An explanation for the isotopic offset between soil and stem water in a temperate tree species

Adrià Barbeta, Teresa Gimeno, Laura Clavé, Bastien Fréjaville, Sam P. Jones, Camille Delvigne, Lisa Wingate, Jérôme Ogée

To cite this version:

Adrià Barbeta, Teresa Gimeno, Laura Clavé, Bastien Fréjaville, Sam P. Jones, et al.. An explanation for the isotopic offset between soil and stem water in a temperate tree species. New Phytologist, 2020, 227 (3), pp.766-779. ⟨10.1111/nph.16564⟩. ⟨hal-03187004⟩

New Phytologist

An explanation for the isotopic offset between soil and stem water in a temperate tree species

Adrià Barbeta[1,2], **Teresa E. Gimeno**[1,3,4], **Laura Clavé**[1], **Bastien Fréjaville**[1], **Sam P. Jones**[1,5], **Camille Delvigne**[1,6], **Lisa Wingate**[1] **and Jérôme Ogée**[1]

[1]INRAE, UMR1391 ISPA, 33140 Villenave d'Ornon, France; [2]BEECA, Department of Evolutionary Biology, Ecology and Environmental Sciences, Universitat de Barcelona, Barcelona, Catalonia, Spain; [3]Basque Centre for Climate Change, 48940 Leioa, Spain; [4]IKERBASQUE, Basque Foundation for Science, 48008 Bilbao, Spain; [5]Instituto Nacional de Pesquisas da Amazônia, Manaus CEP 69060-001, Brazil; [6]Université Catholique de Louvain, Louvain-la-Neuve, Belgium

Authors for correspondence:
Adrià Barbeta
Tel: +34 93 403 13 75
Email: adria.barbeta.margarit@gmail.com

Jérôme Ogée
Tel: +33 5 57 12 24 22
Email: jerome.ogee@inrae.fr

Received: *5 September 2019*
Accepted: *14 March 2020*

New Phytologist (2020) **227:** 766–779
doi: 10.1111/nph.16564

Key words: ecohydrology, *Fagus sylvatica*, plant water sources, root water uptake, water isotopes.

Summary

- A growing number of field studies report isotopic offsets between stem water and its potential sources that prevent the unambiguous identification of plant water origin using water isotopes. We explored the causes of this isotopic offset by conducting a controlled experiment on the temperate tree species *Fagus sylvatica*.
- We measured δ^2H and δ^{18}O of soil and stem water from potted saplings growing on three soil substrates and subjected to two watering regimes.
- Regardless of substrate, soil and stem water δ^2H were similar only near permanent wilting point. Under moister conditions, stem water δ^2H was 11 ± 3‰ more negative than soil water δ^2H, coherent with field studies. Under drier conditions, stem water δ^2H became progressively more enriched than soil water δ^2H. Although stem water δ^{18}O broadly reflected that of soil water, soil–stem δ^2H and δ^{18}O differences were correlated ($r = 0.76$) and increased with transpiration rates indicated by proxies.
- Soil–stem isotopic offsets are more likely to be caused by water isotope heterogeneities within the soil pore and stem tissues, which would be masked under drier conditions as a result of evaporative enrichment, than by fractionation under root water uptake. Our results challenge our current understanding of isotopic signals in the soil–plant continuum.

Introduction

Plant transpiration is the main flux returning water from the land surface to the atmosphere (Jasechko *et al.*, 2013), emphasizing the importance of vegetation in the global water cycle. To trace variations in land–atmosphere water fluxes it is necessary to identify the water pools accessed by plants and how these change over time and space. Analysis of the natural abundance of stable isotopes in water is a commonly used technique for this purpose (Dawson & Ehleringer, 1991; Barbeta & Peñuelas, 2017). This technique is usually applied under the assumption that no isotopic fractionation occurs during root water uptake, as suggested by a series of early observations conducted on plants grown hydroponically (Washburn & Smith, 1934; Zimmermann *et al.*, 1967). Although hydroponic systems do not have the mechanistic complexity and heterogeneity of natural systems (Penna *et al.*, 2018), the evidence for root water uptake not fractionating paved the way for using water-stable isotopes to infer plant water sources (White *et al.*, 1985; Dawson & Ehleringer, 1991), and to assess their spatiotemporal variability (Bertrand *et al.*, 2014; Barbeta *et al.*, 2015) and their ecological implications (Moreno-Gutiérrez *et al.*, 2012; De Deurwaerder *et al.*, 2018).

Improvements in the capability for higher throughput using modern water extraction and isotopic determination techniques have helped collect water isotopic datasets that are more comprehensive than ever before (Stumpp *et al.*, 2018). Perhaps more importantly, plant water source studies are no longer restricted to either oxygen (δ^{18}O) or hydrogen (δ^2H) isotopic composition, but routinely present data for both isotopes. An emerging feature of studies using dual-isotope approaches is that oxygen and hydrogen isotopes do not always agree in the attribution of the source(s) of plant water. This is caused by isotopic offsets between stem water and all potential water sources, that is, the isotopic composition of stem water does not match any of the considered sources in the dual-isotope space. These isotopic offsets have been observed in field sites encompassing a wide range of soil types and biomes, including semiarid shrublands (Wang *et al.*, 2017), conifer forests (Brooks *et al.*, 2010; Geris *et al.*, 2017), broadleaved forests (Bowling *et al.*, 2017; Goldsmith *et al.*, 2018; Barbeta *et al.*, 2019), urban gardens (Oerter and Bowen, 2017), tropical rainforests (Brum *et al.*, 2018; De Deurwaerder *et al.*, 2018) and rice paddy fields (Mahindawansha *et al.*, 2018). In such cases, the use of oxygen or hydrogen isotopes separately can lead to significantly different attributions of plant water sources (Lin & Sternberg, 1993; Evaristo *et al.*, 2017; Brum *et al.*, 2018; Barbeta *et al.*, 2019). Some authors acknowledged this caveat and used either δ^2H or δ^{18}O to infer plant water sources, or proposed and discussed potential mechanisms and implications (Bowling *et al.*, 2017; Evaristo *et al.*, 2017; Barbeta *et al.*, 2019; Oerter & Bowen, 2019; Oerter *et al.*, 2019). However, in many cases, soil–stem isotopic offsets are not addressed.

An isotopic offset between stem water and all potential water sources cannot be attributed solely to methodological issues. For example, contamination of soil- or plant-extracted water by organic compounds can bias measurements of the stable isotopes in water, especially when using laser-based instruments (e.g. Schultz *et al.*, 2011; Martín-Gómez *et al.*, 2015; Millar *et al.*, 2018). However, these contaminations are now routinely dealt with using custom, post-measurement corrections and are unlikely to cause a systematic bias, as isotopic offsets have been found in studies using both mass spectrometers (Brooks *et al.*, 2010; Bowling *et al.*, 2017; Brum *et al.*, 2018; Goldsmith *et al.*, 2018) and laser-based instruments (Geris *et al.*, 2017; De Deurwaerder *et al.*, 2018; Barbeta *et al.*, 2019), and both types of analysers render similar and reproducible results on the same soil water samples (Orlowski *et al.*, 2018). Additional confounding effects related to water extraction techniques should not be overlooked (Thorburn *et al.*, 1993; Walker *et al.*, 1994; Millar *et al.*, 2018; Orlowski *et al.*, 2018). For example, cryogenic vacuum extraction (CVE) is the most widely used technique nowadays but has been shown to give results sensitive to many parameters such as soil texture, water content, extraction time or temperature (Orlowski *et al.*, 2018). Alternative soil and stem water extraction techniques exist (Wassenaar *et al.*, 2008; Munksgaard *et al.*, 2014) and comparative studies have often concluded that contrasting results were caused by differences in extraction yields affecting the isotopic composition of the extracted water of soil (Walker *et al.*, 1994) and stem (Thorburn *et al.*, 1993) via Rayleigh distillation processes, or by large differences in organic contamination of the extracted water (Millar *et al.*, 2018). However, if well conducted, water extraction techniques such as CVE lead to extraction yields > 99% and low amounts of organic contamination (Orlowski *et al.*, 2013; Millar *et al.*, 2018). Another possibility explaining soil–stem isotopic offsets is that the pools of water extracted vary with different techniques, even though some of these pools might be less relevant to the study of plant water sources (e.g. water adsorbed on soil particles or plant storage water). For example, a recent study on wheat showed that the soil–stem isotopic offset was reduced when using direct vapour equilibration on stems, compared with CVE (Millar *et al.*, 2018). Unfortunately, for woody species, direct vapour equilibration presents additional problems related to the interference of volatile organic compounds during isotopic determination and still needs further development and testing (Volkmann *et al.* 2016; Raulerson, 2018). Regardless of the technique, a consistent pattern is observed frequently across studies whereby stem water generally plots below and to the right of any considered water source in the dual-isotope ‘space’ (i.e. a graphical representation of δ^2H vs δ^{18}O values) (Brooks *et al.*, 2010). Such a systematic pattern is unlikely to be attributed solely to soil and stem water extraction artefacts but it remains to be tested whether it is reproducible under controlled conditions.

Isotopic offsets between source and stem water have now been reported in ecologically diverse plant species, but were firstly observed in halophytic and xerophytic plants (Lin & Sternberg, 1993; Ellsworth & Williams, 2007). These drought- and salinity-tolerant plants have a highly developed Casparian strip on the radial cell walls of the root endodermis that impedes apoplastic movement of water, forcing water to move symplastically across cell membranes (Ellsworth & Williams, 2007 and references therein). Water movement through the symplastic route has been hypothesized to fractionate hydrogen isotopes in water, leading to a 3–9‰ depletion of stem water compared with soil water in halophytic and xerophytic plants (Lin & Sternberg, 1993; Ellsworth & Williams, 2007). More recently, Poca *et al.* (2019) showed that arbuscular mycorrhizal associations enhanced the isotopic offset between soil and stem water (up to −15‰) in potted seedlings of the xerophytic species *Acacia caven*. They proposed that isotopic fractionation occurred during transmembrane water transport via aquaporins, and that this mode of transport must be enhanced in presence of mychorrizal associations. However, the impairment of the apoplastic pathway was not demonstrated in this study. More importantly, this mechanism cannot explain why other studies, including our own results from a temperate deciduous forest (Barbeta *et al.*, 2019), found similar isotopic offsets (a soil water excess of 8.4‰) in plant species where root water uptake through the apoplastic route should not be impeded.

It is also increasingly recognized that soil water may exhibit pore-scale isotopic heterogeneity created by water surface interaction effects that leads to an isotopic depletion of adsorbed water compared with bulk soil water (Oerter *et al.*, 2014; Chen *et al.*, 2016; Lin & Horita, 2016; Lin *et al.*, 2018; Penna *et al.*, 2018; Oerter & Bowen, 2019). Then, a depletion of stem water compared with bulk soil water could indicate that trees take up water adsorbed onto soil particles. However, we would expect roots to take up the most mobile (i.e. gravimetric and capillary) soil water (see the discussion in Bowling *et al.*, 2017) which, in contrast to adsorbed water, should be more enriched than bulk soil water (Chen *et al.*, 2016; Barbeta *et al.*, 2019). Under low water availability and high evaporative demand, it has also been shown that stem water loss via evaporation can create significant isotopic enrichment of stem water (Bowling *et al.*, 2017; Martín-Gómez *et al.*, 2017), further complicating the inference of plant water sources from stem water’s isotopic composition. Although such evaporative enrichment of stem water should be easily detectable in the dual-isotope space and is a process that needs to be considered when attempting to derive plant water sources, it cannot explain why stem water is more depleted in δ^2H than any considered water source.

Isotopic heterogeneity in plant water pools was further proposed by Zhao *et al.* (2016) as an alternative explanation for the observed soil–stem water isotopic offsets. They directly extracted sap (i.e. vessel water) from stems of the desert riparian trees *Populus euphratica* and found that its isotopic composition matched that of groundwater. On the other hand, total water from stem or root samples was systematically depleted in ^{2}H with respect to sap water and all likely water sources. They attributed this observation to a putative discrimination during water transport and redistribution within the plant. Unfortunately, repeating this experiment with other species or during the dry season is very challenging, as it requires the xylem sap to be under positive pressure to be collected. Only indirect evidence can be deducted, once all other hypotheses have been discarded.

In this context, we conducted a glasshouse experiment with potted saplings of a temperate deciduous tree (European beech, *Fagus sylvatica* L.) to quantify potential isotopic offsets between plant and source water and to elucidate how these vary with water availability, soil properties and plant physiological performance. We chose this temperate tree species because it had been shown in a previous field study that isotopic separation between source and xylem water was likely (Barbeta *et al.*, 2019). In the field, the total extension of the root system is difficult to assess so the presence of unconsidered water sources can never be completely ruled out (Barbeta *et al.*, 2019; Oerter & Bowen, 2019). By contrast, the advantage of potted plants is that the actual water source can be characterized more thoroughly. We thus wanted to verify that the isotopic offsets observed in the field between xylem and soil water were reproducible under controlled conditions. Our experimental design builds up on the hypotheses formulated by previous studies (Zhao *et al.*, 2016; Martín-Gómez *et al.*, 2017; Vargas *et al.*, 2017; Barbeta *et al.*, 2019; Oerter & Bowen, 2019) and, more importantly, expands the range of soil water availabilities tested to date. The isotopic offset between soil and stem water reported by Vargas *et al.* (2017) was based on a glasshouse experiment with potted *Persea americana* saplings subjected to a relatively mild water shortage (matching the study species demands) on two contrasting soil types. Here we not only explored much harsher water shortages, but we also complemented our experiment with a control treatment whereby plants were regularly watered throughout the experiment to maintain the soil at field capacity. By doing so, we were revisiting the early hydroponic experiments at the origin of the idea that root water uptake is a nonfractionating process, while adding the complexity of textured soils. After several weeks of regular irrigation to compensate for water losses, fractionation during root water uptake (noted ε_U in Fig. 1a) should lead to an isotopic enrichment of soil water above irrigation water ($\delta_{soil} = \delta_P + \varepsilon_U$) while stem water should arrive at isotopic steady state and reflect exactly the isotopic composition of irrigation water ($\delta_{soil} = \delta_P$), leading to a constant isotopic offset between bulk soil and stem waters (Fig. 1a). If soil evaporation is not fully suppressed, soil water will become slightly more enriched than $\delta_P + \varepsilon_U$ but the isotopic difference between soil and stem water should not differ (Supporting Information Fig. S1; Notes S1). Pore-scale isotopic heterogeneity in soil and xylem water pools may be an alternative explanation for the observed soil–xylem isotopic offsets (Fig. 1b). Under well-watered conditions, the adsorbed soil water would represent a small fraction (f_a) of total soil water and thus the isotopic composition of bulk soil water (δ_{soil}) would resemble that of irrigation water (δ_P). In turn, the isotopic composition of bulk stem water (δ_{stem}) would still be more depleted than δ_{soil} (and δ_P) because of isotopic heterogeneity within the stem (i.e. isotopic differences between vessel/sap water and water in nonconductive tissues; Zhao *et al.*, 2016). This would imply that these nonconductive tissues are depleted compared with sap water, as sap would have the signal of the water taken up (δ_P) (Fig. 1b). To test this alternative situation further, we also used three soil textures, including one containing rock fragments, to play on the fraction of adsorbed water in soils and help disentangle the separate roles of soil water content and water potential on the isotopic offsets. To sum up, our aim was to: test whether isotopic offsets between stem water and their sources were reproducible in potted plants with a unique source of water; and to identify the soil physical and/or plant physiological mechanisms producing these isotopic offsets.

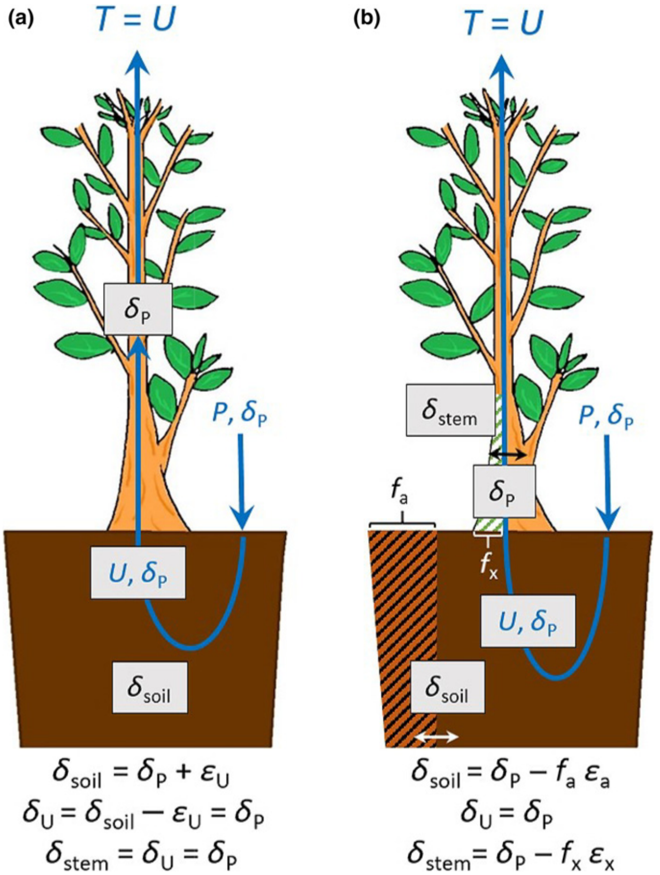

Fig. 1 Illustration of the expected isotopic composition of bulk soil (δ_{soil}) and stem (δ_{stem}) water when irrigation (P, with a constant isotopic composition δ_P) continuously compensates root water uptake (U) and transpiration (T), and evaporation losses are negligible (i.e. control treatments). (a) Scenario when root water uptake fractionates water isotopes (fractionation factor ε_U); (b) scenario when adsorbed water in the soil (fraction f_a) and the stem's storage tissue water (fraction f_x) are depleted with respect to mobile and capillary water in the soil (fractionation ε_a) and the xylem's vessel water (fractionation ε_x). Blue arrows, irrigation–soil–plant–atmosphere water flux; white arrows, exchanges between soil water pools; black arrows, exchanges between plant internal water pools.

Materials and Methods

Plant material and experimental design

From February to July 2018 we grew saplings of *F. sylvatica* L. in a temperature-controlled glasshouse (Talence, France). Climatic conditions inside the glasshouse were monitored with a temperature and humidity probe (HMP60; Vaisala, Vanta, Finland) and a quantum sensor (SQ200; Apogee, Logan, UT, USA). Over the study period (14 May to 20 June 2018, $n = 38$ d), mean air temperature inside the glasshouse (± SE) was 20 ± 0.3°C during the day and 16.3 ± 0.2°C at night. A shading cloth was permanently deployed from 24 April 2018 and mean daily photosynthetic photon flux density (PPFD) was 10 ± 0.9 mol $\mathrm{m^{-2}\,d^{-1}}$.

One-year old beech saplings (mean diameter of 2.1 ± 0.5 cm) were obtained from a commercial nursery (Naudet pépinières, Leuglay, France) grown from seeds originating from the Armorican massif (Bretagne, France). On 20 February 2018, we transplanted 220 plants into 3.5 l squared pots filled with three soil types. The three soil types consisted of a volume mix of soil : sand : commercial substrate (2 : 1 : 1), soil : sand : commercial substrate : crushed rocks (10 : 5 : 5 : 1) and soil : sand : commercial substrate : clay (10 : 2 : 5 : 3). Substrates were: sandy soil from a nearby pine plantation (Jones *et al.*, 2017) (Cestas, France), with a total organic C of 33 g $\mathrm{kg^{-1}}$ and a total N < 1 g $\mathrm{kg^{-1}}$; washed river sand (Gedimat, Levallois-Perret, France); commercial peat substrate for plant growth ('Terrau Gazon'; Soufflet Vigne, Martillac, France); crushed rocks obtained from oven-dried (48 h at 105°C) limestone rocks collected near the Ciron river (Pompéjac, France) and commercial soil conditioner (bentonite clay; Magellan-bio.fr, Cysoing, France). According to texture analyses, the first and second (without the rocks) soil types were classified as coarse sand and the third type was a sandy loam in the limit of sandy clay loam, henceforth sandy clay loam. Soil water retention curves estimated from pedotransfer functions (R package SOILWATER; Cordano *et al.*, 2007) are presented in Fig. S2. We transplanted 100 plants onto the coarse sandy soil, 60 plants onto the coarse sandy soil with rocks and 60 plants onto the sandy clay loam.

From February 2018 until 13 May 2018 all pots were watered regularly to field capacity with tap water (δ^2H = −35.33 ± 0.25 and δ^{18}O = −5.90 ± 0.3) and soil water was allowed to freely evaporate from the surface. Starting on 14 May 2018, we watered all pots daily to field capacity for 3 d consecutively to ensure a homogeneous soil water pool in each pot. A set of 12 plants from each soil type continued to be watered to field capacity regularly (control treatment), while watering was withheld for all other plants from the 17 May 2018 until the end of the drying experiment on 20 June 2018 (drought treatment). A plastic top was placed on all pots to reduce soil water evaporation on 17 May 2018. Mean soil gravimetric water content (GWC) over time for each treatment and soil type was calculated from the weights of 10 and five pots for the drought and control treatments, respectively, for each soil type. Individual GWC and plant water contents for sampled pots and plants were estimated from the soil and stem samples used for cryogenic vacuum distillation. GWC was then converted to volumetric water content (VWC) using the bulk density of each soil type. Based on the retention curve of each soil type, we determined the VWC corresponding to the permanent wilting point (VWC at which soil matric potential is −1500 kPa). For each sampled pot, we calculated θ_{rel}, the difference between the VWC of a given pot minus the VWC at permanent wilting point. Thus, positives θ_{rel} values corresponded to conditions in which soil water can be taken up by roots whilst negative values imply that soil water is not extractable by the plant.

In addition to the two watering treatments, we applied a low vapour pressure deficit (VPD) treatment during the first two sampling campaigns on a subset of plants from the drought treatment with the rock-free coarse sandy soil. This treatment consisted of covering five plants with a semi-transparent plastic bag the evening before the day of sampling. The aim was to reduce transpiration for individual plants over the course of 1 d to assess its impact on potential isotopic offsets between soil and stem water pools.

Ecophysiological measurements and destructive harvests

Over the course of the drying experiment, we performed five campaigns of ecophysiological measurements and destructive harvests for water isotope analysis (1, 8, 15, 28 and 35 d after the last watering event of the drought treatment on 17 May 2018). For each campaign and each soil type, we harvested three plants from the control and five from the drought treatment, except for the first and second campaigns, where five additional plants from the low-VPD treatment (on rock-free sandy soil) were also sampled. For each pot, a soil core from the surface to the bottom of the pot was taken, homogenized in a clean plastic tray and subsampled for isotopic analysis. For each plant we cut two 5-cm-long lignified segments, one from the root and one from the stem (separated by at least 2.5 cm below the above-ground stem) and peeled off the bark and phloem. Soil, root and stem samples were rapidly transferred into screw-cap glass vials, sealed with Parafilm® (Bemis Company Inc., Neenah, WI, USA) and stored in a cool box until transported to the laboratory where they were stored at 4°C until further analysis.

On the day of each destructive harvest (conducted in early afternoon), ecophysiological measurements were also performed on leaves from the harvested plants and included measurements of stomatal conductance to water vapour (g_s) and predawn (Ψ_{pd}) and midday leaf water potential (Ψ_{md}). Leaf water potential was measured with a custom-made Scholander type chamber (DG Meca, Gradignan, France) on one leaf per plant. g_s was measured at mid-morning (10:30–11:30 h, local time) with two cross-calibrated handheld porometers (SC-1 Leaf Porometer; Decagon Inc., Pullman, WA, USA) on one leaf per plant. For the second campaign, we measured g_s with the two hand-held porometers and with an infrared gas analyser (IRGA, Li-Cor 6400; Li-Cor, Lincoln, NE, USA), on the same leaves and matching the conditions inside the gas exchange chamber (temperature, humidity, PPFD and CO_2 concentration) to those prevailing in the glasshouse. The significant correlation between g_s measurements showed that measurements from the hand-held porometers neither overestimated nor underestimated g_s compared with the IRGA ($P = 0.001$, $R^2 = 0.45$, slope = 1.03 ± 0.25).

Cryogenic water extraction and analyses of water isotopic composition

The extraction of water from soil, stem, root and rock samples was performed by cryogenic vacuum distillation using a design and methodology proposed by Orlowski *et al.* (2013), as described in Jones *et al.* (2017). At the onset of the extraction, up to 24 samples kept in sampling glass vials were inserted in larger extraction glass vials connected to a vacuum extraction line and frozen in liquid nitrogen. The extraction line was then evacuated down to an atmospheric (static) pressure of < 1 Pa and composed of 24 glass U-shape tubes that were then inserted in liquid nitrogen to create a cold trap. Samples were then immersed in a water bath at ambient temperature, and the water bath was gradually heated up to 80°C (within 1 h) to start the distillation process. Samples remained in the heated bath at 80°C for 2.5 h. Pressure in the extraction line was continuously monitored with subatmospheric pressure sensors (APG100 Active Pirani Vacuum Gauges; Edwards, Burgess Hill, UK) to check that the lines remained leak-tight throughout the entire extraction and that the water extraction had ended. Samples were weighed before and after the extraction and before and after being oven-dried for 24 h at 105°C to assess extraction efficiency. GWC was estimated from each sample by using the weight measured before and after the cryogenic extraction and again after oven-drying (Newberry *et al.*, 2017).

The isotopic composition (δ^2H and δ^{18}O) of the extracted waters was measured with an off-axis integrated cavity optical spectrometer (TIWA-45EP; Los Gatos Research, San Jose, CA, USA) coupled to a liquid auto-sampler and vaporizer (LC-xt; PAL systems, Zwingen, Switzerland). All isotopic data reported here are calibrated using two internal standards and expressed on the VSMOW-SLAP (Vienna Standard Mean Ocean Water–Standard Light Antarctic Precipitation) scale, as described in Jones *et al.* (2017). Because the presence of organic compounds (ethanol, methanol and/or other biogenic volatile compounds) in water samples can lead to large isotopic discrepancies in laser-based analyses (Martín-Gómez *et al.*, 2015; Wassenaar *et al.* 2018), we developed a post-correction algorithm for the presence of organic compounds based on the narrowband (for methanol) and broadband (for ethanol) metrics of the absorption spectra (Brian Leen *et al.*, 2012). Post-corrections relating how these metrics affect the isotopic composition of waters contaminated with known amounts of ethanol and methanol were developed specifically for our instrument. Overall, these post-corrections were usually higher for stem water than for meteoric or soil water samples but always remained quite small (i.e. < 1.5 ‰ for δ^2H and < 0.7 ‰ for δ^{18}O).

Data analyses

Statistical analysis was conducted in R (R Core Team, 2019) using either general linear models (GLMs) or generalized linear mixed models (for those cases where we set some of the factors as random) from the R package LME4 (Bates *et al.*, 2015). The effect of soil type and drought treatment over the course of the experiment on GWC, Ψ_{pd}, Ψ_{md}, the difference between them ($\Delta\Psi$) and the isotopic composition of the different water pools (soils, stems, roots and rocks) was tested with GLMs with interactions between all factors. In order to determine the most relevant factors explaining the variability in isotopic offsets (Δ^{18}O and Δ^2H, calculated as the difference between plant and soil water isotopic composition), we conducted a stepwise regression model. In the saturated model, we considered, θ_{rel}, soil type, g_s, Ψ_{pd} and Ψ_{md}. Based on the Akaike information criterion (AIC) (Akaike, 1974), we progressively removed those variables that were not significant, deciding to maintain this removal if the AIC decreased (better compromise between goodness of fit and model simplicity).

Results

Manipulation effects on soil water content and plant water use

Soil GWC decreased over time ($P < 0.001$) in the drought treatment, while it was maintained in the control treatment (Figs 2, S3). Soil type had a significant effect on the drying rate ($P = 0.001$ for the soil type × time × treatment interaction), with the fastest drying rates in the rocky sandy soil and the slowest drying rates in the sandy clay loam (Fig. S4).

Ψ_{pd} also decreased over time in the drought treatment while it was maintained in control pots ($P < 0.001$, for the treatment effect; Fig. 2). The impact of the drought treatments on GWC was observed rapidly in the different soils, but differences in Ψ_{pd} ($P > 0.15$ for soil type and its interactions) started to decline only 20 d after the last watering event (Fig. S4). Similarly, plants in the drought treatment had more negative Ψ_{md} and smaller $\Delta\Psi$ than plants in the control treatment ($P = 0.04$ and 0.001 for Ψ_{md} and $\Delta\Psi$, respectively), but with no significant difference between soil types (Fig. S5). Plants in the control treatment had higher g_s than plants in the drought treatment ($P < 0.001$) (Fig. 2) and did not show any difference in g_s between soil types (not shown). The deliberate reduction in VPD promoted by bagging the plants overnight before the first two sampling dates successfully increased g_s, but did not affect Ψ_{pd} (not shown).

Plant water content was not sensitive to the drought treatment. Both control and drought plants showed a progressive decrease in root and stem water content (relative to total weight) over the experiment, with no significant difference between treatments (Fig. 2). Roots always had significantly higher water content than stems. Overall, the drought treatment had a significant influence on the plant water status only for the last two sampling campaigns (i.e. when Ψ_{pd} fell below −1 MPa), coinciding with significantly lower leaf g_s and Ψ_{pd} despite similar root and stem water contents, compared with the control plants.

Manipulation effects on the isotopic composition of water pools

The isotopic composition of soil water was always equal to or more enriched than (i.e. had higher δ^{18}O and δ^2H) irrigation

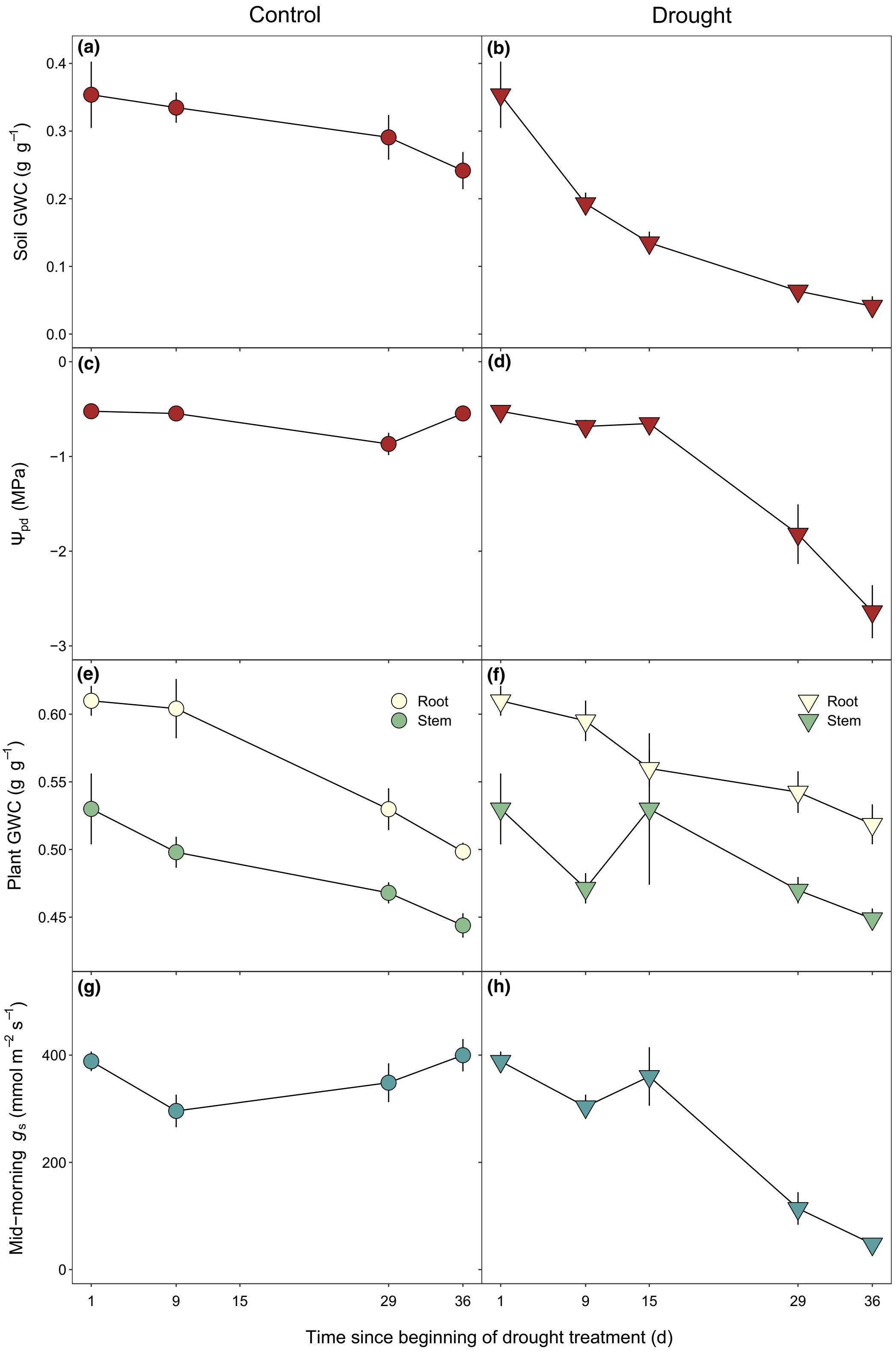

Fig. 2 Time course of soil and *Fagus sylvatica* plant water status over the experiment. (a–h) Mean soil gravimetric water content (GWC) (a, b), leaf predawn water potential (Ψ_{pd}) (c, d), plant water content (roots and stems) (e, f) and stomatal conductance (g_s) (g, h) over the course of the experiment in control (left panels) and drought (right panels) treatments. Error bars are the SE of the mean ($n = 30$ and 15 for soil GWC and 15 and 9 for Ψ_{pd}, plant GWC and g_s for drought and control, respectively).

water, even in the (regularly irrigated) control treatment (Figs 3, 4, S6). This isotopic enrichment above irrigation water of soil water in the control treatment was stronger when the time between the last irrigation and the date of sampling was longer, and comparable to the enrichment in the drought treatment during the first three campaigns. In the drought treatment, and despite our attempt to prevent soil evaporation, the δ^{18}O of soil water increased over time, especially over the last two sampling campaigns (Fig. 3). By contrast, soil water δ^2H did not follow a progressive enrichment as the soil dried, as is expected from soil evaporative enrichment theory (Barnes & Allison, 1983). These patterns were visible and reproducible amongst all soil types (Fig. S7).

Root and stem water δ^{18}O broadly reflected the δ^{18}O of the corresponding soil water (Fig. 3). By contrast, root and stem water δ^2H from the control treatment was always more depleted than soil water δ^2H ($P < 0.001$ and $P < 0.01$; Fig. 3). A similar pattern was also visible in the drought treatment for the first three sampling campaigns (i.e. until the drought treatment had a significant influence on the plant water status; see 'Manipulation effects on soil water content and plant water use' in the Materials and Methods section). However, for the last two campaigns, root and stem water δ^2H started to increase and became more enriched than soil water δ^2H (Fig. 3). No significant difference was found between root and stem water δ^2H.

Differences in the isotopic compositions of stem, root and soil water described earlier were not affected by soil type (Fig. S7). However, water extracted from limestone rocks was more enriched than soil water in response to drought in both δ^{18}O

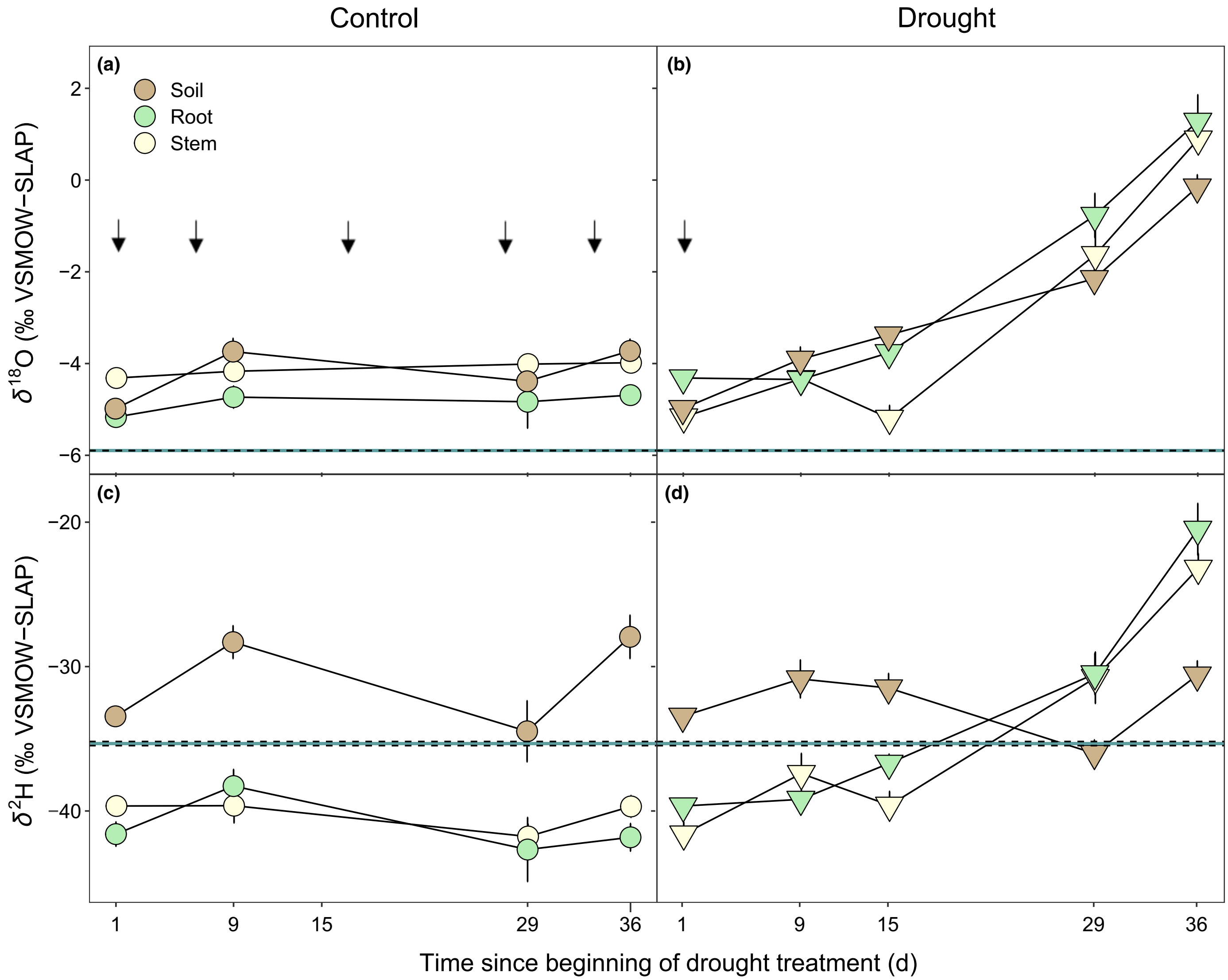

Fig. 3 Time course of soil and *Fagus sylvatica* plant water isotopic composition over the experiment. (a–d) Mean δ^2H and δ^{18}O of soil, stem and root water over the course of the experiment in control (a, c) and drought (b, d) treatments. Error bars are the SE of the mean ($n = 15$ and 9 for drought and control, respectively) and can be masked by the symbol when too small. The solid teal line corresponds to the mean of the isotopic composition of the irrigation water and the dashed lines correspond to its SE. Vertical arrows in the upper panels indicate irrigation times (for both upper and lower panels).

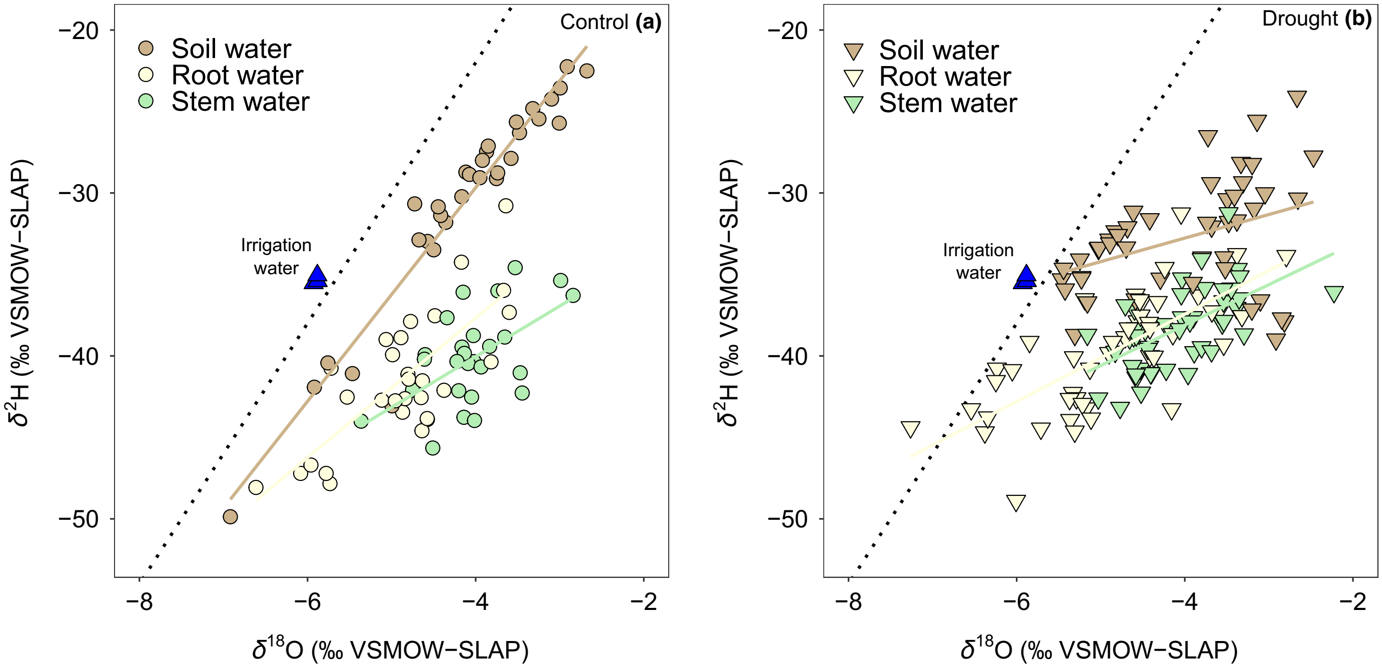

Fig. 4 Dual-isotope representation (δ^{12}H and δ^{18}O) of soil, stem and root water in *Fagus sylvatica* potted plants. (a) Control treatment; (b) drought treatment. Blue triangles indicate the isotopic composition of irrigation water during the experiment and the dotted line represents the local meteoric water line.

($P < 0.05$) and δ^2H ($P < 0.001$) but this did not affect the isotopic composition of plant and soil water pools (Fig. S7).

Isotopic offsets between plant and soil water pools

Although soil, root and stem water δ^{18}O and δ^2H behaved differently upon drying, a strong correlation between δ^{18}O and δ^2H soil–plant offsets (Δ^{18}O and Δ^2H) for both roots and stems was observed (Fig. 5). The slope of the orthogonal distance linear regression (which accounts for errors on both axes) between Δ^{18}O and Δ^2H was 7.9 ± 0.7 and 7.2 ± 0.8 for soil–stem and soil–root offsets, respectively.

The δ^2H soil–stem water offset (Δ^2H) was significantly different from zero ($P < 0.001$) for control plants, with a mean value of 10.6 ± 3.05‰, indicating that stem water was significantly more depleted than soil water (Fig. S8). In the drought treatment, Δ^2H shifted from positive to negative values over time ($P < 0.001$; Fig. S8), indicating that stem water became significantly more enriched in ^{2}H than the corresponding soil water. This shift occurred only when soil water content was below the permanent wilting point, and when the drought treatment started to have significant effects on leaf g_s and Ψ_{pd} (Fig. 2). The δ^{18}O soil–stem water offset (Δ^{18}O) was not significantly different from zero in both treatments (Fig. S8a,c), although Δ^{18}O covaried with Δ^2H (Fig. 4). Therefore, stem δ^{18}O reflected soil water δ^{18}O. Because root and stem water did not differ significantly in their isotopic composition, soil–root isotopic offsets followed similar patterns as soil–stem Δ^2H and Δ^{18}O (Fig. S8b,d). The effect of θ_{rel} was significant and negative for Δ^{18}O, but negligible for Δ^2H (Fig. 6). For both Δ^{18}O and Δ^2H, we found positive effects of Ψ_{md} and $\Delta\Psi$ (Table 1). Leaf $\Delta\Psi$ was the variable that explained the largest part of the variance in Δ^{18}O and Δ^2H. The larger the leaf $\Delta\Psi$, the larger the soil–stem isotopic offsets (Fig. 7; Table 1). Finally, pots exposed to a low-VPD treatment had significantly lower soil–stem Δ^2H than ambient VPD plants ($P < 0.05$), but not significantly different Δ^{18}O (Fig. S9), albeit they tend to have larger g_s (Fig. S10). Plant water content, either in roots or in stems, did not explain the isotopic differences between treatments.

Discussion

Our results from a controlled experiment with potted *F. sylvatica* saplings revealed that hydrogen isotope offsets between soil and plant water pools (Δ^2H) are consistent over a range of soil types but highly dependent on plant water status. As long as soil water remained above the permanent wilting point, stem and root water were significantly more depleted in ^{2}H than their corresponding source water (Figs 3, S5), leading to a soil–stem isotopic offset in Δ^2H of a similar magnitude to those observed in the field for adult *F. sylvatica* and *Quercus robur* trees (Goldsmith *et al.*, 2018; Barbeta *et al.*, 2019) and a number of other species (Lin & Sternberg, 1993; Ellsworth & Williams, 2007; Brooks *et al.*, 2010; Zhao *et al.*, 2016; Evaristo *et al.*, 2017; Brum *et al.*, 2018; Oerter & Bowen, 2019). The reproducibility of this offset in potted and irrigated *F. sylvatica* saplings demonstrates that soil–plant isotopic offset is not restricted to halophytes (Lin & Sternberg, 1993; Ellsworth & Williams, 2007; Eley *et al.*, 2014; Redelstein *et al.*, 2018) or xerophytes (Ellsworth & Williams, 2007; Zhao *et al.*, 2016) but is more general and can also occur

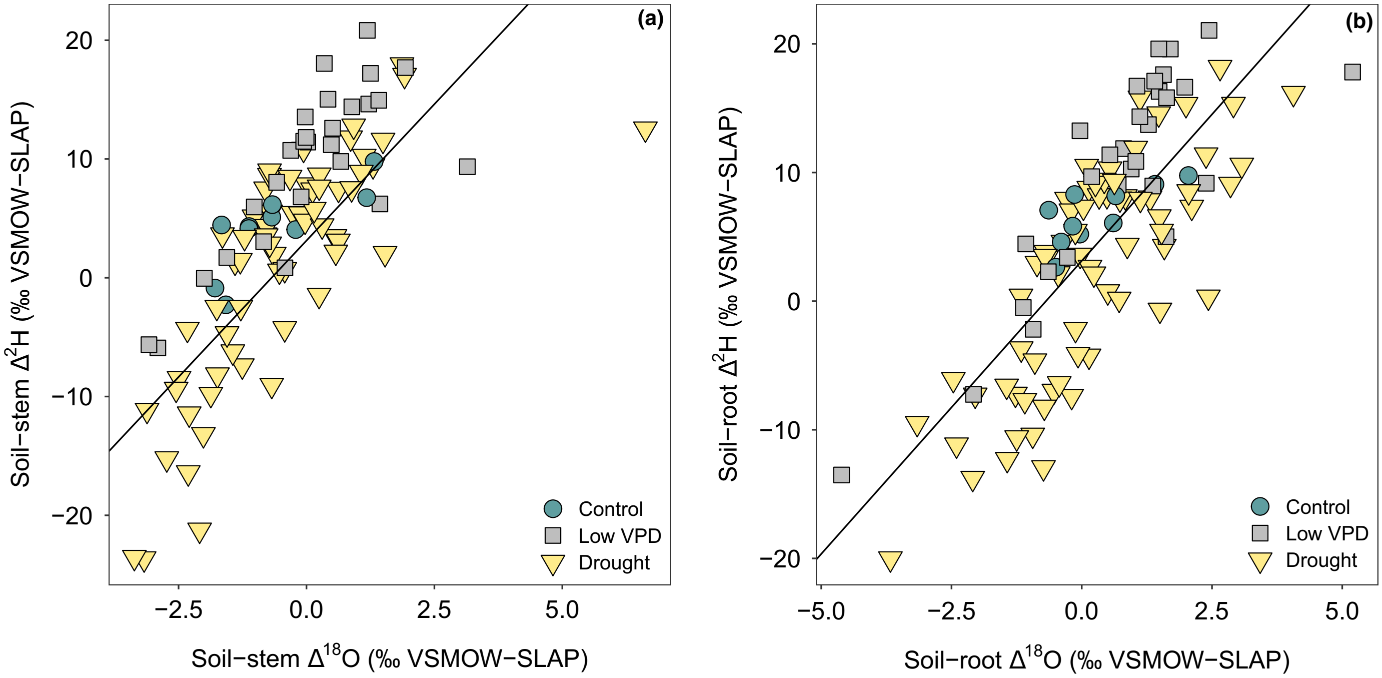

Fig. 5 Correlations between soil–plant $\delta^{18}O$ and $\delta^{2}H$ offsets in *Fagus sylvatica* potted plants. (a) Soil–stem offsets; (b) soil–root offsets. Solid black lines correspond to the orthogonal regression between $\delta^{18}O$ and $\delta^{2}H$ offsets.

in temperate forests. It further suggests that, in the field, soil–plant $\delta^{2}H$ offsets cannot be solely attributed to a missing water source (Oerter & Bowen, 2019; Oerter *et al.*, 2019). Our results showing that the isotope offset can be cancelled or even reversed when Ψ_{pd} drops below −1 MPa in *F. sylvatica* may also explain why some field studies do not observe such an offset, especially in semiarid sites (e.g. Grossiord *et al.*, 2017) or in temperate sites during the dry season (e.g. Bariac *et al.*, 1990). The long-standing principle that there is no isotopic fractionation during root water uptake requires reconsideration, at least for $\delta^{2}H$. Meanwhile, oxygen isotope offsets ($\Delta^{18}O$) between soil and plant water were also present and proportional to $\Delta^{2}H$ (Fig. 5), although not always significant (Fig. S5).

Vargas *et al.* (2017) performed a similar experiment on potted *Persea americana* plants and found soil–stem isotopic offsets that were comparable to those reported here. Their soil–stem $\Delta^{2}H$ and $\Delta^{18}O$ showed a linear relationship with a slope of 10.6 ± 3.8, that is, in the same range as the ones reported here (7.9 ± 0.7 for soil–stem offsets). However, because they explored a narrower range of soil water availability, they did not detect the sign inversion in both $\Delta^{18}O$ and $\Delta^{2}H$ as found here when Ψ_{pd} fell below −1 MPa (Fig. S5). Vargas *et al.* (2017) explained the observed soil–stem isotopic offset by a putative isotope fractionation process during root water uptake (see the Introduction). However, our results from the control treatment do not support this hypothesis, as theoretically this would result in an enrichment of soil water whilst stem water would reflect irrigation water (Fig. 1a; see the theoretical framework in Supporting Information Notes S1). By contrast, we found a strong depletion of stem water $\delta^{2}H$ compared with irrigation water in the control treatments that cannot be explained by root discrimination and/or soil evaporation (see Supporting Information Notes S1). A more likely explanation for the isotopic depletion of bulk stem water compared with soil water in our experiment (and that of Vargas *et al.*) is that storage water in the xylem tissue is depleted compared with vessel water (i.e. sap) (Fig. 1b).

Such isotopic offsets between bulk stem water and vessel (sap) water have been reported in woody plants (Zhao *et al.*, 2016). In addition, large isotopic differences between leaf water pools from the multiple epidermis (storage tissues) (White *et al.*, 1985) and the spongy parenchyma (photosynthetic tissues) of the CAM plant *Peperomia congesta* (HBK) have also been documented (Yakir *et al.*, 1994). The isotopic difference between storage and photosynthetic tissues was comparable to the isotopic offsets reported here between soil and stem water (i.e. > 10‰). Interestingly this isotopic difference was maintained only under turgid conditions and vanished under water limitations (Yakir *et al.*, 1994). This is coherent with our findings that the isotopic offsets between soil and stem water vanish around the permanent wilting point (Fig. 6). However this would imply that, under low transpiration, the mixing of the storage and vessel water in the stem becomes more pronounced, resulting in a lower fractionation between the two water pools (ε_x in Fig. 1b). Such a reduction of ε_x under water limitations would need to be tested using techniques that allow isotopic determination of vessel and stem tissue water separately. However, a reduction in ε_x cannot explain why stem water becomes more enriched than soil water below the permanent wilting point (Fig. 6). A plausible explanation for this pattern is that, when water is limited, stem evaporation (E_x) enriches stem water above the values of soil water, because the transpiration stream cannot replenish the stem tissue at a fast enough rate (Martín-Gómez *et al.*, 2017).

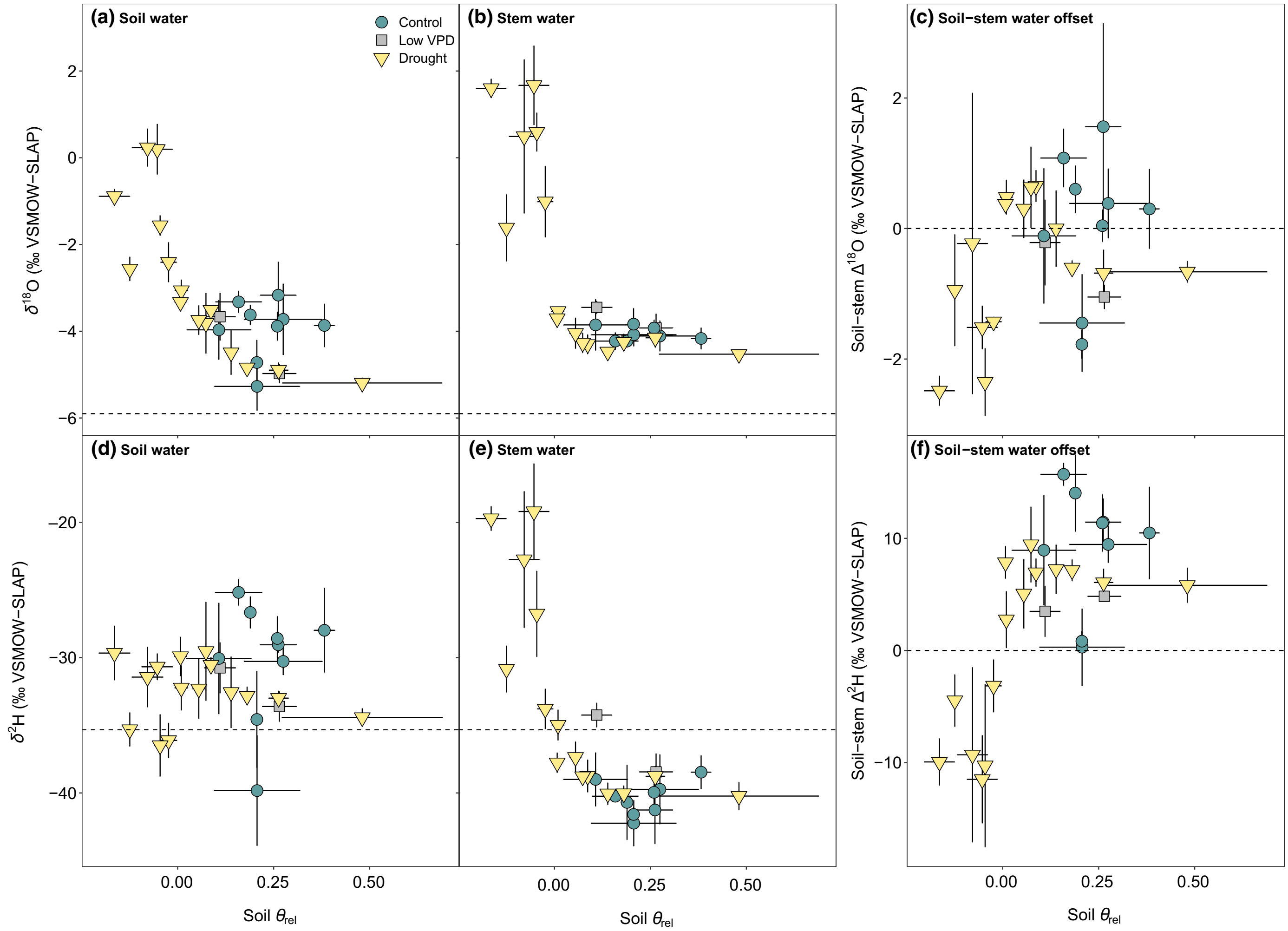

Fig. 6 Effect of soil moisture on soil and *Fagus sylvatica* plant water isotopic compositions. Relationships between soil volumetric water content (VWC) relative to VWC at the permanent wilting point (θ_{rel}) and soil water δ^2H and δ^{18}O (a, d), stem water δ^2H and δ^{18}O (b, e) and the soil–stem isotopic offsets Δ^{18}O and Δ^2H (c, f). Data were averaged by sampling date and irrigation and vapour pressure deficit (VPD) treatments. Error bars are SE of the mean, and the dashed line indicates the isotopic composition of irrigation water (a, b, d, e) or the zero (c, f).

Table 1 Output of the stepwise regression models for the soil–plant isotopic offsets in *Fagus sylvatica*.

		Estimate	SE	*t*-value	*P*-value	R^2
Δ^{18}O	Intercept	0.12	0.43	0.76	0.79	
	θ_{rel}	−2.59	1.01	−2.56	0.01	0.08
	Ψ_{md}	0.81	0.19	4.32	<0.0001	0.19
	$\Delta\Psi$	1.57	0.28	5.60	<0.0001	0.24
	Model R^2					0.36
Δ^2H	Intercept	6.36	2.27	2.81	<0.01	
	θ_{rel}	3.87	5.30	0.73	0.47	0.007
	Ψ_{md}	5.91	0.98	6.05	<0.0001	0.32
	$\Delta\Psi$	9.6	1.47	6.55	<0.0001	0.36
	Model R^2					0.55

Effects include volumetric water content (VWC) relative to VWC at the permanent wilting point (θ_{rel}), leaf midday water potential (Ψ_{md}) and the daily difference between predawn (Ψ_{pd}) and midday water potential ($\Delta\Psi$). SE, SE of the mean.

It remains to be explained why, in the drought treatment, soil water δ^{18}O increases continuously while its δ^2H counterpart remains constant or even decreases once permanent wilting point is reached (Fig. 3). A very similar pattern had already been observed on wheat and sunflower, and had been interpreted as a possible effect of plant organic matter decomposition (Allison *et al.*, 1984). Vargas *et al.* (2017) rejected this idea on the basis that, in their experiment, potted cut stems (that had decaying roots and no transpiring canopy) did not produce any depletion in soil water δ^2H. However, soil water in pots with live plants was not depleted in their experiment either and, as mentioned earlier, they did not explore the full range of water potentials that were explored in this study or in Allison *et al.* (1983). Our data demonstrated that only once the permanent wilting point had been reached and Ψ_{pd} dropped below −1 MPa, did soil water δ^{18}O and δ^2H start to exhibit clear opposite trends. Thus, we hypothesize that Vargas *et al.* did not observe the same trends as

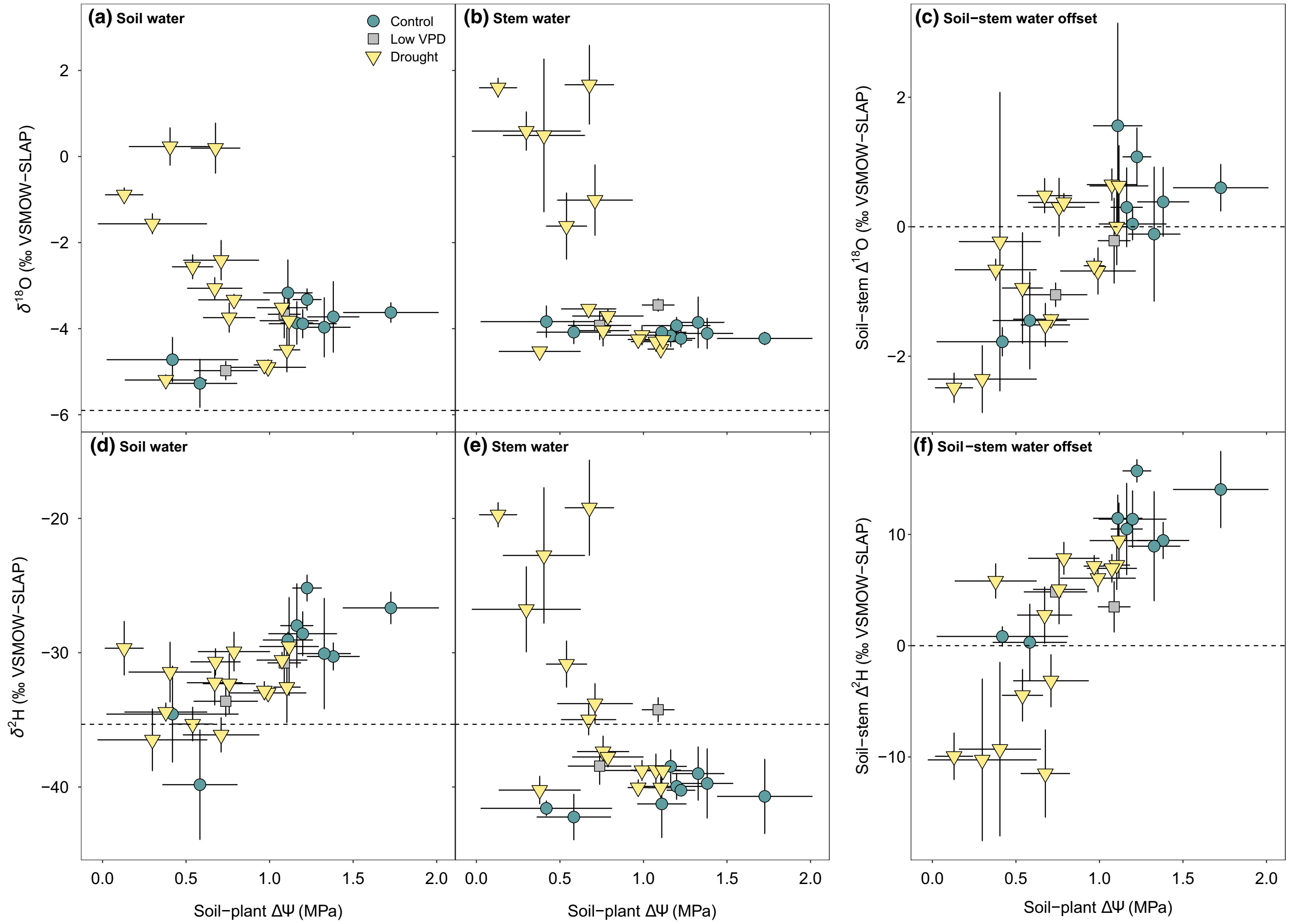

Fig. 7 Effect of *Fagus sylvatica* plant ΔΨ (daily difference between predawn (Ψ_{pd}) and midday leaf water potential (Ψ_{md})) on water isotopic compositions. (a–f) Relationships between plant ΔΨ and soil water δ^2H and δ^{18}O (a, d), stem water δ^2H and δ^{18}O (b, e) and soil–stem isotopic offsets Δ^{18}O and Δ^2H (c, f). Data were averaged by sampling date, and irrigation and vapour pressure deficit (VPD) treatments. Error bars are SE of the mean, and the dashed line indicates the isotopic composition of irrigation water (a, b, d, e) or the zero (c, f).

here because the drought treatment they applied was too mild. In addition, we propose an alternative explanation to that of Allison *et al.* (1983) and suggest that this pattern in soil water isotopes under dry conditions results from surface isotope effects. Indeed, as soil dries, adsorbed water becomes an increasingly larger fraction (f_a) of total soil water (Tuller & Or, 2005; Chen *et al.*, 2016; Lu, 2016). In the two last sampling campaigns of our experiment, soil GWC was $< 0.1\ \mathrm{g\,g^{-1}}$ (11% VWC) in the drought treatment (Fig. 2). According to Lu (2016), adsorbed water can range from 1.7% VWC in sandy soils to 12.8% VWC in silty clay soils. It is thus reasonable to assume that the isotopic fractionation associated with adsorbed water can dominate the isotopic composition of dry soils. Meanwhile, under sustained drought, the remaining bulk soil water would still become progressively enriched because of soil evaporation (E_s). Depending on the balance between the enrichment caused by evaporation and the depletion caused by the higher fraction of adsorbed water, the isotopic composition of bulk soil water could show different trends during drying periods, either positive or negative. Because soil evaporative enrichment creates a relatively stronger enrichment in ^{18}O than in ^{2}H (i.e. the slope of the evaporation line in the dual-isotope space is lower than the slope of the meteoric water line) and surface isotope effects are much stronger for ^{2}H than for ^{18}O (Chen *et al.*, 2016; Lin *et al.*, 2018), it is plausible that soil water δ^{18}O enriches while soil water δ^2H becomes depleted, at least when the soil water balance is dominated by root water uptake. In the field, this opposing trend between soil water δ^{18}O and δ^2H may be harder to observe as capillary rise may compensate water losses, minimizing the influence of adsorbed water, and the depletion of soil water above the evaporation front may be dominated by the back diffusion of (depleted) atmospheric vapour into the soil (Barnes & Allison, 1983).

In conclusion, we propose the following explanation for the dynamics of soil–plant isotopic offsets reported here and in other studies. This is the most plausible explanation, but it is still untested in a qualitative sense. Plants take up mobile and capillary soil water during transpiration. In wet conditions (control treatment), this soil water pool constitutes a large fraction of bulk soil water with an isotopic composition (δ_m) close to that of

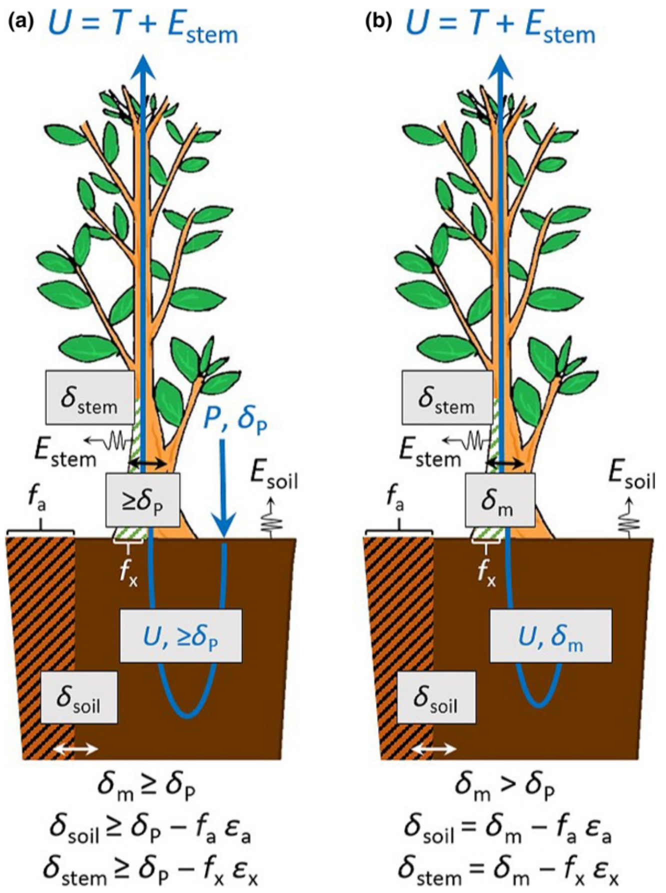

Fig. 8 Illustration of the proposed effects on the isotopic composition (δ) of bulk soil (δ_{soil}) and stem (δ_{stem}) water in the present experiment, where soil (E_{soil}) and stem (E_{stem}) evaporation are not fully suppressed. (a) Control treatment with regular irrigation (P with constant isotopic composition, δ_P) compensating water losses through E_{soil} and root water uptake (U, further lost via transpiration T and E_{stem}). Here, the isotopic composition of soil mobile and capillary water (δ_m) and water inside the xylem vessels are expected to reflect δ_P with a possible enrichment as a result of soil evaporation occurring between irrigations events ($\geq \delta_P$), while soil capillary water (fraction f_a) and stem storage tissue water (fraction f_x) are depleted with respect to mobile soil water (fractionation ε_a) and to water inside the xylem conduits (fractionation ε_x). (b) Drought treatment with water losses via T, E_{soil} and E_{stem} not being compensated with P. Here, δ_m becomes progressively more enriched (as a result of soil evaporative enrichment) while the fraction of soil capillary water (f_a) increases. The resulting δ_{soil} becomes either more enriched (following δ_m) or more depleted (following f_a), depending on the balance between the two processes. Meanwhile, δ_{stem} becomes progressively more enriched, following δ_m because f_x varies proportionally less than f_a along the experiment (Fig. 2). Blue arrows, irrigation–soil–plant–atmosphere water flux; white arrows, exchanges between soil water pools; black arrows, between plant internal water pools.

irrigation water. However, bulk stem water is depleted compared with mobile and capillary soil water (Zhao *et al.*, 2016) because it comprises a mix of vessel water that reflects mobile/capillary soil water, with storage water that is depleted compared with vessel water (Fig. 8a). The origin of this depletion of storage water in the stem is unknown, but could be related to surface processes on plant organic surfaces (Chen *et al.*, 2016). By contrast, during dry conditions (drought treatment), adsorbed water represents an increasingly larger fraction of bulk soil water, creating a significant depletion of bulk soil water compared with mobile/capillary water, and thus compared with vessel water in a transpiring plant. Bulk stem water remains depleted compared with vessel water but, as plant transpiration becomes strongly reduced under prolonged drought, E_x increasingly enriches bulk stem water above the composition of soil mobile/capillary water (δ_m) (Fig. 8b). Our findings that the isotopic offsets between soil and stem water increase with plant transpiration proxies, such as the diurnal amplitude of stem water potential $\Delta\Psi$ (Figs 7, S11) or g_s (Fig. S10), indicate that soil–stem isotopic offsets also reflect the competition between transpiration and stem evaporation (Martín-Gómez *et al.*, 2017) and the matric potential of soil and plant water pools (Gaj & McDonnell, 2019).

Acknowledgements

Many thanks to Steven Wohl, Nicolas Devert, Céline Gire, Lionel Jordan-Meille and the staff at Bordeaux Science Agro for technical support, as well as Nicolas Cornette, Yann Cochet and Kenza Bakouri for assistance in the glasshouse. This work was supported by the French national programme EC2CO-Biohefect (RootWater), the French national research agency (projects Hydrobeech, Climbeech and Micromic within the Cluster of Excellence COTE with grant agreement ANR-10-LABX-45; project ORCA with grant agreement ANR-13-BS06-0005-01), the European Research Council (ERC) under the EU Seventh Framework Program (FP7/2007-2013, with grant agreement no. 338264, awarded to LW) and the Aquitaine Region (project Athene with grant agreement 2016-1R20301-00007218). AB also acknowledges an IdEx Bordeaux postdoctoral fellowship from the Université de Bordeaux (contract no. 22001162).

Author contributions

AB, TEG, LW and JO designed the study; TEG, LC, AB and CD grew plants, applied irrigation treatments and measured soil and plant parameters; AB, BF, SPJ, LW and JO performed stable isotope analyses; and AB and JO wrote the manuscript with contributions from all authors.

ORCID

Adrià Barbeta https://orcid.org/0000-0002-8357-1719
Camille Delvigne https://orcid.org/0000-0003-0013-2022
Teresa E. Gimeno https://orcid.org/0000-0002-1707-9291
Sam P. Jones https://orcid.org/0000-0002-6815-1601
Jérôme Ogée https://orcid.org/0000-0002-3365-8584
Lisa Wingate https://orcid.org/0000-0003-1921-1556

References

Akaike H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19**: 716–723.

Allison GB, Barnes CJ, Hugues MW, Leaney FWJ. 1984. Effect of climate and vegetation on oxygen-18 and deuterium profiles in soils. In: *Isotope hydrology, 1983 : proceedings of an International Symposium on Isotope Hydrology in Water Resources Development*. Vienna, Austria: International Atomic Energy Agency, 105–123.

Barbeta A, Jones SP, Clavé L, Wingate L, Gimeno TE, Fréjaville B, Wohl S, Ogée J. 2019. Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest. *Hydrology and Earth System Sciences* **23**: 1–31.

Barbeta A, Mejía-Chang M, Ogaya R, Voltas J, Dawson TE, Peñuelas J. 2015. The combined effects of a long-term experimental drought and an extreme drought on the use of plant-water sources in a Mediterranean forest. *Global Change Biology* **21**: 1213–1225.

Barbeta A, Peñuelas J. 2017. Relative contribution of groundwater to plant transpiration estimated with stable isotopes. *Scientific Reports* **7**: 1–10.

Bariac T, Jusserand C, Mariotti A. 1990. Evolution de la composition isotopique de l'eau (^{18}O) dans le continuum sol-plante-atmosphere. *Catena* **54**: 413–424.

Barnes CJ, Allison GB. 1983. The distribution of deuterium and ^{18}O in dry soils. *Journal of Hydrology* **60**: 141–156.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**.

Bertrand G, Masini J, Goldscheider N, Meeks J, Lavastre V, Celle-Jeanton H, Gobat JM, Hunkeler D. 2014. Determination of spatiotemporal variability of tree water uptake using stable isotopes (δ^{18}O, δ^2H) in an alluvial system supplied by a high-altitude watershed, Pfyn forest, Switzerland. *Ecohydrology* **7**: 319–333.

Bowling DR, Schulze ES, Hall SJ. 2017. Revisiting streamside trees that do not use stream water: can the two water worlds hypothesis and snowpack isotopic effects explain a missing water source? *Ecohydrology* **10**: 1–12.

Brian Leen J, Berman ESF, Liebson L, Gupta M. 2012. Spectral contaminant identifier for off-axis integrated cavity output spectroscopy measurements of liquid water isotopes. *Review of Scientific Instruments* **83**: 044305.

Brooks JR, Barnard HR, Coulombe R, McDonnell JJ, Renée Brooks J, Barnard HR, Coulombe R, McDonnell JJ. 2010. Ecohydrologic separation of water between trees and streams in a Mediterranean climate. *Nature Geoscience* **3**: 100–104.

Brum M, Vadeboncoeur MA, Ivanov V, Asbjornsen H, Saleska S, Alves LF, Penha D, Dias JD, Aragão LEOC, Barros F *et al.* 2018. Hydrological niche segregation defines forest structure and drought tolerance strategies in a seasonal Amazon forest. *Journal of Ecology*. **107**: 318–333.

Chen G, Auerswald K, Schnyder H. 2016. ^{2}H and ^{18}O depletion of water close to organic surfaces. *Biogeosciences* **13**: 3175–3186.

Cordano E, Andreis D, Zottele F. 2017. *Soilwater: implementation of parametric formulas for soil water retention or conductivity curve. R package v.1.0.5.* https://CRAN.Rproject.org/package=soilwater [accessed 3 September 2018]

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

De Deurwaerder H, Hervé-Fernández P, Stahl C, Burban B, Petronelli P, Hoffman B, Bonal D, Boeckx P, Verbeeck H. 2018. Liana and tree below-ground water competition—evidence for water resource partitioning during the dry season. *Tree Physiology* **38**: 1071–1083.

Eley Y, Dawson L, Black S, Andrews J, Pedentchouk N. 2014. Understanding ^{2}H/^{1}H systematics of leaf wax *n*-alkanes in coastal plants at Stiffkey saltmarsh, Norfolk, UK. *Geochimica et Cosmochimica Acta* **128**: 13–28.

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

Evaristo J, McDonnell JJ, Clemens J. 2017. Plant source water apportionment using stable isotopes: a comparison of simple linear, two-compartment mixing model approaches. *Hydrological Processes* **31**: 3750–3758.

Gaj M, McDonnell JJ. 2019. Possible soil tension controls on the isotopic equilibrium fractionation factor for evaporation from soil. *Hydrological Processes* **33**: 1629–1634.

Geris J, Tetzlaff D, McDonnell JJ, Soulsby C. 2017. Spatial and temporal patterns of soil water storage and vegetation water use in humid northern catchments. *Science of the Total Environment* **595**: 486–493.

Goldsmith GR, Allen ST, Braun S, Engbersen N, Romero González-Quijano C, Kirchner JW, Siegwolf RTW. 2018. Spatial variation in throughfall, soil, and plant water isotopes in a temperate forest. *Ecohydrology* **12**: e2059.

Grossiord C, Sevanto S, Dawson TE, Adams HD, Collins AD, Dickman LT, Newman BD, Stockton EA, McDowell NG. 2017. Warming combined with more extreme precipitation regimes modifies the water sources used by trees. *New Phytologist* **213**: 584–596.

Jasechko S, Sharp ZD, Gibson JJ, Birks SJ, Yi Y, Fawcett PJ. 2013. Terrestrial water fluxes dominated by transpiration. *Nature* **496**: 347–350.

Jones SP, Ogée J, Sauze J, Wohl S, Saavedra N, Fernández-Prado N, Maire J, Launois T, Bosc A, Wingate L. 2017. Non-destructive estimates of soil carbonic anhydrase activity and associated soil water oxygen isotope composition. *Hydrology and Earth System Sciences* **21**: 6363–6377.

Lin G, Sternberg L SL. 1993. Hydrogen isotopic fractionation by plant roots during water uptake in coastal wetland plants. In: Ehleringer J, Hall A, Farquhar G, eds. *Stable isotopes and plant carbon-water relations*. New York, NY, USA: Academic Press, 497–510.

Lin Y, Horita J. 2016. An experimental study on isotope fractionation in a mesoporous silica-water system with implications for vadose-zone hydrology. *Geochimica et Cosmochimica Acta* **184**: 257–271.

Lin Y, Horita J, Abe O. 2018. Adsorption isotope effects of water on mesoporous silica and alumina with implications for the land-vegetation-atmosphere system. *Geochimica et Cosmochimica Acta* **223**: 520–536.

Lu N. 2016. Generalized soil water retention equation for adsorption and capillarity. *Journal of Geotechnical and Geoenvironmental Engineering* **142**. doi: 10.1061/(ASCE)GT.1943-5606.0001524

Mahindawansha A, Orlowski N, Kraft P, Rothfuss Y, Racela H, Breuer L. 2018. Quantification of plant water uptake by water stable isotopes in rice paddy systems. *Plant and Soil* **429**: 281–302.

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 Phytologist* **207**: 914–927.

Martín-Gómez P, Serrano L, Ferrio JP. 2017. Short-term dynamics of evaporative enrichment of xylem water in woody stems: implications for ecohydrology. *Tree Physiology* **37**: 511–522.

Millar C, Pratt D, Schneider D, McDonnell JJ. 2018. A comparison of extraction systems for plant water stable isotope analysis. *Rapid Communications in Mass Spectometry* **32**: 1031–1044.

Moreno-Gutiérrez C, Dawson TE, Nicolás E, Querejeta JI. 2012. Isotopes reveal contrasting water use strategies among coexisting plant species in a Mediterranean ecosystem. *New Phytologist* **196**: 489–496.

Munksgaard NC, Cheesman AW, Wurster CM, Cernusak LA, Bird MI. 2014. Microwave extraction-isotope ratio infrared spectroscopy (ME-IRIS): a novel technique for rapid extraction and in-line analysis of δ^{18}O and δ^2H values of water in plants, soils and insects. *Rapid Communications in Mass Spectrometry* **28**: 2151–2161.

Newberry SL, Nelson DB, Kahmen A. 2017. Cryogenic vacuum artifacts do not affect plant water-uptake studies using stable isotope analysis. *Ecohydrology* **10**: e1892.

Oerter EJ, Bowen G. 2017. In situ monitoring of H and O stable isotopes in soil water reveals ecohydrologic dynamics in managed soil systems. *Ecohydrology* **10**: e1841.

Oerter EJ, Bowen GJ. 2019. Spatio-temporal heterogeneity in soil water stable isotopic composition and its ecohydrologic implications in semiarid ecosystems. *Hydrological Processes* **33**: 1724–1738.

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. *Journal of Hydrology* **515**: 1–9.

Oerter EJ, Siebert G, Bowling DR, Bowen G. 2019. Soil water vapour isotopes identify missing water source for streamside trees. *Ecohydrology* **12**: e2083.

Orlowski N, Breuer L, Angeli N, Boeckx P, Brumbt C, Cook CS, Dubbert M, Dyckmans J, Gallagher B, Gralher B *et al.* 2018. Inter-laboratory comparison of cryogenic water extraction systems for stable isotope analysis of soil water. *Hydrology and Earth System Sciences* **22**: 3619–3637.

Orlowski N, Frede H-G, Brüggemann N, Breuer L. 2013. Validation and application of a cryogenic vacuum extraction system for soil and plant water extraction for isotope analysis. *Journal of Sensors and Sensor Systems* **2**: 179–193.

Penna D, Hopp L, Scandellari F, Allen ST, Benettin P, Beyer M, Geris J, Klaus J, Marshall JD, Schwendenmann L *et al.* 2018. Ideas and perspectives: tracing terrestrial ecosystem water fluxes using hydrogen and oxygen stable isotopes – challenges and opportunities from an interdisciplinary perspective. *Biogeosciences* **15**: 6399–6415.

Poca M, Coomans O, Urcelay C, Zeballos SR, Bodé S, Boeckx P. 2019. Isotope fractionation during root water uptake by *Acacia caven* is enhanced by arbuscular mycorrhizas. *Plant and Soil* **441**: 485–497.

R Core Team. 2019. *R: A Language and Environment for Statistical Computing. v.3.6.1.* Vienna, Austria: R Foundation for Statistical Computing. [WWW document] URL https://www.R-project.org [accessed 5 July 2019].

Raulerson SA. 2018. *Toward a diffusive, non-destructive approach to measuring stable isotopes of water within tree stems.* Atlanta, GA, USA: Georgia State University. [WWW document] URL https://scholarworks.gsu.edu/geoscience_theses/115/ [accessed 1 March 2019].

Redelstein R, Coners H, Knohl A, Leuschner C. 2018. Water sources of plant uptake along a salt marsh flooding gradient. *Oecologia* **188**: 607–622.

Schultz NM, Griffis TJ, Lee X, Baker JM. 2011. Identification and correction of spectral contamination in $^{2}H/^{1}H$ and $^{18}O/^{16}O$ measured in leaf, stem, and soil water. *Rapid Communications in Mass Spectrometry* **25**: 3360–3368.

Stumpp C, Brüggemann N, Wingate L. 2018. Stable isotope approaches in vadose zone research. *Vadose Zone Journal* **17**: 1–7.

Thorburn PJ, Walker GR, Brunel J-P. 1993. Extraction of water from Eucalyptus trees for analysis of deuterium and oxygen-18: laboratory and field techniques. *Plant, Cell & Environment* **16**: 269–277.

Tuller M, Or D. 2005. Water films and scaling of soil characteristic curves at low water contents. *Water Resources Research* **41**: 1–6.

Vargas AI, Schaffer B, Yuhong L, Sternberg LSL. 2017. Testing plant use of mobile vs immobile soil water sources using stable isotope experiments. *New Phytologist* **215**: 582–594.

Volkmann THM, Haberer K, Gessler A, Weiler M. 2016. High-resolution isotope measurements resolve rapid ecohydrological dynamics at the soil–plant interface. *New Phytologist* **210**: 839–849.

Walker GR, Woods PH, Allison GB. 1994. Interlaboratory comparison of methods to determine the stable isotope composition of soil water. *Chemical Geology* **111**: 297–306.

Wang J, Fu B, Lu N, Zhang L. 2017. Seasonal variation in water uptake patterns of three plant species based on stable isotopes in the semi-arid Loess Plateau. *Science of the Total Environment* **609**: 27–37.

Washburn EW, Smith ER. 1934. The isotopic fractionation of water by physiological processes. *Science* **79**: 188–189.

Wassenaar LI, Douence C, Altabet MA, Aggarwal PK. 2018. N and O isotope ($\delta^{15}N\alpha$, $\delta^{15}N\beta$, $\delta^{18}O$, $\delta^{17}O$) analyses of dissolved NO_3^- and NO_2^- by the Cd-azide reduction method and N_2O laser spectrometry. *Rapid Communications in Mass Spectrometry.* **32**: 184–194.

Wassenaar LI, Hendry MJ, Chostner VL, Lis GP. 2008. High resolution pore water $\delta^{2}H$ and $\delta^{18}O$ measurements by $H_2O_{(liquid)}$-$H_2O_{(vapor)}$ equilibration laser spectroscopy. *Environmental Science and Technology* **42**: 9262–9267.

White J, Cook E, Lawrence J, Broecker W. 1985. The D/H ratios of sap in trees – implications for water sources and tree-ring D/H ratios. *Geochimica Et Cosmochimica Acta* **49**: 237–246.

Yakir D, Ting I, DeNiro M. 1994. Natural abundance $^{2}H/^{1}H$ ratios of water storage in leaves of *Peperomia congesta* HBK during water stress. *Journal of Plant Physiology* **144**: 607–612.

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 & Environment* **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: proceedings of a symposium.* Vienna, Austria. International Atomic Energy Agency, 576–585.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Effect of soil evaporation on the theoretical isotope ratios of soil and stem water.

Fig. S2 Soil water retention curves.

Fig. S3 Boxplot of the temporal course of soil and plant conditions.

Fig. S4 Boxplot of the temporal course of soil and plant conditions in each soil type.

Fig. S5 Midday leaf water potentials and daily amplitude in leaf water potentials.

Fig. S6 Boxplot of the temporal course of the isotopic composition of soil and plant water.

Fig. S7 Boxplot of the isotopic composition of water pools in the different soils.

Fig. S8 Soil–stem and soil–root isotopic offsets.

Fig. S9 Effect of the low-VPD treatment on soil–stem isotopic offsets.

Fig. S10 Effect of leaf stomatal conductance on soil and stem water isotopic compositions.

Fig. S11 Effect of midday leaf water potential on soil and stem water isotopic compositions.

Notes S1 Sensitivity analysis on the control treatment.