) with taproots bridging the gap between upper and lower (taproot) compartments (*left*). Receiver pine seedlings growing in upper mesocosm compartments can be seen under donor saplings. Donor sapling taproots bridging the gap between upper compartments and lower (taproot) compartments were protected from direct radiation using PVC tubing (removed for the picture to allow observation of taproots). During the 9-day drying cycle, deuterium labeled water was added daily to lower (taproot) compartments until soil saturation. *Right*: close-up view of an upper compartment containing 18-month old pine seedlings (receiver plants) growing under a pine sapling (donor plant)

and taproot compartments were protected from direct sunlight using PVC tubing. These 5-year old pine saplings thereafter served as the donor plants conducting hydraulic redistribution. In November 2010, one-year old heavily ectomycorrhizal *P. halepensis* seedlings obtained from a local nursery were transplanted into the upper compartments in all mesocosms and served as receiver plants (four seedlings per mesocosm). *Pinus halepensis* seedlings from the nursery were extensively colonized by *Thelephora terrestris* and *Suillus granulatus* ectomycorrhizal fungi, with mean EMF root colonization around 90–100 %.

Fungicide application

Five years after mesocosm establishment, the fungicide Bumper® ES (ADAMA Agricultural Solutions, Raleigh, NC, USA) was applied to upper compartments in five mesocosms (fungicide-treated) to reduce mycorrhizal colonization of roots and the abundance of mycorrhizal mycelium in the soil, while the remaining six mesocosms were left undisturbed (control). Bumper® ES is a liquid, propiconazole-based fungicide (41.8 %) that provides a broad-spectrum activity against Ascomycetes, Deuteromycetes, and Basidiomycetes by inhibiting mycelial growth (Teste et al. 2006). A

suspension of Bumper® ES was prepared (1 ml per l of deionized water) and applied three times (April, May and June 2011; 500 ml in each application) to fungicide-treated mesocosms. The same quantity of deionized water was added to control mesocosms on each fungicide application date (500 ml x 3 dates = 1500 ml total) so that both treatments received the same amount of water over the experimental period. In June 2011, by the end of the experiment, we measured sapling and seedling heights (mm) to ensure that these did not differ between fungicide-treated and control mesocosms (Tables 1 and S1).

Application of deuterium-labeled water

Following the protocol described in Querejeta et al. (2012), sixty-seven months after mesocosm establishment (June 2011) the upper compartments of all mesocosms were irrigated to field capacity (500 ml of water added) after which irrigation to upper compartments was withheld for nine days to create the necessary soil water potential gradient for hydraulic redistribution. During this nine-day soil drying period, the soil in taproot compartments was maintained at or near saturation by daily irrigation with deuterium-labeled water (Fig. 1). Irrigation took place after the sunset to favor

Table 1 Differences in plant, fungal and soil variables measured in upper compartments between fungicide-treated ($n = 4$) and control ($n = 4$) mesocosms

		Fungicide treated	Control	P-value
Donor saplings	Height (mm)	61 ± 6	65 ± 6	n.s
	Stem water content (%)	54.4 ± 2.0	53.6 ± 1.3	n.s
	δD (‰)	966 ± 23	1007 ± 14	n.s
	Specific root length (m g^{-1})	16.0 ± 0.6	11.3 ± 1.0	0.009
	Root length density (cm cm^{-3})	6.04 ± 1.80	4.74 ± 0.39	n.s
	EMF roots (tips g$_{root}$$^{-1}$)	421 ± 22	640 ± 67	0.021
	Thelephora terrestris (tips g$_{root}$$^{-1}$)	284 ± 24	364 ± 85	0.054
	Suillus granulatus (tips g$_{root}$$^{-1}$)	121 ± 12	268 ± 61	0.056
	Non-identified EMF (tips g$_{root}$$^{-1}$)	16 ± 3	8 ± 4	0.588
Soil	Total hyphal length (m g$_{soil}$$^{-1}$)	50.8 ± 10.9	103.2 ± 21.7	0.005
	Active hyphal length (m g$_{soil}$$^{-1}$)	0.7 ± 0.2	14 ± 3.6	<0.001
	Soil water content (%)	6.2 ± 0.4	7.5 ± 0.3	0.043
	δD (‰)	53.4 ± 2.8	51.3 ± 6.2	n.s
Receiver seedlings	Height (mm)	101 ± 15	75 ± 6	n.s
	Stem water content (%)	53.7 ± 1.3	53.9 ± 0.5	n.s
	Stem Ψ_{pd} (MPa)	-1.55 ± 0.22	-0.80 ± 0.14	0.029
	δD (‰)	35.2 ± 6.7	27.6 ± 4.2	n.s

Values are means ± 1 standard error. Significance (p-values) of Student T test analyses is shown. Plant variables include plant height, stem water content and predawn water potential (Ψ_{pd}), stem water deuterium isotopic composition (δD), the specific root length of donor saplings (SRL), the number of EMF-colonized root tips (EMF roots) and the number of root tips colonized by *Suillus granulatus* and *Thelephora terrestris*. Fungal and soil variables include total and active soil hyphal lengths, the isotopic composition of soil water (δD) and gravimetric soil water content

the nocturnal redistribution of the labeled-water through hydraulic redistribution. Deuterium-labeled tracer water was prepared by adding 3.30 ml of pure D_2O (99.8 % deuterium enrichment, Sigma Chemical Co.) to 10 liters of tap water (δD = -43.2‰). The resulting deuterium-labeled water used to irrigate the taproot compartments had a δD ≈ 2000‰. Deuterium-labeled water has been used as an effective tracer of HRW movement in many studies (e.g. Egerton-Warburton et al. 2007; Warren et al. 2008; Prieto et al. 2012; Querejeta et al. 2012). The extra mesocosm used to control for evaporative isotopic fractionation of soil water during a drying cycle (Allison et al. 1983) received no addition of fungicide or deuterium-labeled water.

Sampling and laboratory procedures

Before dawn (4-5 h GTM) of the ninth day after irrigation withdrawal from upper root compartments, we measured the stem water potential (Ψ_{pd}) of the tallest seedling in each of the mesocosms. Predawn stem water potentials of seedlings were determined using a Scholander-type pressure bomb (Soil Moisture Equipment Corp. Model 3000, Santa Barbara, CA, USA). The predawn water potential of seedlings was assumed to be in equilibrium with the soil water potential in upper mesocosm compartments (Richter 1997). Thereafter, the whole aboveground biomass of the receiver seedlings was collected. We also collected stem sections from donor pines and bulk soil samples from upper root compartments in all the mesocosms. Water for hydrogen stable isotope analyses was extracted from plant (seedlings and donor pine stem sections) and soil samples using a cryogenic vacuum distillation line (Ehleringer and Osmond 1989). Plant and soil sample water contents were determined based on the difference between the fresh and dry sample weights obtained before and after cryogenic vacuum distillation, respectively. Hydrogen isotope ratios of plant and soil water samples were measured using Wavelength Scanned Cavity Ring Down Spectrometry (WS-CRDS, L2120-i, Picarro Inc., USA). Ten injections of each sample were made, of which the first five injections were used for equipment rising and thus discarded for data

analysis. Measurements of a matching set of international standards and laboratory reference waters (range: -428‰ to 1250‰) included in each analysis batch were used to correct raw data for sample-to-sample memory effects and through-run drift. Hydrogen stable isotope analyses were conducted at the Isotope Bioscience Laboratory at Ghent University (Belgium).

At the end of the 9-day soil drying cycle, one soil sample (100 cm^3) per mesocosm was collected from the top 10 cm in upper compartments and kept cold (4 °C) until processed. All roots contained in the sample were collected by washing the soil with tap water using a 0.1 mm sieve. Roots were then rinsed to eliminate adhered soil particles, spread out in distilled water onto a mesh tray and finally transferred on a transparent acetate sheet and scanned at 400 dpi (Hummel et al. 2007). The resulting image was processed with image analysis software (Winrhizo, version 2009; Regent Instrument, Quebec, QC, Canada) to determine total root length (L). After scanning, roots were oven-dried at 60 °C for 72 h and then weighed to determine their dry mass (DM). Root length density (cm cm^{-3}) was calculated as the ratio between L and soil volume (100 cm^3) and SRL (m g^{-1}) was calculated as the ratio between L and DM.

Ectomycorrhizal fungi

Total and active soil hyphal lengths were analyzed according to Bardgett (1991) and Sylvia (1992) modified as follows. Ten g of soil were shaken in 1500 ml of water in a blender for 30 s followed by 2 min rest to allow sedimentation of dense particles. Then, 500 ml of the supernatant were passed through a sieve of 44 μm mesh. The retained material was suspended in 10 ml of phosphate buffer (pH = 7.4) and homogenized. An aliquot of 5 ml of this suspension was treated with fluorescein diacetate, an esterase substrate, which, after hydrolysis, fluoresces when excited under UV, indicating the active part of the mycelium. After incubation for five minutes in the dark, the aliquot was filtered under vacuum onto a 0.45 μm pore and 46 mm diameter nitrocellulose membrane. Counting was performed at 400-x magnification under UV in a microscope adapted with a grid line ocular in 64 fields on the membrane surface. The total soil hyphal length was measured in the same sample under white light. The hyphae (active or total) that contact the grid line of the ocular are counted

and the mycelial length (mm) was quantified by using the modified grid-line method (Tennant 1975).

To assess the ectomycorrhizal colonization of pine roots, the methods described in Grand and Harvey (1982) and Amaranthus and Perry (1989) were followed. Homogeneous samples of fine roots (<2 mm) were collected from upper mesocosm compartments. Root tips that appeared ectomycorrhizal and viable were counted and EMF morphotypes were identified according to the descriptions provided by Agerer (1987–2006), Torres and Honrubia (1991; 1994) and Unestam and Sun (1995), with the aid of a binocular dissecting scope.

In order to confirm the identity of the EMF morphotypes identified visually, the fungal ITS region was sequenced for ectomycorrhizal root tips categorized according to morphological criteria. DNA was isolated from approximately 8–10 individual ectomycorrhizal root tips per morphotype. Each ectomycorrhizal root tip was placed into a 2-ml screw-cap propylene tube together with two tungsten carbide balls (3 mm) and ground (3 min, 13,000 r.p.m.) using a mixer mill (MM 400, Retsch, Haan, Germany). Total DNA was extracted using a DNeasy plant mini Kit following the manufacturer's recommendations (Qiagen). The extracted DNA was resuspended in 20 μl of water and stored at -20 °C. Dilutions (1/10, 1/100 and 1/1000) of each homogenate or DNA extract were prepared using sterile nanopure water, and 2 μl were used as template. PCR amplification, using the forward primer ITS1F (CTTGGTCATTTAGAGGAAGTAA, Gardes and Bruns 1993), and the reverse primer ITS4 (TCCTCCGCTTATTGATATGC, White et al. 1990) was carried out in a final volume of 25 μl using PureTaq^{TM} Ready-To-Go PCR beads (Amersham Pharmacia Biotech), and 0.5 μM of each primer (PCR conditions: 94 °C for 2 min, then 30 cycles at 94 °C for 40 s, 53 °C for 40 s, 72 °C for 90 s, followed by a final extension period at 72 °C for 5 min).

The PCR products were purified using a Gel extraction Kit (Qiagen) cloned into pGEM-T Easy vector (Promega) and transformed into Escherichia coli (Xl1 blue). Putative positive transformants were screened in each resulting ITS library, using 0.7 unit of RedTaq DNA polymerase (Sigma) and the supplied reaction buffer to a final volume of 25 μl and a re-amplification with ITS1F and ITS4 primers with the same cycling parameters described above. Product quality and size were checked in agarose gels as described above. Several clones having inserts of the correct size

(≈690 bps) in each library were sequenced using the universal primers SP6 and T7 by Laboratorio de Sistemas Genómicos (Valencia, Spain). The 21 sequences of the EMF clones generated in this study have been deposited in the European Nucleotide Archive (http://www.ebi.ac.uk/)) with accession numbers LT174709 to LT174729.

Statistical analyses

Plant, fungal and soil variables were analyzed with Student T-tests to detect significant differences ($p < 0.05$) between treatments. To evaluate the relationships among plant, fungal and soil variables, a principal components analysis (PCA) was performed using 11 variables (the δD composition of donor and seedling stem water and seedling water potentials; the number of ectomycorrhizal root tips and the frequency of Thelephora terrestris and Suillus granulatus root tips and specific root length in donor plants; the total and active hyphal lengths in the soil, soil water content and the δD composition of soil water). In addition to the PCA, individual correlation analyses (Pearson) among variables were conducted within treatments, and the prediction intervals were calculated. Since we observed some seedling mortality in the mesocosms, a Student's T test was performed at the end of the experiment to test for potential differences between treatments, and found that mortality (%) was similar in both of them (30 ± 6.8 vs. 24 ± 9.8 % in control and fungicide-treated mesocosms, respectively, $p = 0.61$). For plant seedling height a mean value was calculated using all seedlings (2-3) in each mesocosm. The contribution of deuterium-labeled water redistributed by donor saplings to soil water in upper compartments was derived from soil water δD values at the end of the 9-day drying cycle and calculated with two-end linear mixing models (Flanagan and Ehleringer 1991). For these calculations, we used the mean δD value of stem water in donor saplings as one end, and the δD of soil water from the extra mesocosm (with no addition of deuterium labeled water) as the other end, which allowed us to account for the (confounding) effects of evaporative isotopic fractionation and enrichment of soil water during the drying cycle (Allison et al. 1983). Likewise, the contribution of deuterium-labeled redistributed water to seedling water uptake was derived from their stem water δD values at the end of the 9-day drying cycle and calculated with two-end linear mixing models, using the mean δD of

stem water in donor saplings as one end, and the δD of stem water in seedlings from the extra mesocosm (with no addition of tracer water) as the other end. Student T tests and Pearson correlations were conducted using the SPSS 22.0 program (SPSS Inc., Chicago, IL, USA). Values throughout the text are expressed as mean ± 1 s.e.m.

Results

The stem water δD values of P. halepensis saplings revealed that three of them (out of eleven replicates) failed to take up the deuterium-labeled water added to the taproot compartments, indicating that their taproots were no longer functional at the time of the experiment (Table S1). These three failed mesocosms were thus discarded from all subsequent analyses. The remaining eight mesocosms (4 fungicide-treated and 4 controls) showed similarly high uptake of the deuterium-labeled water added to taproot compartments by the pine saplings, thus demonstrating good functionality of their taproots. Calculations using two-end, linear mixing models indicated that across treatments 8 % of soil water in upper compartments and 5.5 % of stem water in receiver seedlings were derived from HRW at the end of the drying cycle.

Fungicide effects on plant mycorrhizal status and root traits

Mean fine root densities in upper compartments across treatments were 4.04 ± 0.56 mg cm$^{-3}_{soil}$ and 5.39 ± 0.88 cm cm$^{-3}_{soil}$ expressed by mass or length of roots per soil volume, respectively. The roots of P. halepensis saplings and seedlings were heavily colonized by EMF across treatments, with two overwhelmingly dominant morphotypes which together accounted for 97 % of total ectomycorrhizal root tips present in the mesocosms: Thelephora terrestris and Suillus granulatus (see Figs. S1 and S2 for pictures and detailed descriptions of the two morphotypes). Based on the presence and type of emanating hyphae and presence/absence of rhizomorphs, Thelephora terrestris had a short-distance hyphal exploration type, whereas Suillus granulatus had a long-distance exploration type.

Fungicide-treated mesocosms had 35 % lower frequency of EMF root tips in upper compartments than control mesocosms (Table 1). The frequencies of

Thelephora terrestris and *Suillus granulatus* EMF root tips were reduced by 22 and 55 %, respectively, by fungicide application. Extraradical hyphal length in soil (total) was reduced by over 50 % in fungicide-treated mesocosms compared to control mesocosms, whereas viable hyphal length was reduced by 95 %.

Specific root length (SRL) was 30 % higher in fungicide-treated mesocosms than in the controls, but root length density (RLD) was unaffected by fungicide addition. Across treatments and mesocosms ($n = 8$), SRL was negatively associated with both total EMF root tip frequency and *Suillus granulatus* root tip frequency as well as with active soil hyphal length ($r = -0.78$; $p = 0.022$ and $r = -0.90$; $p = 0.002$, $r = -0.74$; $p = 0.036$, respectively; see also Fig. S3 and Table S2).

Fungicide effects on soil and plant water relations

The stem water contents and δD values of donor saplings at the end of the 9-day soil drying cycle were similar between fungicide-treated and control mesocosms (Table 1). However, mean soil water content in upper compartments and seedling predawn water potentials were both lower in fungicide-treated mesocosms than in control mesocosms at the end of the drying cycle (Table 1). Contrary to expectations, neither soil water δD nor seedling stem water δD values differed significantly between fungicide-treated and control mesocosms at the end of the drying period (Table 1).

Across treatments and mesocosms ($n = 8$), soil water content (SWC) at the end of the drying cycle correlated positively with soil hyphal length (Fig. 2 and S3, $r = 0.72$, $p = 0.043$). The predawn water potentials of receiver seedlings at the end of the drying cycle also increased with total and active soil hyphal length across treatments ($r = 0.71$; $p = 0.048$ and $r = 0.72$; $p = 0.042$, respectively; see also Fig. S3 and Table S2). Higher stem water δD values in donor saplings at the end of the drying cycle (indicating greater uptake of deuterium-labeled water from taproot compartments) were associated with higher predawn water potentials in receiver seedlings across mesocosms ($r = 0.77$; $p = 0.030$), which suggests that the intensity of competition for upper soil water between saplings and seedlings decreased with increasing taproot water uptake by saplings (Fig. S3 and Table S2).

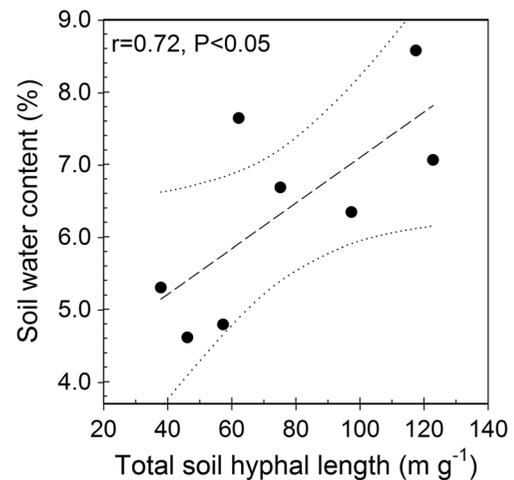The stem water δD values of receiver seedlings at the end of the drying cycle increased with soil hyphal length

Fig. 2 Soil water content as a function of total soil hyphal length in upper compartments across fungicide-treated and control (non-fungicide-treated) mesocosms (n = 8). Regression line (*dashed*) and prediction intervals (*dotted lines*) as well as Pearson correlation coefficient (r) and significance (p-value) are shown

for both control and fungicide-treated mesocosms separately, although these relationships were marginally significant, likely due to low replicate numbers ($r = 0.92$; $p = 0.082$ and $r = 0.93$; $p = 0.065$ for control and fungicide-treated mesocosms, respectively; $n = 4$ for both treatments, Tables S3 and S4). Interestingly, in fungicide treated mesocosms the difference in δD values between soil water and the stem water of receiver seedlings at the end of the drying cycle (which is expected to decline with increasing effectiveness of EMF hyphal pathways to transfer the deuterium-labeled HRW from saplings to seedlings) decreased sharply with increasing soil hyphal length (Fig. 3, $r = -0.99$, $p = 0.007$).

Across control mesocosms ($n = 4$), the stem water δD values of receiver seedlings at the end of the soil drying cycle increased with increasing frequency of EMF root tips colonized by *Thelephora terrestris* (Fig. 4, $r = 0.95$, $p = 0.047$). Upper soil water content at the end of the drying cycle decreased with increasing frequency of root tips colonized by *Suillus granulatus* across control mesocosms (Fig. 5, $r = -0.95$, $p = 0.045$).

Discussion

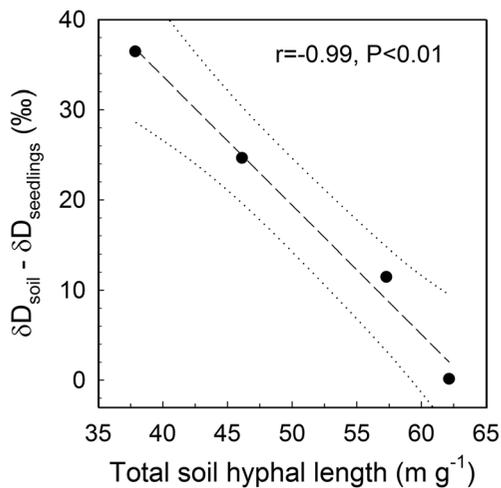Our study provides evidence that soil EMF hyphal density modulates the transfer of HRW from *Pinus*

Fig. 3 Difference between the deuterium isotopic composition of soil and seedling stem water ($\delta D_{soil} - \delta D_{seedlings}$) as a function of the total soil hyphal length in upper compartments across fungicide-treated mesocosms ($n = 4$). Regression line (dashed) and prediction intervals (dotted lines) as well as Pearson correlation coefficient (r) and significance (p-value) are shown

Fig. 5 Soil water content as a function of the number of root tips colonized by *Suillus granulatus* root tips in upper compartments across control (non-fungicide-treated) mesocosms ($n = 4$). Regression line (dashed) and prediction intervals (dotted lines) as well as Pearson correlation coefficient (r) and significance (p-value) are shown

halepensis saplings to conspecific seedlings. Moreover, we found indications that this process may be dependent on the functional traits of the dominant EMF in the rhizosphere, as increasing frequency of EMF with hydrophilic hyphae (*Thelephora terrestris*) enhances interplant transfer of HRW.
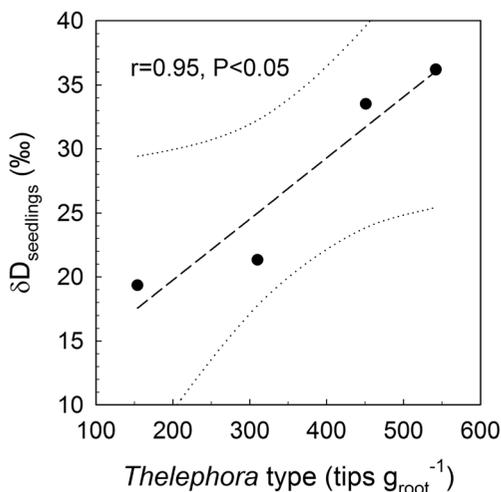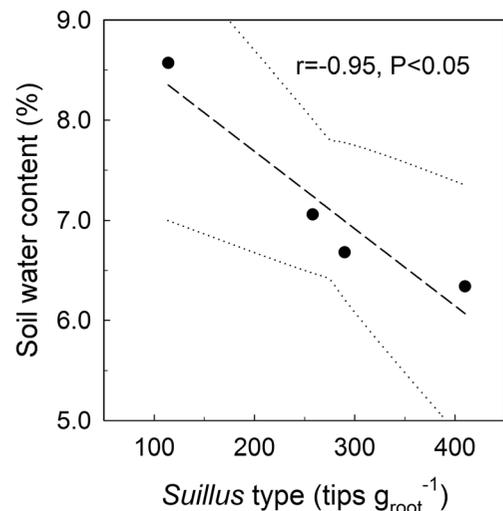

Fig. 4 Deuterium isotopic composition of seedling stem water ($\delta D_{seedlings}$) as a function of the number of root tips colonized by *Thelephora terrestris* root tips in upper compartments across control (non-fungicide-treated) mesocosms ($n = 4$). Regression line (dashed) and prediction intervals (dotted lines) as well as Pearson correlation coefficient (r) and significance (p-value) are shown

Extraradical hyphal role in interplant HRW transfer from saplings to seedlings

Repeated fungicide application effectively reduced the frequency of ectomycorrhizal root tips, the length density of hyphae in soil and the abundance of active hyphae in upper compartments, which allowed us to evaluate the influence of root and soil mycorrhizal status on the transfer of HRW from pine saplings to seedlings during a soil drying cycle. The stem water δD values of receiver seedlings increased (marginally) with soil hyphal length for both treatments separately, suggesting that the transfer of deuterium-labeled HRW from donor to receiver plants was enhanced by soil hyphae (largely EMF hyphae) across a wide range of soil hyphal length values (38–123 m g^{-1}). In fungicide-treated mesocosms, the difference in δD values between the stem water of receiver seedlings and soil water in upper compartments decreased sharply with increasing soil hyphal densities (Fig. 4), thus supporting a key role of EMF hyphae in the direct transfer of deuterium-labeled HRW from donor saplings to receiver seedlings and/or in the uptake of HRW from soil by seedlings at relatively low soil hyphal densities (38–63 m g^{-1}). However, this negative correlation was not observed for control mesocosms with higher soil hyphal densities (75–123 m g^{-1}) suggesting that, above a certain threshold of hyphal

abundance, additional increases in soil hyphal length may not further enhance interplant HRW transfer significantly.

The two dominant ectomycorrhizal morphotypes in the mesocosms, *Thelephora terrestris* (60 % of the total EMF root tips) and *Suillus granulatus* (40 % of total EMF root tips), are known to have contrasting hyphal exploration types and water repellence properties. Whereas the ectomycorrhizal roots and external mycelia of *Thelephora*- type EMF are hydrophilic, those of *Suillus*- type EMF are highly hydrophobic (Unestam 1991; Unestam and Sun 1995). Across control mesocosms, increasing frequency of hydrophilic *Thelephora terrestris* enhanced interplant transfer of deuterium labeled HRW, as indicated by increasing δD stem water values in receiver seedlings with increasing frequency of *Thelephora terrestris* root tips in upper soil (Fig. 5). Hydrophilic fungi form spreading mycelia that can take up and transport water both via the hyphal symplast and via the hyphal walls or apoplast (Unestam and Sun 1995), thus potentially enhancing the transfer of HRW through hyphae. In contrast, *Suillus granulatus* forms thick mycelial cords or rhizomorphs composed of several hyphae glued together by hydrophobic surfaces (Unestam 1991). Whereas *Suillus*-type rhizomorphs can transfer significant amounts of water to roots in wet soil (Duddridge et al. 1980; Brownlee et al. 1983), they may be less effective at translocating small amounts of HRW in drying soil due to their strongly hydrophobic properties (Unestam 1991), which suggests a less important role in hydraulic redistribution and interplant HRW transfer. Moreover, the larger-diameter vessel hyphae present in rhizomorphs are probably more prone to "cavitation" and loss of water transport capacity in drying soil, which may be another reason why hydrophobic EMF may have played a less important role in interplant HRW transfer in our study. At high densities in soil, the hydrophobic EMF roots and rhizomorphs of *Suillus granulatus* form dense mycelial patches that repel water (Unestam 1991), which may hamper the capacity of the soil matrix to hold and retain moisture, as suggested by the negative association between the frequency of root tips colonized by *Suillus granulatus* and soil water content across control mesocosms (Fig 6). Although these results are based on a rather limited number of replicate mesocosms, and thus must be interpreted with caution, they suggest that the hyphal exploration type and water repellence properties of the dominant EMF in

roots and rhizosphere soil may have contrasting impacts on hydraulic redistribution magnitude and interplant HRW transfer. Further research on a larger number of EMF morphotypes will be needed to fully elucidate these effects.

Contrary to expectations, there were no significant differences in upper soil water δD values or seedling stem water δD values between control and fungicide treated mesocosms at the end of the 9-day drying cycle. However, it is important to note that higher soil water contents in the upper compartments of control mesocosms reduced the driving force for hydraulic redistribution (i.e. the gradient in water potentials between the drying soil in upper compartments and the water-saturated soil in taproot compartments; Caldwell et al. 1998), thus interfering with and impeding direct comparisons between treatments. In control mesocosms, the enhancing effect of high EMF hyphal densities on interplant HRW transfer was likely offset by the hampering effects of a smaller water potential gradient between compartments at the end of the drying cycle (0.80 ± 0.14 MPa), relative to fungicide-treated mesocosms (1.55 ± 0.22 MPa) (Querejeta et al. 2012).

Fungicide impacts on soil water content and seedling water status

Mean soil water content in upper compartments at the end of the 9-day drying cycle was 1.3 % lower in fungicide-treated mesocosms than in control mesocosms, which may have been due to: a) lower initial soil water content at the onset of the drying cycle in the upper compartments of fungicide-treated mesocosms due to decreased soil water holding capacity caused by reduced soil hyphal lengths (Augé et al. 2001; Augé 2004; Querejeta et al. 2012); and/or, b) faster depletion of soil water in upper compartments during the drying cycle in fungicide-treated mesocosms through enhanced water uptake by plants with higher SRL (Eissenstat 1991). The data provide support for a combination of both decreased soil water holding capacity (a) and faster soil water depletion by plants with higher SRL (b) as plausible explanations for the lower soil water contents in the upper compartments of fungicide-treated mesocosms. First, upper soil water content at the end of the drying cycle and total soil hyphal length were correlated across mesocosms and treatments, indicating that reduced hyphal length in fungicide-treated mesocosms reduced the soil water

retention capacity, as previously found in other studies (Augé et al. 2001; Augé 2004; Querejeta et al. 2012). In addition, the higher SRL of *P. halepensis* in fungicide-treated mesocosms may have further contributed to a drier upper soil at the end of the drying cycle, as roots with high SRL can extract water from soil more efficiently and rapidly than roots with low SRL (Eissenstat 1991; Huang and Eissenstat 2000).

Consistent with the higher water content of upper soil in control mesocosms, the predawn water potentials of receiver seedlings (with roots confined to upper compartments) were also higher in control than in fungicide-treated mesocosms at the end of the 9-day drying cycle. In addition, higher EMF root colonization and more abundant EMF extraradical hyphae may have further contributed to an enhanced seedling water status in control mesocosms through increased EMF root and hyphal water uptake during progressive soil drying (Duddridge et al. 1980; Brownlee et al. 1983; Muhsin and Zwiazek 2002; Bogeat-Triboulot et al. 2004; Marjanović et al. 2005; Lehto and Zwiazek 2011).

Magnitude of hydraulic redistribution in the mesocosms

The proportion of HRW taken up by the receiver seedlings in the mesocosms across treatments (5.5 % of their stem water derived from hydraulic redistribution at the end of the drying cycle) falls within the wide range of magnitudes of HRW transfer from conifer trees to seedlings in field studies (from less than 1 % to up to 21.6 % of stem water in *Pinus ponderosa* and *Pseudotsuga menziesii*; Brooks et al. 2002; Schoonmaker et al. 2007; Warren et al. 2008). Mean fine root density in the mesocosms (4.04 ± 0.56 mg cm$^{-3}_{soil}$) was similar to that found in young *P. halepensis* stands (2.5–7.6 mg cm$^{-3}_{soil}$; Querejeta et al. 2001; Fuentes et al. 2010) or in other Mediterranean-type ecosystems (Kummerow et al. 1978; Roberts 1976), so the magnitudes of hydraulic redistribution and interplant HRW transfer found in the mesocosms should be comparable to those occurring under field conditions, despite large differences in rooting depth and volume between both scenarios (Filella and Peñuelas 2003).

Concluding remarks

This study shows that interplant transfer of HRW is enhanced by increasing hyphal densities in ectomycorrhizal *Pinus halepensis* across a wide range of soil hyphal densities. Moreover, increasing frequency of the hydrophilic EMF *Thelephora terrestris* favored the transfer of HRW from donor saplings to receiver seedlings at high soil hyphal densities. The contrasting effects of hydrophilic (*Thelephora terrestris*) vs hydrophobic (*Suillus granulatus*) EMF on hydraulic redistribution patterns suggest that changes in the abundance and/or composition of EMF communities in response to anthropogenic disturbance or climate change could alter interplant HRW transfers, with potential impacts on the establishment and growth of seedlings dependent on HRW for their survival during drought periods (Prieto et al. 2011). For example, climate change-induced alteration of EMF abundance and community composition in Mediterranean pine woodlands (e.g. declines in EMF abundance and/or increasing dominance of drought-tolerant, hydrophobic EMF relative to drought-sensitive, hydrophilic EMF with climate warming and drying; Unestam and Sun 1995; Agerer 2001; Querejeta et al. 2009) could reduce the strength of plant facilitative interactions through changes in hydraulic redistribution patterns, with detrimental feedback effects on understory seedling regeneration.

References

Agerer R (ed) (1987–2006) Colour Atlas of Ectomycorrhizae. Einhorn-Verlag, Schwäbisch Gmünd

Agerer R (2001) Exploration types of ectomycorrhizae. Mycorrhiza 11:107–114

Allison GB, Barnes CJ, Hughes MW (1983) The distribution of deuterium and ^{18}O in dry soils 2. Experimental. J Hydrol 64: 377–397

Amaranthus MP, Perry D (1989) Rapid root tip and mycorrhiza formation and increased survival of Douglas-fir seedlings after soil transfer. New Forest 3:259–264

Armas C, Kim JH, Bleby TM, Jackson RB (2012) The effect of hydraulic lift on organic matter decomposition, soil nitrogen cycling, and nitrogen acquisition by a grass species. Oecologia 168:11–22

Augé RM (2004) Arbuscular mycorrhizae and soil/plant water relations. Can J Soil Sci 84:373–381

Augé RM, Stodola AJW, Tims JE, Saxton AM (2001) Moisture retention properties of a mycorrhizal soil. Plant Soil 230:87–97

Bardgett RD (1991) The use of the membrane filter technique for comparative measurements of hyphal lengths in different grassland sites. Agr Ecosyst Environ 34:115–119

Bauerle TL, Richards JH, Smart DR, Eissenstat DM (2008) Importance of internal hydraulic redistribution for prolonging the lifespan of roots in dry soil. Plant Cell Environ 31: 177–186

Bingham MA, Simard S (2012) Ectomycorrhizal networks of Pseudotsuga menziesii var. glauca trees facilitate establishment of conspecific seedlings under drought. Ecosystems 15: 188–199

Bogeat-Triboulot MB, Bartoli F, Garbaye J, Marmeisse R, Tagu D (2004) Fungal ectomycorrhizal community affect root hydraulic properties and soil adherence to roots of Pinus pinaster seedlings. Plant Soil 267:213–223

Booth MG, Hoeksema JD (2010) Mycorrhizal networks counteract competitive effects of canopy trees on seedling survival. Ecology 91:2294–2302

Brooks JR, Meinzer FC, Coulombe R, Gregg J (2002) Hydraulic redistribution of soil water during summer drought in two contrasting Pacific Northwest coniferous forests. Tree Physiol 22:1107–1117

Brownlee C, Duddridge JA, Malibari A, Read DJ (1983) The structure and function of mycelial systems of ectomycorrhizal roots with a special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. Plant Soil 71:433–443

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320:37–77

Caldwell MM, Richards JH (1989) Hydraulic lift: water efflux from upper roots improves effectiveness of water uptake by deep roots. Oecologia 79:1–5

Caldwell MM, Dawson TE, Richards JH (1998) Hydraulic lift: consequences of water efflux from the roots of plants. Oecologia 113:151–161

Cardon ZG, Stark JM, Herron PM, Rasmussen JA (2013) Sagebrush carrying out hydraulic lift enhances surface soil nitrogen cycling and nitrogen uptake into inflorescences. Proc Natl Acad Sci USA 110:18988–18993

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

Dawson TE (1996) Determining water use by trees and forests from isotopic, energy balance, and transpiration analyses: the roles of tree size and hydraulic lift. Tree Physiol 16:263–272

Domec JC, King JS, Noormets A, Treasure E, Gavazzi MJ, Sun G, McNulty SG (2010) Hydraulic redistribution of soil water by roots affects whole-stand evapotranspiration and net ecosystem carbon exchange. New Phytol 187:171–183

Duddridge JA, Malibari A, Read DJ (1980) Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. Nature 287:834–836

Egerton-Warburton LM, Querejeta JI, Allen MF (2007) Common mycorrhizal networks provide a potential pathway for the transfer of hydraulically lifted water between plants. J Exp Bot 58:1473–1483

Egerton-Warburton LM, Querejeta JI, Allen MF (2008) Efflux of hydraulically lifted water from mycorrhizal fungal hyphae during imposed drought. Plant Signal Behav 3:68–71

Ehleringer JR, Osmond CB (1989) Stable isotopes. In: Pearcy RW, Ehleringer JR, Mooney HA, Rundel PW (eds) Plant physiological ecology. Kluwer Academic Publishers, London, pp 281–300

Eissenstat DM (1991) On the relationship between specific root length and the rate of root proliferation: a field study using citrus rootstocks. New Phytol 118:63–68

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

Flanagan LB, Ehleringer JR (1991) Stable isotope composition of stem and leaf water: applications to the study of plant water use. Funct Ecol 5:270–277

Fuentes D, Valdecantos A, Llovet J, Cortina J, Vallejo VR (2010) Fine-tuning of sewage sludge application to promote the establishment of Pinus halepensis seedlings. Ecol Eng 36: 1213–1221

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Grand LF, Harvey AE (1982) Quantitative measurements of ectomycorrhizae on plant roots. methods and principles of mycorrhizal research. Am Phytopathol Soc, St Paul

Hoagland DR, Arnon DI (1950) The water-culture method of growing plants without soil. Calif Aes Bull 347:31

Huang B, Eissenstat DM (2000) Linking hydraulic conductivity to anatomy in plants that vary in specific root length. J Am Soc Hortic Sci 125:260–264

Hummel I, Vile D, Violle C, Devaux J, Ricci B, Blanchard A, Garnier E, Roumet C (2007) Relating root structure and anatomy to whole-plant functioning in 14 herbaceous Mediterranean species. New Phytol 173:313–321

Kummerow J, Krause D, Jow W (1978) Seasonal changes of fine root density in the Southern Californian chaparral. Oecologia 37:201–212

Lehto T, Zwiazek J (2011) Ectomycorrhizas and water relations of trees: a review. Mycorrhiza 21:71–90

Maestre FT, Cortina J (2004) Are Pinus halepensis plantations useful as a restoration tool in semiarid Mediterranean areas? For Ecol Manag 198:303–317

Marjanović Ž, Uehlein N, Kaldenhoff R, Zwiazek JJ, Weiß M, Hampp R, Nehls U (2005) Aquaporins in poplar: what a difference a symbiont makes! Planta 222:258–268

Muhsin TM, Zwiazek JJ (2002) Ectomycorrhizas increase apoplastic water transport and hydraulic conductivity in Ulmus americana seedlings. New Phytol 153: 153–158

Plamboeck AH, Dawson TE, Egerton-Warburton LE, North M, Bruns TD, Querejeta JI (2007) Water transfer via ectomycorrhizal fungal hyphae to conifer seedlings. Mycorrhiza 17:439–447

Prieto I, Ryel RJ (2014) Internal hydraulic redistribution prevents the loss of root conductivity during drought. Tree Physiol 34: 39–48

Prieto I, Padilla FM, Armas C, Pugnaire FI (2011) The role of hydraulic lift on seedling establishment under a nurse plant species in a semi-arid environment. Perspect Plant Ecol Evol Syst 13:181–187

Prieto I, Armas C, Pugnaire FI (2012) Water release through plant roots: new insights into its consequences at the plant and ecosystem level. New Phytol 193:830–841

Querejeta JI, Roldan A, Albaladejo J, Castillo V (2001) Soil water availability improved by site preparation in a *Pinus halepensis* afforestation under semiarid climate. For Ecol Manag 149:115–128

Querejeta JI, Egerton-Warburton LM, Allen MF (2003) Direct nocturnal transfer from oaks to their mycorrhizal symbionts during severe soil drying. Oecologia 134:55–64

Querejeta JI, Egerton-Warburton LM, Allen MF (2009) Topographic position modulates the mycorrhizal response of oak trees to interannual rainfall variability. Ecology 90: 649–662

Querejeta JI, Egerton-Warburton LM, Prieto I, Vargas R, Allen MF (2012) Changes in soil hyphal abundance and viability can alter the patterns of hydraulic redistribution by plant roots. Plant Soil 355:63–73

Richter H (1997) Water relations of plants in the field: some comments on the measurement of selected parameters. J Exp Bot 48:1–7

Roberts J (1976) A study of root distribution and growth in a *Pinus sylvestris* L. (Scots Pine) plantation in East Anglia. Plant Soil 44:607–621

Schoonmaker A, Teste F, Simard S, Guy R (2007) Tree proximity, soil pathways and common mycorrhizal networks: their influence on the utilization of redistributed water by understory seedlings. Oecologia 154:455–466

Sylvia DM (1992) Quantification of external hyphae of vesicular-arbuscular mycorrhizal fungi. Method Microbiol 24:53–65

Tennant D (1975) A test of the modified root intersect method of estimating root length. J Ecol 63:995–1001

Teste F, Simard S (2008) Mycorrhizal networks and distance from mature trees alter patterns of competition and facilitation in dry Douglas-fir forests. Oecologia 158:193–203

Teste F, Karst J, Jones MD, Simard S, Durall DM (2006) Methods to control ectomycorrhizal colonization: effectiveness of chemical and physical barriers. Mycorrhiza 17:51–65

Torres P, Honrubia M (1991) In vitro synthesis of ectomycorrhizae between *Suillus collinitus* (Fr.) O Kuntze and *Rhizopogon roseolus* (Corda) Th M Fr. with *Pinus halepensis* Miller. Mycotaxon 41:437–443

Torres P, Honrubia M (1994) Ectomycorrhizal associations proven for *Pinus halepensis*. Israel J Plant Sci 42:51–58

Unestam T (1991) Water repellency, mat formation and leaf-stimulated growth of some ectomycorrhizal fungi. Mycorrhiza 1:13–20

Unestam T, Sun YP (1995) Extramatrical structures of hydrophobic and hydrophilic ectomycorrhizal fungi. Mycorrhiza 5: 301–311

Warren JM, Brooks JR, Meinzer FC, Eberhart JL (2008) Hydraulic redistribution of water from *Pinus ponderosa* trees to seedlings: evidence for an ectomycorrhizal pathway. New Phytol 178:382–394

White TJ, Bruns T, Lee S et al. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ and White TJ (Eds.). PCR protocols. a guide to methods and applications, San Diego, pp. 315–322