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REGULAR ARTICLE

Isotope fractionation during root water uptake by *Acacia caven* is enhanced by arbuscular mycorrhizas

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Abstract
Aim A growing number of studies show a discrepancy between the isotopic composition of xylem water and plant water sources. We tested the effect of arbuscular mycorrhizal fungi (AMF) on the isotopic composition of *Acacia caven* xylem water. As the most common plant-fungal association, AMF might explain this isotopic mismatch.
Methods Seedlings were grown with and without AMF and irrigated with the same water. After 120 days, stem and soil samples were collected and following cryogenic distillation, H and O isotopic composition of xylem and soil water, as well as irrigation water, was measured.
Results Xylem water of non-mycorrhizal seedlings was significantly depleted in ^{2}H compared to soil water (differences up to −15.6‰). When AMF were present, the depletion was significantly higher and appeared for both H and O (differences up to −24.6‰ for $\delta^{2}H$ and −2.9‰ for $\delta^{18}O$ between soil and xylem water).
Conclusions Results suggest that isotopic fractionation occurred during water uptake in this xerophytic species. To explain this, we propose an aquaporin-driven mechanism mediating water transport via transmembrane passage. Furthermore, we show for the first time, that AMF enhance the observed discrimination against heavy isotopes, probably by enforcing water passage through aquaporins. Given their ubiquity, AMF could question the fractionation-free assumption during root water uptake.

Keywords Aquaporins · Ecohydrology · Isotope fractionation · Mycorrhiza · Plant-soil-water interactions · Stable isotopes

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Introduction

The stable isotopic composition of hydrogen ($^{2}H/^{1}H$, $\delta^{2}H$) and oxygen ($^{18}O/^{16}O$, $\delta^{18}O$) serve as fingerprints of the water molecule within the hydrological cycle, providing information on its source or processes to which it has been exposed (Clark and Fritz 1997; Gat 1996). For the water molecule, most notable changes in the ratio of the abundance of heavy-to-light isotopes relative to a standard -i.e. isotopic fractionation- are associated with phase changes from solid to liquid to vapor and vice versa (Michener and Lajtha 2008). Fractionation during phase transitions is a result of the

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difference in physical properties between water isotopologues -i.e. isotopically different water molecules such as $^{1}H_2{}^{16}O$, $^{1}H^{2}H^{16}O$ and $^{1}H_2{}^{18}O$ - (Leibundgut et al. 2009). Xylem isotopic composition has been used for tracing plant water sources under the assumption that isotope fractionation does not occur during root water uptake (Dawson and Ehleringer 1991; Ehleringer and Dawson 1992; Zimmermann et al. 1967). It has been further assumed that there is no fractionation during xylem transport until it reaches the upper canopy (Dawson and Ehleringer 1993).

Nevertheless, a growing body of evidence reveals a mismatch between xylem water and assumed plant water sources. Studies that provide evidence for enrichment -i.e. more positive δ-values given the ratio is more abundant in heavy isotopes- in the xylem compared to water sources, argue that either it occurred via reverse flow of evaporated water in leaves (Dawson and Ehleringer 1993), xylem-phloem water exchange (Cernusak et al. 2005), redistribution of enriched foliar water uptake (Eller et al. 2013), bark evaporation (Ellsworth and Sternberg 2015), or a decline in the sap flow rate (Martín-Gómez et al. 2016). Studies that found depletion -i.e. more negative δ-values given the ratio is less abundant in heavy isotopes- in xylem compared to plant water sources, concluded that fractionation occurred during water uptake. Initial findings by Lin and da SL Sternberg (1993) and Ellsworth and Williams (2007) focused on salt and drought tolerant species and suggested that $\delta^{2}H$ fractionation occurred during root water uptake. The presence of highly suberized and lignified cell walls in the roots of these species, would favor water movement through the symplastic pathway - i.e. cell-to-cell water transport through plasmodesmata- which is hypothesized to cause fractionation. Later on, Zhao et al. (2016) suggested that H isotopic fractionation occurred between xylem sap and root or stem tissue water in a xerophytic species. Recently, Vargas et al. (2017) showed that a none xerophytic species, avocado plants (*Persea americana* Mill.cv Lula), discriminates against both ^{2}H and ^{18}O during water uptake. Xylem water isotopic depletion increased as soil dries and is less sandy. Altogether these studies suggest that isotope fractionation during root water uptake might be more common than previously thought.

More recently, Barbeta et al. (2019) found ^{2}H depletion in the xylem of two tree species in South-Western France, arguing that fractionation occurred either in the soil-root interface, within plant tissues or within the unsaturated soil zone. Evidence of water isotope fractionation in the soil solution due to interactions with clay particles and cations has been shown before (Oerter et al. 2014; Oshun et al. 2016). Additionally, several ecohydrological studies have found a mismatch between the isotopic composition of xylem and stream water (e.g. Brooks et al. 2010; Evaristo et al. 2015; Goldsmith et al. 2012; Hervé-Fernández et al. 2016). Brooks et al. (2010) explained their early findings by suggesting that soil water is separated into two water compartments which do not fully mix: a tightly bound water pool from which plants take up water, and a mobile water pool, which flows through preferential paths and eventually feeds streams. The so called Two Water Worlds (TWW) hypothesis has stirred significant debate in the scientific community and several authors have questioned if ecohydrological separation (i.e. TWW) is actually possible, proposing alternative explanations (see Berry et al. 2018 and McDonnell 2014 for an overview). Notably, the TWW hypothesis was proposed under the assumption that water extracted from plant stems reflect the isotopic composition of plant water sources.

In the quest of understanding this mismatch of xylem and plant water sources, as well as enlightening the TWW discussion, some authors have questioned if mycorrhizal associations would influence water isotopic composition during root uptake (Berry et al. 2018; Evaristo et al. 2016; McDonnell 2014). Mycorrhizas are symbiotic associations between plant roots and soil fungi (Parniske 2008). The role of mycorrhizas in water movement is less well understood than their contribution to carbon and nutrient cycles (Allen 2007). Mycorrhizal associations, and particularly arbuscular mycorrhizas (AM), are the rule rather than the exception for most plants in most ecosystems (Smith and Read 2008; Soudzilovskaia et al. 2015). If AM cause isotope fractionation during water uptake, then xylem water would not represent the isotopic composition of its potential water sources. This would have important implications for studies in ecophysiology and ecohydrology that rely on water isotopes as a tracer. The aim of our study was to assess if arbuscular mycorrhizal fungi (AMF) influence the isotopic composition of water during its passage from the soil to the plant. For this purpose, we set up a pot experiment in which *Acacia caven* (Mol.) Mol., a widespread tree species of the seasonally dry mountain Chaco forest in South America, was grown during 120 days with and without AMF.

Materials and methods

Experimental design

A greenhouse experiment was conducted at the Instituto Multidisciplinario de Biología Vegetal (IMBIV) in Córdoba, Argentina. *Acacia caven* (note that *Vachellia caven* (Molina) Seigler & Ebinger is also an accepted name for this species), was chosen because it is a native pioneer, relatively fast-growing species, dominant in the seasonally dry, subtropical mountain Chaco forest (Cabido et al. 2018). This was needed to ensure suberized and sufficient amount of plant material for water extractions. Moreover, this nitrogen fixing species is known to form AM and to be positively affected by them (Perez and Urcelay 2009).

Seeds were surface-sterilized with 5% bleach, scarified with sand paper (Högberg et al. 1999; Venier et al. 2017) and incubated in a germination chamber with a thermal period of 25/15 °C and 12/12 h light/darkness (Venier et al. 2017). Once germinated, a single seedling was transplanted into a 0.5 l free-draining pot filled with a sterilized sand:soil-mixture (2:1, *v*/v), giving a total of 48 pots (i.e. 48 monocultures). Sterilization was previously carried out in an autoclave at 1.5 atm during 60 min to ensure an AM-free medium for all pots before applying the treatment. The sand-soil mixture physicochemical characteristics were: bulk density of 1.25 ± 0.02 g cm^{-3} (measured using the core method, Blake and Hartge 1986), 9.2% clay, 6.1% loam and 84.7% of sand (determined by the pipette method, Pansu and Gautheyrou 2006) and pH was 7.13 ± 0.03 (measured with a pH-meter in a 1:5 *v*/v soil/water suspension). Total carbon and nitrogen contents were of 0.18 and 1.81%, respectively (measured using Elemental Analysis). Olsen-P was of 23.11 ± 3 mg PO_4-P kg^{-1} (measured according to Olsen 1954).

Following Perez and Urcelay (2009), in half of the pots which were randomly selected (AM pots, $n = 24$), a volume of 25 cm^3 arbuscular mycorrhizal fungi (AMF) inoculum was applied in a small pit surrounding each plant. The inoculum consisted of soil known to contain propagules of a native AMF community from a nearby area where *A. caven* grows naturally (Longo et al. 2014). To balance soil quantity in both treatments, the same amount of autoclaved inoculum (i.e. sterile) was added to non-mycorrhizal plants (non-AM pots, $n = 24$). In order to equalize the microbial community in both treatments (excluding AMF), non-AM pots were supplemented with 30 ml of microbial slurry. The slurry was prepared by filtering a 1:5 (v/v) suspension of the inoculum through a 36 μm mesh to remove AMF propagules. Finally, in order to equilibrate the initial soil moisture in both treatments, 30 ml of water were finally added to the AM pots. Figure 1 provides a conceptual overview of the two treatments.

All seedlings were grown for 120 days in the greenhouse without any addition of fertilizer. Mean (± SD) daytime temperature in the greenhouse was 23 ± 4 °C and the photoperiod was the natural photoperiod in Córdoba from December 2017 till the end of April 2018 (progressively decreasing from 14 h12 to 11 h04, Servicio de Hidrografía Naval de Argentina). The position of the pots was shifted once a week to avoid any influence of gradients within the greenhouse. Tap water was supplied regularly three times per day (at 09:00, 13:00 and 18:00 h) to maintain the seedlings under well-watered conditions. Plants of both treatments were irrigated at the same time and with the same water source. The mean (± SD) irrigation regime was 3.65 ± 1.06 mm day^{-1}, supplied through a mist nozzle irrigation system. Irrigation water was collected throughout the entire experiment ($n = 30$) by a pluviometer provided with an oil layer to avoid any evaporative enrichment of the water. During the first three months of the experiment, irrigation water samples were collected from the pluviometer every week. The sampling frequency was increased to a sample per day during the last twenty days of the experiment. Samples were kept in HDPE scintillation vials sealed with Parafilm® to avoid modifications in the isotopic signal due to evaporation and isotope exchange. They were stored in a refrigerator until analyzed.

Sampling procedures

To avoid any cross contamination, all non-mycorrhizal plants were measured and harvested first. At the moment of harvest (day 120), the volumetric soil moisture content was 46 ± 1.5% ($n = 6$). The intensity of mycorrhizal colonization is generally expressed as the percentage of root length colonized by AMF (Smith and Read 2008; McGonigle et al. 1990). This was estimated by microscopic observation of AM structures (hyphae, arbuscules and vesicles) after staining of a root subsample of all pots ($n_{AM} = 17$ and $n_{non\text{-}AM} = 20$, note that replicates are less than the original number because some plants were lost during the experiment). The

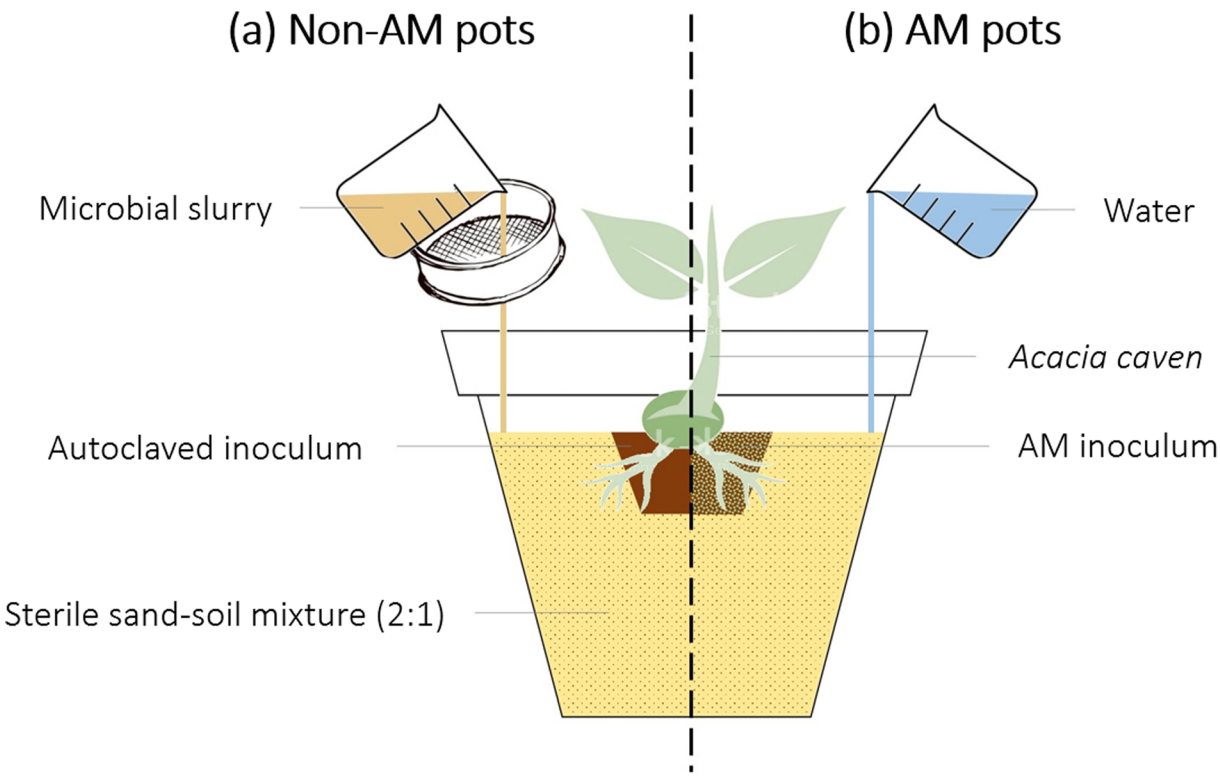

Fig. 1 Conceptual scheme of the non-mycorrhizal (Non-AM, left) and mycorrhizal (AM, right) treatments applied to -and xylem and soil samples for water extractions taken from- two different groups of 24 randomly selected single-seedling pots each. The microbial slurry in the non-AM pots consisted of a 1:5 (*v*/v) inoculum/water suspension. It was filtered through a 36 μm mesh to provide the non-AM treatment with the same microbial population as the AM treatment, but free of AM fungi propagules

staining procedure consisted of several steps after Koske and Gemma (1989) and Grace and Stribley (1991). First, the root system was isolated and oven dried (60 °C, 72 h). Second, roots were washed in 20% KOH at 90 °C for 25 min, followed by thorough rinsing in water. Third, they were acidified in 0.1 N HCl for 1 h. Fourth, roots were stained with a 0.05% aniline blue solution (*w*/*v*, in lactic acid) at 90 °C during 35 min. Finally, we mounted on a separate microscope slide each stained root subsample and quantified the AMF colonization (hyphae, arbuscules and vesicles) under optical microscope (Nikon Eclipse E200, ×40 magnification) using the magnified intersection method (McGonigle et al. 1990).

A subset of pots was used to assess the plants' responsiveness to mycorrhizal colonization in order to check that the treatment was successful ($n_{AM} = 5$ and $n_{non\text{-}AM} = 5$). For this, we measured total dry biomass (g) by oven drying shoots (60 °C, 72 h) which were weighted together with their corresponding root system (isolated and dried before the staining procedure). Biomass alone might be an inconclusive indicator of responsiveness, given that negative growth depressions are sometimes observed in young plants when rapidly infected by AMF (Smith 1980). Therefore, we further determined aboveground P content (g PO_4^{-3}-P kg^{-1} dry matter) by grinding and dry-ashing aboveground plant material (method by Chapman and Pratt 1962 modified by Ryan et al. 2001). Higher P concentrations in AM plants is usually observed regardless of the overall growth responsiveness to colonization (Ibijbijen et al. 1996; Smith 1980).

The rest of the pots were subjected to stem and soil water extraction for isotopic analysis ($n_{AM} = 12$ and $n_{non\text{-}AM} = 15$). If present, the upper not completely suberized, green section of the stem was discarded, for it is likely subject to cuticular evaporation. For the same reason, leaves were removed from the collected

stem segments at the exact moment of harvest. A soil aliquot covering the complete profile was sampled from each pot. Soil and stem samples were kept in glass vials with airtight screw caps and immediately frozen until water extraction. Total stem water, including bark and bast water, was extracted. However, for the sake of simplicity, stem water will henceforth be called xylem water. Water from soil and xylem was extracted by cryogenic vacuum distillation (West et al. 2006). Extraction temperature and time were 105 °C and 6 h, respectively (Araguás-Araguás et al. 1995). The extracted water samples were filtered (0.45 μm) and stored in 2 ml or 300 μl glass chromatography vials covered by an airtight cap with silicone/PTFE septa. Samples were kept inside a refrigerator until analyzed for δ^2H and $\delta^{18}O$. For isotopic determinations, the final number of replicates was less than the original number: for AM soil and xylem water samples $n = 8$, and for non-AM soil water samples $n = 11$, while xylem water samples $n = 10$. The reason for this is either because: (1) samples had unsuccessfully received the corresponding treatment (AM-samples that had lower than 40% of root length colonized by AMF or non-AM samples that presented higher than 2% root length colonized by AMF were discarded), (2) samples lost vacuum during the extraction process (samples which exhibited, for example, water on the bridge were automatically discarded regardless the water recovery), or (3) water extraction efficiency was below the established threshold. Extraction efficiency was determined by comparing the difference in weight of samples before and after extraction, with the difference in weight before extraction and after a final oven drying (105 °C for 48 h). Soil water samples that reached or surpassed the 98% water recovery threshold were selected (Araguás-Araguás et al. 1995). For xylem water, this threshold was the upper end of extraction efficiency and was not always achieved, so we set the lower end to 95.1% in order to avoid losing too many samples and statistical power (median water extraction efficiency = 96.6%). We checked that the latter decisions did not compromise the quality of the data. We found no significant association between δ-values of xylem water and the percentage of water recovery ($r = 0.12$, $P = 0.65$ for δ^2H and $r = 0.21$, $P = 0.40$ for $\delta^{18}O$, for both $n = 18$). When considering xylem samples for each treatment separately there were no significant statistical associations neither ($r = 0.17$, $P = 0.66$ for δ^2H and $r = 0.38$, $P = 0.31$ for $\delta^{18}O$ for AM xylem samples; $r = 0.30$, $P = 0.40$ for δ^2H and $r = 0.36$, $P = 0.30$ for $\delta^{18}O$ for non-AM xylem samples). It can hence be discarded that the differences recorded within and between treatments are an eventual artefact of water extraction efficiency threshold of xylem water samples. Extractions were performed by batches of maximum 20 samples. There was no systematic pattern on sample losses per or within batches. Given the extraction parameters we used, it is surprising that water recovery was in this range for xylem samples. The small size of the 4-months old stem samples could be the cause, given small amounts of water was extracted from them.

Isotopic analysis

The δ^2H and $\delta^{18}O$ compositions were analyzed by Isotope-ratio infrared spectroscopy (IRIS) using a Wavelength Scanned-Cavity Ringdown Spectrometer (WS-CRDS, L2120-i, Picarro, USA), coupled with a vaporizing module (A0211 high-precision vaporizer) and a Micro Combustion Module (MCM). It has been shown that MCMs can accurately remove the noise from organic contaminants found in soil and xylem water (Martín-Gómez et al. 2015). Each sample was measured eight times. Calibration was performed with three internal laboratory references. Measurements were corrected for drift and memory effects by means of the Water Stable Isotope Calibration Program SICalib version 2.16f, provided by the IAEA (Gröning 2011). After correction, all eight measurements were considered for the estimation of the final isotopic values of the analyzed water sample. The ±1σ measurement uncertainty of the WS-CRDS was ±1.0 and ± 0.2 for δ^2H and $\delta^{18}O$, respectively. All O and H isotope values are expressed relative to VSMOW2 (in ‰).

Statistical analysis

The non-parametric Mann-Whitney *U*-test was selected for all analyses because data was not normally distributed, nor was the variance homogeneous. This test was applied to examine potential differences between AM and non-AM treatments in % AMF colonization, aboveground P content and dry biomass (total, above- and belowground). Since we irrigated the plants with a single water source, we applied simple statistical analyses instead of multisource linear mixing models. Thus, the

Mann-Whitney *U*-test was used for detecting differences of isotopic values ($\delta^{18}O$ and $\delta^{2}H$) between and within treatments (i.e. AM versus non-AM, irrigation versus soil water and soil versus xylem water). All tests were carried out in a two-sided way with the statistical significance level (α) set to 0.05. Furthermore, in order to assess the magnitude of differences in case they were detected, we calculated the difference in the composition of H and O isotopes between soil and xylem ($\Delta\delta^{2}H = \delta^{2}H_{soil\ water} - \delta^{2}H_{xylem\ water}$ and $\Delta\delta^{18}O = \delta^{18}O_{soil\ water} - \delta^{18}O_{xylem\ water}$) on paired samples for each treatment (AM and Non-AM pots). This analysis was done only for those pots for which both soil and xylem samples passed the selection criteria for isotopic determinations ($n_{AM} = 5$ and $n_{non\text{-}AM} = 10$). Data analysis was performed using the R software package, version 3.5.1 (R Core Team 2013).

Results

Non-AM seedlings showed little to no mycorrhizal colonization, while AM seedlings exhibited high AMF root colonization levels; values differed significantly between treatments ($U = 340$, $p < 0.001$; Fig. 2a). Aboveground P content values in AM seedlings were significantly higher than those for non-AM seedlings ($U = 25$, $p < 0.01$; Fig. 2b). Total dry biomass values were slightly lower for AM seedlings, though not statistically significant ($U = 7$, $p = 0.31$; Fig. 2c). When analyzing above- and belowground plant biomass separately, no statistical difference was found between treatments for neither of them ($U = 6$, $p = 0.22$ and $U = 12$, $p = 1$ for above- and belowground biomass, respectively).

The $\delta^{2}H$ and $\delta^{18}O$ values of soil and xylem water for both treatments, as well as irrigation water, are plotted in a dual isotope plot together with the Global Meteoric Water Line (GMWL) (Fig. 3). Lateral box plots in Fig. 3 show isotopic data dispersion for irrigated water, as well as soil and xylem water for each of both treatments. The isotopic composition of soil water did not differ significantly from that of the irrigation water of the last three days before harvest ($U = 6$, $p = 0.1264$ for $\delta^{2}H$ and $U = 22$, $p = 0.456$ for $\delta^{18}O$). Moreover, the $\delta^{2}H$ and $\delta^{18}O$ values of soil water did not differ significantly between AM and non-AM treatments ($U = 32$, $p = 0.351$ and $U = 20$, $p = 0.051$, respectively). In non-AM seedlings $\delta^{2}H$-values for xylem water were significantly more depleted from those for soil water ($U = 108$, $p < 0.001$); while for $\delta^{18}O$ values this was not significant, but close to be ($U = 83$, $p = 0.051$). The difference between soil and xylem isotopic values was enhanced and statistically significant for both isotopes when AMF were present ($U = 64$, $p < 0.001$ for both $\delta^{2}H$ and $\delta^{18}O$). Table 1 shows the magnitude of the isotopic differences ($\Delta\delta^{2}H$ and $\Delta\delta^{18}O$) between soil and xylem water for both treatments (AM and Non-AM pots), by presenting maximum and minimum values of the calculated differences. Accordingly, xylem isotopic values for AM seedlings were significantly more depleted in both ^{2}H and ^{18}O, compared to non-AM seedlings ($U = 13$, $p < 0.01$ for $\delta^{2}H$ and $U = 11$, $p < 0.05$ for $\delta^{18}O$).

Discussion

Fractionation during root water uptake by *Acacia caven* seedlings

We found xylem water isotopic composition in non-AM *A. caven* seedlings significantly depleted in ^{2}H compared to soil water. Incomplete extraction of water during cryogenic distillation could fractionate water isotopes (Orlowski et al. 2016) and potentially explain our results. However, we followed rigorously the extraction procedure and checked that the selected % of water recovery threshold introduced no bias on the δ-values within and between treatments (see Sampling Procedure on Methods section). One could argue that small soil pores could have been filled with an isotopically lighter water source at a given moment of the experiment, as suggested by the two soil water compartments in the context of the TWW (Brooks et al. 2010; McDonnell 2014). Even though we did not collect the water percolating out of the pots (draining water) which would 'correspond' to groundwater recharge and stream water in the TWW; we did record the isotopic composition of the irrigated water since the beginning of the experiment, and it never reached δ-values as negative as the ones measured in xylem water of the non-AM seedlings ($\delta^{2}H$-values reached −26.7‰ and −43.3‰ for irrigation and xylem water, respectively; $\delta^{18}O$-values reached −4.8‰ and −6.7‰ for irrigation and xylem water, respectively). Furthermore, it could be hypothesized that this depleted xylem water was present in soil micropores before the start of the experiment. But any liquid water that possibly remained in the sand-soil

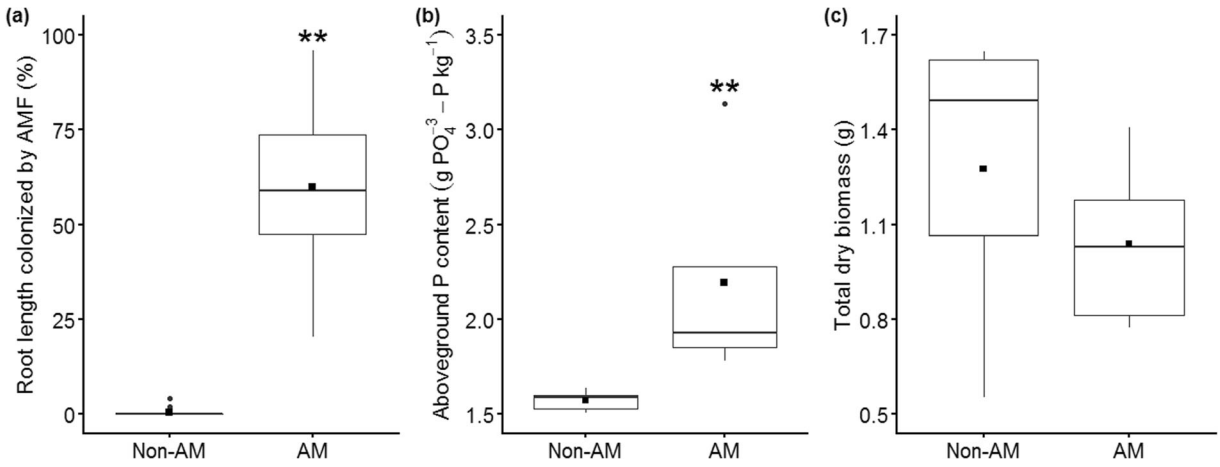

Fig. 2 Box plots representing median, interquartile range (Q1 and Q3) and outliers of: (**a**) root length colonized by arbuscular mycorrhizal fungi (AMF) (%), (**b**) aboveground P content (g PO_4^{-3}-P kg^{-1} dry matter), and (**c**) total dry biomass (**g**) for non-arbuscular mycorrhizal (non-AM) and arbuscular mycorrhizal (AM) seedlings. Asterisks denote significant differences between treatments for the different variables (Mann-Whitney *U*-test, $^{**}p < 0.01$)

mixture after it was autoclaved would have been enriched in δ^2H and $\delta^{18}O$ values instead of depleted due to steam sterilization. Thus, it seems very unlikely that the depleted isotopic signal measured in the xylem water of the non-AM plants is a result of the existence of a second more depleted water pool in the soil. One could

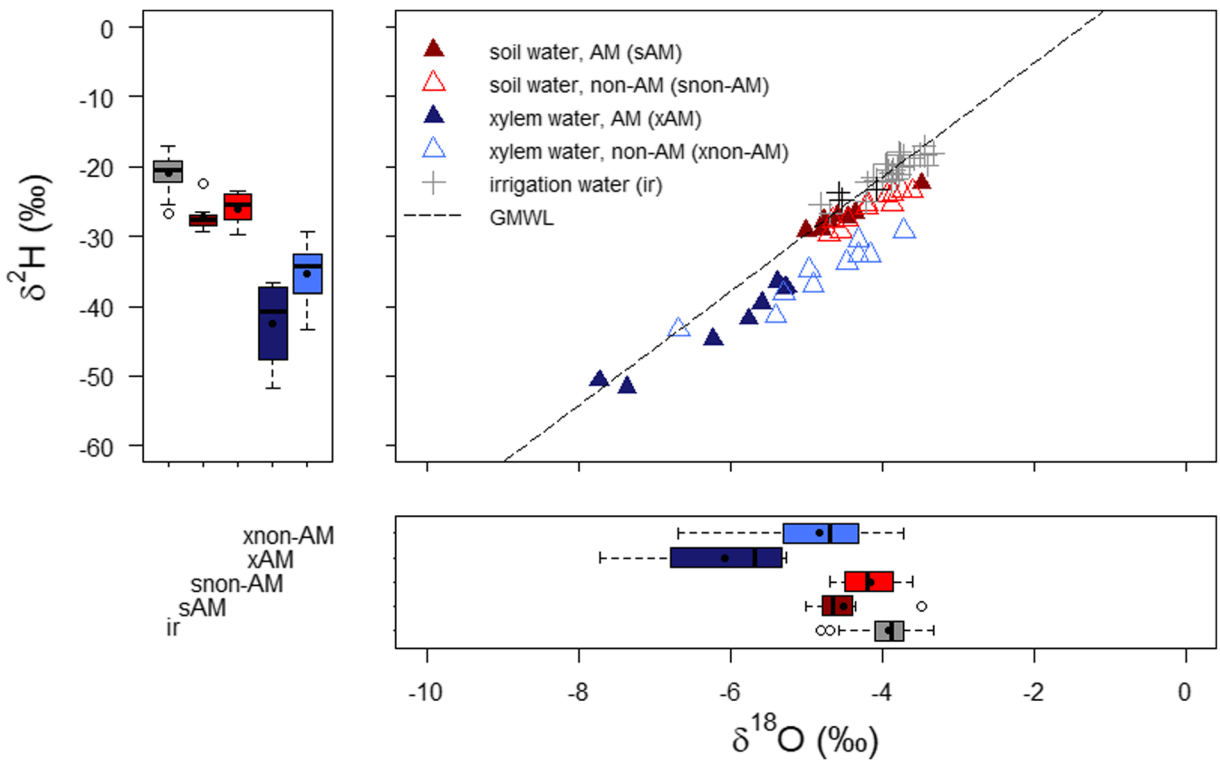

Fig. 3 Dual isotope plot showing δ^2H and $\delta^{18}O$ values of irrigation water (crosses), soil water (red triangles) and xylem water (blue triangles) for the non-mycorrhizal (non-AM, open-light red/blue) and mycorrhizal (AM, closed-dark red/blue) seedlings. The last three irrigation samples taken before harvest (24, 25 and 26 of April 2018) are indicated in black crosses. The global meteoric water line (GMWL) according to Rozanski et al. (1993) is represented by the dotted black line. Vertical and horizontal boxplots display the distribution of δ^2H and $\delta^{18}O$ values respectively, for the different groups of water samples

Table 1 Minimum and maximum absolute (Min | Max) differences between the isotopic composition ($\Delta\delta^2H$ and $\Delta\delta^{18}O$) of soil and xylem for both treatments (AM: arbuscular mycorrhizal pots and Non-AM: non-arbuscular mycorrhizal pots). Differences were calculated based on paired samples (i.e. xylem and soil samples coming from the same pot)

	$\Delta\delta^2H$ soil-xylem	$\Delta\delta^{18}O$ soil-xylem
Non-AM	−2.9 | -15.6	0.8 | -2.0
AM	−8.8 | -24.6	0.6 | -2.9

further argue that belowground physicochemical fractionation processes -such as interactions with clay particles and adsorbed cations- may create isotopic heterogeneity within the soil matrix, leading to distinct isotopic values (Oerter et al. 2014; Oshun et al. 2016). Nevertheless, the growth medium consisted of a sandy soil (2:1, *v*/v), so the effect of such interactions is likely to be small. Moreover, when soil moisture is high, as it was in our pots (> 46%), isotopic variation in the soil is supposed to be lower; thus, the isotopic signal of water extracted is more representative of the pool (Oerter et al. 2014).

Our findings suggest that isotope fractionation is likely to have occurred during water uptake by *A. caven* seedlings. Since xylem water was depleted in heavy isotopes relative to soil water, it can be excluded that it was caused by relocation of evaporated water in leaves, cuticular evaporation or temporal decline in the sap flow rates (Dawson and Ehleringer 1993; Ellsworth and Sternberg 2015; Martín-Gómez et al. 2016). Fractionation was more apparent on δ^2H than on $\delta^{18}O$, which is not surprising given the higher fractionation factor (α) of ^2H relative to ^{18}O, owing to the higher relative mass difference between $^2H/^1H$ compared to $^{18}O/^{16}O$ (Michener and Lajtha 2008; Rundel et al. 2012). The fact that no strong enrichment signal was observed in the soils of neither of both treatments is probably due to the high irrigation frequency during the experiment, which caused dilution and draining of the pots. Even though, we do not have the isotopic composition of the draining water from the pots to corroborate this, field studies of Hervé-Fernández et al. (2016) and Geris et al. (2015) also argued that high-water availability in their study areas might explain low enrichment patterns in the soil. Moreover, our observations of water isotope discrimination by plants under well-watered conditions and sandy soils challenges what Vargas et al. (2017) recently proposed. Through their results, the authors argued that root water uptake fractionation increases as soils are less sandy and have lower water content.

Acacia caven, our model species, is known to be adapted to xeric environments (Raab et al. 2015), such as a wide number of conspecifics (e.g. Eamus et al. 2013; Otieno et al. 2005). It has been hypothesized that in drought resistant and salinity tolerant species, water isotope fractionation could be caused by the predominant movement of water through the symplastic pathway (Ellsworth and Williams 2007; Lin and da SL Sternberg 1993). This was even hypothesized for other species from the genus *Acacia*, such as *A. constricta* and *A. greggii* (Ellsworth and Williams 2007). However, the mechanism behind this remains unclear. In our search for a more mechanistic explanation, we propose that the element that could explain fractionation through the cell-to-cell pathway is aquaporins. Aquaporins are channel proteins that facilitate transmembrane water flow (i.e. cell-to-cell water transport crossing membranes) in living organisms (Tyerman et al. 2002; Hacke and Laur 2016). These proteins have been suggested to regulate the cell-to-cell pathway of water movement in plants, which dominates when the apoplastic pathway (i.e. water transport through cell walls without crossing membranes) is hampered. This hampering could be caused by suberization or lignification in xerophytic species or even by the presence of the Casparian strip. The single pore of an aquaporin has a minimum diameter of 2.8 Å, which approximates the mean diameter of a water molecule (Kozono et al. 2002). As a result, water molecules have to move in a 'single-file' configuration through the center of an aquaporin. During this passage, water dipoles are reoriented through fine-tuned interactions involving hydrogen bonds between the water molecule and molecules forming the channel. This, in turn, causes the breakage of hydrogen bonds between adjacent water molecules (Kozono et al. 2002). Since water molecules containing ^2H and ^{18}O form stronger hydrogen bonds than those containing ^1H and ^{16}O (Clark and Fritz 1997; Michener and Lajtha 2008; Rundel et al. 2012), a significant discrimination against heavy isotopes could take place during transmembrane water transport. Given that substitution of ^{16}O for ^{18}O in a water molecule causes only a small relative decrease in vibrational energy compared to the substitution of ^1H for ^2H, fractionation during transmembrane transport (i.e. passage through aquaporins) is expected to be more pronounced for H than O (Ellsworth and Williams 2007), as

observed in our work (see Table 1 and Fig. 3). Evidence that could support our hypothesis comes from model simulations and experimental work of Mamonov et al. (2007), who observed a slightly lower osmotic permeability of $^{2}H_{2}{}^{16}O$ compared to $^{1}H_{2}{}^{16}O$ in a type of aquaporin (AQ1). Also, under water stress aquaporins have been described to regulate root and leaf hydraulics (e.g. Aroca et al. 2007; Rodríguez-Gamir et al. 2019). Furthermore, aquaporins have been suggested to contribute to water movement between xylem and phloem (Hacke and Laur 2016), which could also potentially alter xylem isotopic composition. This is far from being conclusive, and the extent to which this mechanism could cause a fractionation effect comparable to the isotopic differences between soil and xylem water reported here, is unknown. We rather suggest that the passage of water through aquaporins deserves further investigation, as a mechanism likely to explain fractionation during the transmembrane water movement in plants.

Mycorrhizal effect on xylem water isotopic composition

Mycorrhizal and non-mycorrhizal treatments were successfully implemented and the higher concentration of P in the inoculated seedlings showed that AMF community effectively interacted with their host. Increased organic C required by AMF leads to C-limitation in early growth stages of the plant (Ibijbijen et al. 1996; Smith and Read 2008), explaining the slightly, though not significant, lower total biomass found in mycorrhizal seedlings. Arbuscular mycorrhizal associations seem to have enhanced the preferential uptake of light isotopologues by *A. caven* seedlings as evidenced by the remarkable differences between soil and xylem isotopic composition. For $\Delta\delta^{2}H$, depletion in AM seedlings ranged from 6‰ to 10‰ more than the already observed in the non-AM seedlings. Depletion was furthered observed in $\Delta\delta^{18}O$, which was hardly significant for the non-AM pots (see Table 1 and Fig. 3). To our best knowledge, our experiment is the first to show that arbuscular mycorrhizal fungi colonization can significantly alter the isotopic composition of soil water during plant water uptake by roots.

One could argue that AMF could induce fractionation through a mass-effect, by increasing water uptake of preferentially lighter isotopologues due to augmentation of root biomass. However, we found no statistical difference between treatments in belowground biomass. Thus, it is unlikely that xylem isotopic patterns are a result of the enhancement of root biomass by the colonization of AMF allowing plants the access to more (depleted) water. Moreover, it is still unclear which is the magnitude of the actual contribution of hyphal water to total root water uptake (Wu et al. 2013). As reviewed by Augé (2001), in cases where specific water uptake at roots was measured, rates did not differ between plants with and without AMF (Bryla and Duniway 1997, 1998; Fitter 1988). While AMF certainly have a substantial effect in the transport of limiting nutrients such as P, their capacity to directly supply a significant amount of the water requirements of plants, which are 10^{5} to 10^{6} times higher in terms of mass than those of P (Schlesinger and Bernhardt 2013), is more questionable.

Intraradical AM fungal structures occupy the apoplastic compartment of plant roots (Parniske 2008). Hence, instead of having a direct impact on isotopic fractionation through massive uptake and supply of depleted water to the plant, AMF may rather enhance fractionation indirectly by hampering water movement through the apoplast. Considering that our species is a xerophytic, what we hypothesized might have happened in seedlings without mycorrhizas, could have been enhanced by the presence of mycorrhizas: proportionally more water would be transported through the transmembrane pathways, thereby crossing aquaporins. AM symbiosis has been reported to increase the expression of some aquaporin encoding genes (Krajinski et al. 2000) and/or activity (Ruiz-Lozano and Aroca 2010). This could provide indirect evidence supporting the enforcement of the 'aquaporin hypothesis' by AMF. It has been suggested that the presence of AMF may have the ability of switching root water pathways depending on environmental conditions; however, an enhancement of apoplastic water transport in AM tomato and maize plants under well-watered conditions has also been shown (Bárzana et al. 2012). This raises the question if the combination of a xerophytic species with AMF, like in our experiment, brings a particular case of water isotopologue uptake. Research combining the use of tracers for tracking water pathways and isotopic analysis of water in soil and xylem of plants with and without mycorrhizas and under water availability and stress might shed light upon these confounding pieces of evidences.

Altogether, more research dedicated to the 'aquaporin hypothesis' that we here propose and its link to AM colonization is needed, mostly considering AM are

widespread (Smith and Read 2008; Soudzilovskaia et al. 2015). This further raises the question on the effect of ectomycorrhiza (EM) on the isotopic composition of water during its passage from the soil to the plant. EM fungi are the second most common type of mycorrhizal fungi. They have been reported to fractionate stable C and N isotopes during exchange processes with their host plant (Hobbie et al. 1999; Högberg et al. 1999), but their effect on O and H isotopes remains unknown. In the context of mycorrhizal ubiquity, we investigated the mycorrhizal dependence of the plant species used in three of the most emblematic studies supporting the TWW hypothesis with direct field measurements (Brooks et al. 2010; Goldsmith et al. 2012; Hervé-Fernández et al. 2016). Out of the eleven reported plant species, four are known to form AM, two are likely to form AM, four species form EM, and one species form both AM and EM (Table 2). Hence, and based on our findings, isotopic fractionation could potentially have occurred in 64% of the cases (i.e. 7/11), maybe more if EM behave in the same way as AM apparently do. This at least raises the question if mycorrhiza could explain the observed isotopic deviation in xylem water in studies under field conditions. However, caution should be taken before generalizing our results, since we cannot out rule that patterns here evidenced are a specific result of the interaction between xerophytic species and mycorrhizas. Further physiological and water-isotopic research under controlled conditions of multiple plant species with and without mycorrhiza and for longer periods (more mature plants) is crucial before any generalization can be done.

Conclusions

Our results from a single species pot experiment add evidence to previous studies which indicate that the mismatch between xylem and plant water sources can be explained by isotope fractionation during water uptake (Vargas et al. 2017; Zhao et al. 2016; Ellsworth and Williams 2007; Lin and da SL Sternberg 1993). This process might be more common than previously thought. We further propose a mechanistic explanation to isotope fractionation, hypothesizing that heavy isotope discrimination could be induced by aquaporins which mediate water transport via the transmembrane passage. In addition, we show for the first time, that

Table 2 Mycorrhizal status and corresponding reference of the tree species for which the Two Water Worlds (TWW) hypothesis has been suggested from direct field measurements.

TWW Reference	Species	Mycorrhizal status	
Hervé-Fernández et al. (2016)	*Aextoxicom punctatum*	AM[a]	
	Laureliopsis philippiana	AM[a]	
	Eucryphia cordifolia	AM[a]	
	Eucalyptus nitens		EM[b]
Goldsmith et al. (2012)	*Quercus lancifolia*		EM[c]
	Quercus ocoteifolia		EM[+ d]
	Alchornea latifolia	AM[e]	
	Clethra mexicana	AM[+ e]	
	Alnus jorullensis		EM[f]
	Miconia glaberrima	AM[+ g]	
Brooks et al. (2010)	*Pseudotsuga menziesii*	AM[d]	EM[d]

AM arbuscular mycorrhiza, *EM* ectomycorrhiza, [+] status reported for the genus but not yet confirmed for the species

[a] Castillo et al. (2006)

[b] Pennington et al. (2011). Chu-Chou and Grace (1982)

[c] Corrales et al. (2016)

[d] Wang and Qiu (2006)

[e] Vega-Frutis et al. (2015)

[f] Daft et al. (1985)

[g] Urcelay et al. (2005)

AMF could enhance discrimination against heavy isotopologues. This could be explained through the 'aquaporin hypothesis' given AMF colonization would enforce water passage through aquaporins. If further experiments show similar results, mycorrhizal ubiquity would question the validity of the fractionation-free root water uptake assumption in most natural environments. Besides, we stress the relevance of the use of stable isotopes to unravel plant-mycorrhizal-water relations. We further highlight the need of more research on more mature and multiple plant species, plant functional types, mycorrhizal and soil types and environmental factors to test the generality of our results. Finally, to test the 'aquaporin hypothesis', we call for studies that combine experimental, isotopic and molecular approaches.

The fact that fractionation can take place along the pathway of root water uptake does not make isotopic methods useless for tracing water within the soil-plant-atmosphere continuum. As with physical fractionation in the atmospheric part of the hydrological cycle, modeling plant and mycorrhizal δ^2H and $\delta^{18}O$ fractionation would allow to predict and trace back the isotopic composition of water during uptake by the plant. Kuppel et al. (2018) recently published a model that simulates stable O and H isotope dynamics in catchments (EcH2O-iso) and includes a module for root water uptake. Our results, together with the already existing evidences of isotope fractionation during water uptake in other species (Vargas et al. 2017; Zhao et al. 2016; Ellsworth and Williams 2007; Lin and da SL Sternberg 1993), provide key insights and data on the behavior of H and O isotopes in the soil-plant-atmosphere continuum that could improve and further develop such type of models.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Allen MF (2007) Mycorrhizal fungi: highways for water and nutrients in arid soils. Vadose Zone J 6:291–297

Araguás-Araguás L, Rozanski K, Gonfiantini R, Louvat D (1995) Isotope effects accompanying vacuum extraction of soil water for stable isotope analyses. J Hydrol 168:159–171

Aroca R, Porcel R, Ruiz-Lozano JM (2007) How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in Phaseolus vulgaris under drought, cold or salinity stresses? New Phytol 173:808–816

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Barbeta A, Jones S, Clavé L et al (2019) Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest. Hydrol Earth Syst Sc Discuss:1–31. https://doi.org/10.5194/hess-2018-631

Bárzana G, Aroca R, Paz JA, Chaumont F, Martinez-Ballesta MC, Carvajal M, & Ruiz-Lozano JM (2012). Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. Annals of Botany 109(5):1009-1017.

Berry ZC, Evaristo J, Moore G, Poca M, Steppe K, Verrot L, Asbjornsen H, Borma L, Bretfeld M, Hervé-Fernández P, Seyfried M, Schwendenmann L, Sinacore K, De Wispelaere L, McDonnell JJ (2018) The two water worlds hypothesis: addressing multiple working hypotheses and proposing a way forward. Ecohydrology 11(3):e1843

Blake GR, Hartge KH (1986) Bulk density 1. *Methods of Soil Analysis: Part 1 - Physical and Mineralogical Methods*, pp 363–375

Brooks JR, Barnard HR, Coulombe R, McDonnell JJ (2010) Ecohydrologic separation of water between trees and streams in a Mediterranean climate. Nat Geosci 3:100–104

Bryla DR, Duniway JM (1997) Water uptake by safflower and wheat roots infected with arbuscular mycorrhizal fungi. New Phytol 136:591–601

Bryla DR, Duniway JM (1998) The influence of the mycorrhiza Glomus etunicatum on drought acclimation in safflower and wheat. Physiol Plant 104:87–96

Cabido M, Zeballos SR, Zak M, Carranza ML, Giorgis MA, Cantero JJ, Acosta AT (2018) Native woody vegetation in Central Argentina: classification of Chaco and Espinal forests. Appl Veg Sci 21:298–311

Castillo CG, Borie F, Godoy R, Rubio R, Sieverding E (2006) Diversity of mycorrhizal plant species and arbuscular

mycorrhizal fungi in evergreen forest, deciduous forest and grassland ecosystems of southern Chile. J Appl Bot Food Qual 80:40–47

Cernusak LA, Farquhar GD, Pate JS (2005) Environmental and physiological controls over oxygen and carbon isotope composition of Tasmanian blue gum, Eucalyptus globulus. Tree Physiol 25:129–146

Chu-Chou M, Grace LJ (1982) Mycorrhizal fungi of Eucalyptus in the north island of New Zealand. Soil Biol Biochem 14:133–137

Clark ID, Fritz P (1997) Environmental isotopes in hydrogeology. CRC press

Corrales A, Mangan SA, Turner BL, Dalling JW (2016) An ectomycorrhizal nitrogen economy facilitates monodominance in a neotropical forest. Ecol Lett 19:383–392

Daft MJ, Clelland DM, Gardner IC (1985) Symbiosis with endomycorrhizas and nitrogen-fixing organisms. P Roy Soc Edinb B 85:283–298

Dawson TE, Ehleringer JR (1991) Streamside trees that do not use stream water. Nature 350:335–337

Dawson TE, Ehleringer JR (1993) Isotopic enrichment of water in the" woody" tissues of plants: implications for plant water source, water uptake, and other studies which use stable isotopic composition of cellulose. Geochi Cosmochim Ac 57:3487–3487, 3492

Eamus D, Cleverly J, Boulain N, Grant N, Faux R, Villalobos-Vega R (2013) Carbon and water fluxes in an arid-zone Acacia savanna woodland: an analyses of seasonal patterns and responses to rainfall events. Agric For Meteorol 182: 225–238

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

Eller CB, Lima AL, Oliveira RS (2013) Foliar uptake of fog water and transport belowground alleviates drought effects in the cloud forest tree species, *Drimys brasiliensis* (Winteraceae). New Phytol 199:151–162

Ellsworth PZ, Sternberg LS (2015) Seasonal water use by deciduous and evergreen woody species in a scrub community is based on water availability and root distribution. Ecohydrology 8:538–551

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

Evaristo J, Jasechko S, McDonnell JJ (2015) Global separation of plant transpiration from groundwater and streamflow. Nature 525:91–94

Evaristo J, McDonnell JJ, Scholl MA, Bruijnzeel LA, Chun KP (2016) Insights into plant water uptake from xylem-water isotope measurements in two tropical catchments with contrasting moisture conditions. Hydrol Process 30:3210–3227

Fitter AH (1988) Water relations of red clover Trifolium pratense L. as affected by VA mycorrhizal infection and phosphorus supply before and during drought. J Exp Bot 39:595–603

Gat JR (1996) Oxygen and hydrogen isotopes in the hydrologic cycle. Annu Rev Earth Pl Sc 24:225–262

Geris J, Tetzlaff D, McDonnell J, Anderson J, Paton G, Soulsby C (2015) Ecohydrological separation in wet, low energy northern environments? A preliminary assessment using different soil water extraction techniques. Hydrol Process 29:5139–5152

Goldsmith GR, Muñoz-Villers LE, Holwerda F, McDonnell JJ, Asbjornsen H, Dawson TE (2012) Stable isotopes reveal linkages among ecohydrological processes in a seasonally dry tropical montane cloud forest. Ecohydrology 5:779–790

Grace C, Stribley DP (1991) A safer procedure for routine staining of vesicular-arbuscular mycorrhizal fungi. Mycol Res 95: 1160–1162

Gröning M (2011) Improved water δ^2H and $\delta^{18}O$ calibration and calculation of measurement uncertainty using a simple software tool. Rapid Comm Mass Sp 25:2711–2720

Hacke UG, Laur J (2016) Aquaporins: channels for the molecule of life. In: *eLS*. 10(9780470015902), a9780470001289

Hervé-Fernández P, Oyarzún C, Brumbt C, Huygens D, Bodé S, Verhoest NEC, Boeckx P (2016) Assessing the 'two water worlds' hypothesis and water sources for native and exotic evergreen species in south-Central Chile. Hydrol Process 30: 4227–4241

Hobbie EA, Macko SA, Shugart HH (1999) Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. Oecologia 118:353–360

Högberg P, Högberg MN, Quist ME, Ekblad ALF, Näsholm T (1999) Nitrogen isotope fractionation during nitrogen uptake by ectomycorrhizal and non-mycorrhizal Pinus sylvestris. New Phytol 142:569–576

Ibijbijen J, Urquiaga S, Ismaili M, Alves BJR, Boddey RM (1996) Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition and nitrogen fixation of three varieties of common beans (*Phaseolus vulgaris*). New Phytol 134:353–360

Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect VA mycorrhizas. Mycol Res 92:486–488

Kozono D, Yasui M, King LS, Agre P (2002) Aquaporin water channels: atomic structure molecular dynamics meet clinical medicine. J Clin Invest 109:1395–1399

Krajinski F, Biela A, Schubert D, Gianinazzi-Pearson V, Kaldenhoff R, Franken P (2000) Arbuscular mycorrhiza development regulates the mRNA abundance of Mtaqp1 encoding a mercury-insensitive aquaporin of Medicago truncatula. Planta 211:85–90

Kuppel S, Tetzlaff D, Maneta M, Soulsby C (2018) EcH2O-iso 1.0: water isotopes and age tracking in a process-based, distributed ecohydrological model. Geosci Model Dev 11: 3045–3069

Leibundgut C, Maloszewski P, Külls C (2009) Tracers in hydrology. Wiley-Blackwell

Lin G, da SL Sternberg, L (1993) hydrogen isotopic fractionation by plant roots during water uptake in coastal wetland plants. In Stable isotopes and plant carbon-water relations (pp. 497–510). Elsevier

Longo S, Nouhra E, Goto BT, Berbara RL, Urcelay C (2014) Effects of fire on arbuscular mycorrhizal fungi in the mountain Chaco Forest. Forest Ecol Manag 315:86–94

Mamonov AB, Coalson RD, Zeidel ML, Mathai JC (2007) Water and deuterium oxide permeability through aquaporin 1: MD predictions and experimental verification. J Gen Physiol 130: 111–116

Martín-Gómez P, Barbeta A, Voltas J, Peñuelas J, Dennis K, Palacio S, Dawson TE, Ferrio JP (2015) Isotope-ratio infrared spectroscopy: a reliable tool for the investigation of plant-water sources? New Phytol 207:914–927

Martín-Gómez P, Serrano L, Ferrio JP (2016) Short-term dynamics of evaporative enrichment of xylem water in woody stems: implications for ecohydrology. Tree Physiol 37:511–522

McDonnell JJ (2014) The two water worlds hypothesis: ecohydrological separation of water between streams and trees? WIRES Water 1:323–329

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. New Phytol 115:495–501

Michener R, Lajtha K (eds) (2008) Stable isotopes in ecology and environmental science. John Wiley & Sons

Oerter E, Finstad K, Schaefer J, Goldsmith GR, Dawson T, Amundson R (2014) Oxygen isotope fractionation effects in soil water via interaction with cations (mg, ca, K, Na) adsorbed to phyllosilicate clay minerals. J Hydrol 515:1–9

Olsen SR (1954) *Estimation of available phosphorus in soils by extraction with sodium bicarbonate.* United States Department of Agriculture, Washington

Orlowski N, Breuer L, McDonnell JJ (2016) Critical issues with cryogenic extraction of soil water for stable isotope analysis. Ecohydrology 9:1–5

Oshun J, Dietrich WE, Dawson TE, Fung I (2016) Dynamic, structured heterogeneity of water isotopes inside hillslopes. Water Resour Res 52:164–189

Otieno DO, Schmidt MWT, Adiku S, Tenhunen J (2005) Physiological and morphological responses to water stress in two Acacia species from contrasting habitats. Tree Physiol 25:361–371

Pansu M, Gautheyrou J (2006) Pipette method after Robinson-köhn or andreasen. In: Handbook of soil analysis: mineralogical, organic and inorganic methods. Springer Science & Business Media, pp 35–42

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 6:763–775

Pennington HG, Bidartondo MI, Barsoum N (2011) A few exotic mycorrhizal fungi dominate eucalypts planted in England. Fungal Ecol 4:299–302

Perez M, Urcelay C (2009) Differential growth response to arbuscular mycorrhizal fungi and plant density in two wild plants belonging to contrasting functional types. Mycorrhiza 19:517–523

R Core Team. (2013). *R: A language and environment for statistical computing*. Vienna, Austria

Raab N, Meza FJ, Franck N, Bambach N (2015) Empirical stomatal conductance models reveal that the isohydric behavior of an Acacia caven Mediterranean Savannah scales from leaf to ecosystem. Agric For Meteorol 213:203–216

Rodríguez-Gamir J, Xue J, Clearwater MJ, Meason DF, Clinton PW, Domec JC (2019) Aquaporin regulation in roots controls plant hydraulic conductance, stomatal conductance, and leaf water potential in Pinus radiata under water stress. Plant Cell Environ 42:717–729

Rozanski K, Araguás-Araguás L, Gonfiantini R (1993) Isotopic patterns in modern global precipitation. In: Climate change in continental isotopic records, vol 78, pp 1–36

Ruiz-Lozano JM, Aroca R (2010) Modulation of aquaporin genes by the arbuscular mycorrhizal symbiosis in relation to osmotic stress tolerance. In: Seckbach J, Grube M. eds. Symbioses and stress: joint ventures in biology, cellular origin, life in extreme habitats and astrobiology. Dordrecht: springer science+business. Media:359–374

Rundel PW, Ehleringer JR, Nagy KA (Eds.) (2012) *Stable isotopes in ecological research* (Vol. 68). Springer Science & Business Media

Ryan J, Estefan G, Rashid A (2001) *Soil and plant analysis laboratory manual.* International center for agricultural research in the dry areas (ICARDA), Aleppo, Syria

Schlesinger WH, Bernhardt ES (2013) Biogeochemistry: an analysis of global change. Academic press

Smith SSE (1980) Mycorrhizas of autotrophic higher plants. Biol Rev 55:475–510

Smith SSE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, New York, ISBN, 440026354, 605

Soudzilovskaia NA, Douma JC, Akhmetzhanova AA, van Bodegom PM, Cornwell WK, Moens EJ, Treseder KK, Tibbett M, Wang YP, Cornelissen JHC (2015) Global patterns of plant root colonization intensity by mycorrhizal fungi explained by climate and soil chemistry. Glob Ecol Biogeogr 24:371–382

Tyerman SD, Niemietz CM, Bramley H (2002) Plant aquaporins: multifunctional water and solute channels with expanding roles. Plant Cell Environ 25:173–194

Urcelay C, Tecco PA, Chiarini F (2005) Micorrizas arbusculares del tipo 'Arum' y 'Paris' y endófitos radicales septados oscuros en Miconia ioneura y Tibouchina paratropica (Melastomataceae). B Soc Argent Bot 40:151–155

Vargas AI, Schaffer B, Yuhong L, Sternberg LDSL (2017) Testing plant use of mobile vs immobile soil water sources using stable isotope experiments. New Phytol 215(2):582–594

Vega-Frutis R, López JC, Flandes C, Guevara R (2015) Have male trees of the tropical rain forest evolved to minimize the interactions with mycorrhizal symbionts? Perspect Plant Ecol 17:444–453

Venier P, Cabido M, Funes G (2017) Germination characteristics of five coexisting neotropical species of Acacia in seasonally dry Chaco forests in Argentina. Plant Spec Biol 32:134–146

Wang B, Qiu YL (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363

West AG, Patrickson SJ, Ehleringer JR (2006) Water extraction times for plant and soil materials used in stable isotope analysis. Rapid Comm Mass Sp 20:1317–1321

Wu QS, Srivastava AK, Zou YN (2013) AMF-induced tolerance to drought stress in citrus: a review. Sci Hortic 164:77–87

Zhao L, Wang L, Cernusak LA, Liu X, Xiao H, Zhou M, Zhang S (2016) Significant difference in hydrogen isotope composition between xylem and tissue water in Populus euphratica. Plant Cell Environ 39:1848–1857

Zimmermann U, Ehhalt D, Münnich KO (1967) Soil-water movement and evapotranspiration: changes in the isotopic composition of the water. In: Isotopes in hydrology. In *Conference on isotopes in hydrology*, pp 567–585