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ORIGINAL ARTICLE

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# Water taken up through the bark is detected in the transpiration stream in intact upper-canopy branches

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## Abstract

Alternative water uptake pathways through leaves and bark complement water supply with interception, fog or dew. Bark water-uptake contributes to embolism-repair, as demonstrated in cut branches. We tested whether bark water-uptake could also contribute to supplement xylem-water for transpiration. We applied bandages injected with $^{2}H$-enriched water on intact upper-canopy branches of *Pinus sylvestris* and *Fagus sylvatica* in a boreal and in a temperate forest, in summer and winter, and monitored transpiration and online isotopic composition ($\delta^{2}H$ and $\delta^{18}O$) of water vapour, before sampling for analyses of $\delta^{2}H$ and $\delta^{18}O$ in tissue waters. Xylem, bark and leaf waters from segments downstream from the bandages were $^{2}H$-enriched whereas $\delta^{18}O$ was similar to controls. Transpiration was positively correlated with $^{2}H$-enrichment. Isotopic compositions of transpiration and xylem water allowed us to calculate isotopic exchange through the bark via vapour exchange, which was negligible in comparison to estimated bark water-uptake, suggesting that water-uptake occurred via liquid phase. Results were consistent across species, forests and seasons, indicating that bark water-uptake may be more ubiquitous than previously considered. We suggest that water taken up through the bark could be incorporated into the transpiration stream, which could imply that sap-flow measurements underestimate transpiration when bark is wet.

**KEYWORDS**
bark, deuterium, drought, European beech, frost, hydrogen, oxygen, Scots pine, water stable isotopes, xylem

## 1 | INTRODUCTION

Vegetation transpiration constitutes the largest flux of water between the atmosphere and the terrestrial biosphere (Jasechko et al., 2013). Transpiration regulates the climate both locally and globally (Ellison et al., 2017; Seneviratne et al., 2010) and is the process that ultimately determines the amount of water available for human consumption (Schlesinger & Jasechko, 2014; Ukkola et al., 2016). Estimates of transpiration under current and future climate scenarios inherently assume that plants take up water from the soil through their roots. This water is then transported via the xylem to the leaves where it evaporates into the atmosphere, mainly through stomata (Sellers et al., 1997). Along this soil-plant-atmosphere continuum, roots would be responsible for the bulk of water absorption, but there exist

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alternative water uptake pathways through leaves and stems. Water uptake through stems or leaves allows plants to bypass soil water uptake and benefit from diverse water inputs, including dew, fog or small precipitation events that wet the canopy, but not the soil (Breshears et al., 2008; Hill et al., 2021). In ecosystems where the vegetation accesses this so-called 'occult precipitation', neglecting leaf or stem water uptake can hinder closure of the water balance in hydrological studies (Binks et al., 2019). In such ecosystems, incorporating water uptake via alternative pathways could help reconcile estimates of transpiration from sap-flux and eddy covariance measurements (Nelson et al., 2020) or avoid overestimations of rooting depth and root water uptake derived from satellite products and modelling approaches (Cabon et al., 2018; Yang et al., 2016).

Water uptake by leaves has been demonstrated for various species across a wide range of biomes (see: Berry et al., 2019; for a review). For example, in water-limited ecosystems, foliar water uptake allows the vegetation to take advantage of dew or canopy interception (Breshears et al., 2008; Hill et al., 2021) and maintain photosynthesis (Coopman et al., 2021; Munne-Bosch et al., 1999). Foliar water uptake has also been measured in tropical biomes including mangroves, montane and lowland forests (Binks et al., 2019; Coopman et al., 2021; Eller et al., 2013; Goldsmith et al., 2013). In contrast, branch water uptake has only been reported in a handful of species and mainly on cut branches or under artificial conditions. Branch water uptake was reported for the first time by Katz et al. (1989) on cut branches of Norway spruce (*Picea abies*). Katz et al. (1989) hypothesized that branch water uptake could complement plant water and nutrient supply. Their results showed that bark water uptake improved branch hydrological status, but they did not find clear evidence to support mineral nutrient uptake through the bark (Katz et al., 1989). More recent studies have confirmed that water uptake through bark increases both branch and leaf water potential, but more importantly, these studies found that bark water uptake can contribute to embolism repair in some conifers (*Sequoia sempervivens*, Earles et al., 2016; and *Picea abies*, Mayr et al., 2014). Lastly, Liu et al. (2019) found that bark water uptake sustained corticular photosynthesis in an angiosperm (*Salix matsudana*). So far, studies on bark water uptake have focused on the physiological significance of this process for two very specific processes (embolism repair or corticular photosynthesis), but none has considered the potential contribution of bark water uptake to the transpiration flux.

Water uptake from sources alternative to the soil has been pointed out as a relevant process that can help maintain transpiration and avoid hydraulic failure in trees. For example, in redwood (*Sequoia sempervirens*), foliar uptake of fog water decreases dehydration and risk of hydraulic failure (Burgess & Dawson, 2004). Foliar water uptake has also been suggested to help resume physiological activity by allowing trees to benefit from small precipitation pulses during drought spells (Dietrich & Kahmen, 2019). Tree branches also intercept fog, dew and precipitation. However, in contrast to leaves, the bark covering these branches is not protected by a hydrophobic cuticle and can therefore store significant amounts of water (indeed mean bark relative water content is approximately 40%, according to Rosell et al., 2014; considering bark as all tissues outside the vascular cambium). Thus, bark water uptake could also help maintain transpiration under high evaporative demand and low soil water availability. For example, bark water uptake could be relevant under very negative xylem water tensions in upper-canopy branches of tall trees or in winter, when low temperatures increase cavitation risk and water viscosity (Ambrose et al., 2009; Olson et al., 2018). Yet, bark water uptake has not been assessed in such scenarios under natural conditions, beyond its contribution to embolism repair.

Here, we aimed to detect and quantify water uptake through the bark in intact upper-canopy branches of two tree species: Scots pine (*Pinus sylvestris*) and European beech (*Fagus sylvatica*). We used measurements of water isotopic composition from different tissues (xylem, bark and leaves) to track the uptake and transport of isotopically enriched water through the bark. We combined analyses of bulk tissue water with online measurements of water vapour isotopic composition to assess the contribution of bark water uptake to transpiration. Our measurement setup allowed the calculation of liquid water uptake through the bark, distinguishing this process from isotopic exchange through the bark in the absence of mass flow (Goldsmith et al., 2017). We expected to find evidence of bark water uptake particularly during periods with high evaporative demand or cold temperatures. We took advantage of measurements of transpiration and bark water uptake during an unusually hot summer in Northern Sweden (July 2018) and again in a later replicated campaign characterized by cooler and moist conditions (September 2018). To test for the impacts of cold temperatures on bark water uptake, we conducted the same experiment in a temperate forest in Northern Spain the following winter, on the same study species (*P. sylvestris*). Finally, species vary greatly both in hydraulic wood traits and bark functional traits (Johnson et al., 2012; Rosell et al., 2014), particularly between angiosperms and gymnosperms (Yang et al., 2022), yet previous studies on bark water uptake have focused exclusively on gymnosperms (with the exception of Liu et al., 2019; performed under artificial laboratory conditions). Here, we tested for bark water uptake in an evergreen conifer (*P. sylvestris*) and in a broadleaved deciduous angiosperm (*Fagus sylvatica*), growing in the same temperate forest in Spain. The aims of these experiments were: (*i*) to detect the uptake of water through the bark on intact upper-canopy branches, (*ii*) to quantify the rate of bark water uptake under contrasting climatic conditions, and (*iii*) to estimate the impact of seasonal climatic variability and plant species identity on bark water uptake.

## 2 | MATERIALS AND METHODS

### 2.1 | Study sites and experimental design

We conducted our measurements in two study sites. The first site was a boreal forest located in Northern Sweden (Rosinedalsheden,

64.33 N, 19.75E, 153 m a.s.l.). This site consists of an approximately 100 year-old, naturally regenerated stand of *P. sylvestris* L. (Scots pine, Stangl et al., 2019). The climate is boreal, with a mean (±SE) annual precipitation of 585 ± 26 mm and a mean annual temperature of 2.84 ± 0.15°C. Winters are cold and long (the area is covered by snow from late October until early May and mean temperature of the coldest month, January, is: −7.7 ± 0.4°C) and summers are cool and wet (mean temperature of the warmest month, July, is: 15.7 ± 0.31°C). The conditions during the summer campaign in this study (July 2018) were unusually hot and dry; indeed, in 2018, the number of summer days and hours of sunshine was the highest on record according to the closest meteorological stations (1989–2019, Vindeln-Sunnansjönäs, 64.14 N, 19.76E, 237 m a.s.l for temperature and 2002–2019, Umeå airport, 63.79 N, 20.29E, 14 m a.s.l for precipitation and others, Swedish Meteorological and Hydrological Institute). The soil is a sandy Podsol with a thin (2–5 cm) layer of organic matter (Hasselquist et al., 2012). We performed two sampling campaigns on this site: one in summer (19–25 July 2018) and the second one in early autumn (3–17 September 2018). In each campaign, we selected 1–3 upper-canopy (16 m) branches (mean diameter: 0.64 ± 0.08 cm) per tree in 3–5 *P. sylvestris* accessible from the top of a scaffolding tower.

The second site was a temperate forest located in the Natural Monument of Monte Santiago in the province of Burgos (Spain). This area falls within the transition between a temperate oceanic and a Mediterranean continental climate. Mean (±SE) annual precipitation is 1028 ± 68.2 mm and mean annual temperature is 9.33 ± 0.23°C. Winters are cold and wet (mean precipitation and temperature of the coldest month, February, are 106 ± 18.4 mm and 3.1 ± 0.5°C, respectively) summers are moderately dry and warm (mean precipitation and temperature of the driest month, July, are 36.8 ± 4.5 mm and 15.5 ± 0.4°C, respectively); these are all according to records from the nearest meteorological station (2001–2013, Puerto de Orduña, 42.956 N. 3.022 W, 900 m a.s.l., Euskalmet). The vegetation is dominated by a mature forest of European beech (*Fagus sylvatica* L.), surrounded by open pastures and scattered plantations of *P. sylvestris*. Persistence of *F. sylvatica* in the study area is presumed to be favoured by recurrent fog episodes of oceanic origin, which would alleviate vapour pressure deficit in the summer afternoons (Barbeta et al., 2019). The soils are shallow, poorly developed, rocky and originated from calcareous rocks (see Fernández-Marín et al., 2015 for further details on the description of the study site). In this area, we selected two sampling locations, one within a *P. sylvestris* plantation and one in the middle of the *F. sylvatica* forest. In each location, we selected five adult individuals accessible with a hydraulic aerial lifting platform mounted on a truck. Mean (±se, $n = 5$) DBH of the selected *P. sylvestris* and *F. sylvatica* trees was 31 ± 3 and 61 ± 6 cm and mean height was 11 ± 1 and 17 ± 2 m, respectively. On this site, we performed two sampling campaigns in two-three consecutive sunny and cloudless days in winter (13–14 February 2019, five individuals of *P. sylvestris*) and in summer (3–5 July 2019, five individuals of *P. sylvestris* and five individuals of *F. sylvatica*).

## 2.2 | Labelling experiment

At the boreal forest in Sweden, on each selected branch (1–3 upper-canopy branches per tree in 3–5 trees), we applied one bandage soaked in $\delta^2H$-enriched water. The bandage consisted of a 10 × 10 cm cotton pad, wrapped around the branch and covered in Parafilm®. On average, the bandages covered a bark surface area of 23 $cm^2$ (calculated for a 10 cm long bandage covering a branch with an average diameter of 0.7 cm). The two ends of the bandage were tightened around the branch and held in place with tape and cable ties (Figure 1). All bandages were injected with 25 mL of isotopically enriched water. Isotopically enriched water consisted of a 8:1000 mix (v:v) of deuterated ($^2H_2O$, 99.9%) and tap water (with a $\delta^2H$ of −90‰), resulting in a $\delta^2H$ of 102 000‰, that is, nearly eight orders of magnitude greater than expected natural abundance. In the summer campaign (19–25 July 2018), bandages were applied on three different dates and branches were collected 1, 4 and 5 days after; in the autumn campaign (3–17 September 2018), branches were collected 14 days after the bandage application and bandages were re-injected with isotopically enriched water 10 days after initial application. In addition to all branches with bandages, we collected one control branch (no bandage) per tree, in the autumn campaign.

At the temperate forest in Spain, on each campaign, we applied two-four bandages (similar to those described above) per tree, in the morning (9–11 AM, local time), in the upper third of the canopy (mean height of branches with bandages: 7.5 ± 0.4 and 9.1 ± 0.7 m, for *P. sylvestris* and *F. sylvatica*, respectively), facing SE-SW. Bandages were injected with a 8:1000 mix (v:v) of deuterated ($^2H_2O$, 99.9%) and mineral water ($\delta^2H$ of −43‰), the resulting $\delta^2H$ of the mix was 102411‰, similar to that applied at the boreal forest in Sweden. Branches with bandages were collected 24 h after application, together with one branch per tree without bandage (control). Mean diameter of labelled branches in the winter campaign (*P. sylvestris*) was 0.87 ± 0.04 cm and in the summer campaign branch diameter was: 1.03 ± 0.04 and 0.89 ± 0.05 cm, for *P. sylvestris* and *F. sylvatica*, respectively.

Branches with bandages were cut together with one branch without bandage of similar characteristics (size and position within the crown) per tree (control). For each branch with bandage collected, we sampled the upstream and downstream segments, cut 3–5 cm away from the edge of the bandage. We defined upstream segments as those in between the main trunk and the bandage and downstream segments as those in between the bandage and the terminal foliage (Figure 1). Upon collection, we measured the length and diameter of the segment beneath the bandage (i.e., directly exposed to the injected enriched water). For control branches, one central segment was collected. For all sampled segments, we carefully peeled off the bark and phloem and collected the xylem into screw-cap glass vials sealed with Parafilm®. In addition, for one branch with bandage per tree, for both the upstream and downstream segments, and for the control branches, we also sampled bark (as peeled off, therefore including

**FIGURE 1** Application of two bandages soaked in $\delta^2$H-enriched water on two upper-canopy branches of *Pinus sylvestris* at the temperate forest in Spain. The cyan and red squares depict the sampled branch segments upstream and downstream from the bandages, respectively.

cambium and phloem) and leaves. All samples were stored in a cool-box until they were transported to the laboratory, where they were stored at −20°C until further analyses.

## 2.3 | Cryogenic water extraction and analyses of liquid water isotopic composition

Water extraction from the xylem, bark and leaves was performed by cryogenic vacuum distillation using a design and methodology proposed by Orlowski et al. (2013), following the methodology detailed in Jones et al. (2017). Briefly, the cryogenic extraction line consisted of six independent lines each with four vacuum extraction sub-lines connected on one end to the glass vials containing the samples and on the other end to U-shaped tubes. At the onset of the extraction, samples in the glass vials were quickly frozen by immersing them into liquid N. The extraction line was then evacuated down to an atmospheric (static) pressure < 1 Pa, and then the U-shape tubes were immersed in liquid nitrogen to create a cold trap. At the same time, samples were immersed in a water bath at ambient temperature. First, the water bath was kept at ambient temperature for 1 h. Next the water bath was gradually heated up to 80°C (within 1 h). Finally, samples remained in the heated bath at 80°C for 1 h. Pressure in the extraction line was continuously monitored with sub-atmospheric 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 was complete. Samples were weighed before and after the extraction and before and after being oven-dried for 24 h at 105°C to assess the water extraction efficiency and to calculate sample water content.

We measured the isotopic composition ($\delta^2$H and $\delta^{18}$O) of the extracted water samples with an off-axis integrated cavity optical spectrometer (TIWA-45EP, Los Gatos Research) coupled to a liquid auto-sampler and vaporiser (LC-xt, PAL systems). All measured values were calibrated using two internal standards and expressed on the VSMOW-SLAP scale (Jones et al., 2017). When analysing water samples extracted from plant tissues with laser-based instruments, the presence of organic compounds (ethanol and methanol mainly) can lead to large isotopic biases (Martín-Gómez et al., 2015). Therefore, we developed a post-correction algorithm to correct for the presence of organic compounds based on the narrowband (for methanol) and broadband (for ethanol) metrics of the absorption spectra (Leen et al., 2012; Schultz et al., 2011). We computed linear (broadband) and exponential (narrowband) relationships between these metrics and the isotopic deviation in water samples of known isotopic composition spiked with incremental quantities of ethanol and ethanol. For each of these contaminated samples, we corrected the isotopic deviation in $\delta^{18}$O and $\delta^2$H corresponding to its broadband and narrowband metrics to build our own post-correction algorithm specific for our instrument. The reliability of these corrections was confirmed in a recent study conducted with the same instrument, by means of analyses of a subset of water samples analysed with this instrument and with an isotopic ratio mass spectrometer (Barbeta et al., 2022).

## 2.4 | Online measurements of gas exchange and water vapour isotopic composition

At the boreal forest in Northern Sweden, during both campaigns (summer and autumn 2018), we measured gas-exchange ($CO_2$ and $H_2O$, although the former was not used here) and online water vapour isotopic composition ($\delta^2$H and $\delta^{18}$O), continuously, on upper-canopy shoots of two (summer 2018) and four (autumn 2018) *P. sylvestris* trees. We used the scaffolding tower (16 m tall) to reach the shoots and secure the equipment. Our measurement system was similar to that described in Stangl et al. (2019) and consisted of a custom-made gas-exchange system (GUS, Wallin et al., 2001) coupled to a cavity ring-down spectrophotometer (CRDS, L2130-I, Picarro Inc.). Briefly, the GUS system consisted of a set of four temperature-controlled, custom-built shoot cuvettes (330 mL each) made of acrylic transparent plastic (Plexiglas). Cuvettes were connected to a multichannel gas-exchange system equipped with infra-red gas analysers (IRGA, CIRAS-1, PP systems Hitchin Herts) to measure $CO_2$ and $H_2O$ partial pressure in the air drawn from the shoot cuvettes and the reference channels. The

polyethylene tubing connecting the cuvettes and the gas analyser was insulated and heated to avoid condensation. The GUS cycled through the four (two) cuvettes and two reference lines, each channel was measured for 7 min and the average of the last 5 min was recorded. A zero calibration and a cross-calibration protocol, to match values in the sample and reference channels, were run every hour. In addition, the IRGAs were calibrated using a dew-point generator (LI-610, Li-Cor) and gas bottles of known [$CO_2$] twice a year. The CRDS was connected to the GUS central line, in parallel to the sample IRGA. The CRDS measured the concentration and isotopic composition ($\delta^2H$ and $\delta^{18}O$) of the water vapour in the air drawn from each branch chamber or reference line. The CRDS was self-calibrated every five hours with the Picarro Standard Delivery Module using two internal standards of known isotopic composition ($\delta^2H$: −94.28‰ and 225‰ and $\delta^{18}O$: −12.87‰ and 12.7‰) analysed on an Isotopic Ratio Mass Spectrometer (IRMS, Thermo-Finnigan LLC). Each standard was measured for 20min at two flow rates (80 and 50 mL $s^{-1}$). All values of $\delta^2H$ and $\delta^{18}O$ of water vapour were expressed on the VSMOW-SLAP scale using the δ-notation:

$$\delta = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right)1000. \quad (1)$$

## 2.5 | Calculation of deuterium-excess and bark water uptake

Online measurements of water vapour isotopic composition were used to calculate deuterium-excess (*d*-excess) of the air coming in and out of the cuvettes, according to (Dansgaard, 1964):

$$d - excess = \quad \delta^2H - 8 \quad \delta^{18}O. \quad (2)$$

The rate of bark water uptake was calculated from gas-exchange and $\delta^2H$ and $\delta^{18}O$ of water vapour. First, we calculated transpiration rate per unit of leaf area ($E_{\text{leaf}}$ in mmol $m^{-2}$ $s^{-1}$) according to:

$$E_{\text{leaf}} = \frac{f_o w_o - f_i w_i}{S_{\text{leaf}}}, \quad (3)$$

where $S_{\text{leaf}}$ is the leaf surface area enclosed in the cuvette (in $m^2$, quantified at the end of each measurement period); $f_i$ and $f_o$ are air flows (in mol $s^{-1}$), $w_i$ and $w_o$ are water mole fractions (in mmol $mol^{-1}$). Next, we calculated the $\delta^2H$ and $\delta^{18}O$ of transpired water ($\delta_E$ in ‰) according to:

$$\delta_E = 1000\frac{f_o w_o \frac{\delta_o}{1000} - f_i w_i \frac{\delta_i}{1000}}{f_o w_o - f_i w_i}. \quad (4)$$

In Equation (3), $\delta_i$ and $\delta_o$ are isotope compositions (of either $^2H$ or $^{18}O$, in ‰), coming into and out of the chamber, respectively. In steady-state, $\delta_E$ should match that of the xylem ($\delta_x$). In branches where a bandage was applied, if water was being taken up through the wet bark and incorporated into the transpiration stream, $\delta_E$ should carry the tracer signal, assuming that water taken up through the bark was incorporated to the xylem and not lost via evaporation or stored outside the xylem within the woody matrix (Barbeta et al., 2022). Hence, in steady-state (i.e., assuming that the deuterium mole concentrations of transpired water, $^2C_E$, and that of xylem water, $^2C_{\text{x-up}}$, do not differ: $^2C_E = {}^2C_{\text{x-up}}$), we can estimate the rate of water uptake through the wet bark ($U_{\text{bark}}$) according to:

$$U_{\text{bark}} = \frac{{}^2C_E - {}^2C_{\text{x-up}}}{{}^2C_{\text{tracer}} - {}^2C_{\text{x-up}}} E_{\text{branch}}, \quad (5)$$

where $^2C_E$, $^2C_{\text{x-up}}$ and $^2C_{\text{tracer}}$ are deuterium ($^2H$) mole concentrations of: transpired water ($^2C_E$), upstream xylem water ($^2C_{\text{x-up}}$) and tracer injected to the bandages ($^2C_{\text{tracer}}$). Deuterium mole concentrations were calculated as:

$${}^2C = \frac{{}^2R_{sample}}{{}^2R_{sample} + 1}, \quad (6)$$

$^2R_{\text{sample}}$ was calculated from Equation (1) and taking $^2R_{\text{standard}}$ as that of VSMOW (155.76 $10^{-6}$ mol $mol^{-1}$). In Equation (5), $E_{\text{branch}}$ is whole-branch transpiration (in mmol $s^{-1}$) calculated from $E_{\text{leaf}}$ (in mmol $m^{-2}$ $s^{-1}$) and total branch leaf surface area ($S_{\text{leaf-branch}}$, in $m^2$):

$$E_{\text{branch}} = E_{\text{leaf}} S_{\text{leaf-branch}}. \quad (7)$$

We estimated $S_{\text{leaf-branch}}$ from the branch cross-sectional area and using an allometric equation specific for branches of *P. sylvestris* (Cermak et al., 1998). We calculated $U_{\text{bark}}$ only under steady state and when $\delta^2H_E \geq \delta^2H_{\text{xylem}}$. To determine whether our measurements were in steady-state, we compared the oxygen isotopic compositions of transpiration ($\delta^{18}O_E$) and of xylem water ($\delta^{18}O_x$): we assumed steady-state when: $|\delta^{18}O_E - \delta^{18}O_x| \leq |2\sigma_{18O\text{-}x}|$ ($\sigma_{18O\text{-}x}$ being the standard deviation of all $\delta^{18}O_x$ measurements within a campaign, Figures S1 and S2).

Goldsmith et al. (2017) demonstrated that isotopic exchange between plant water pools and water vapour of the surrounding air can occur in the absence of mass flow. To get an estimate of the magnitude of this flux, we calculated vapour-phase diffusion flow through the bark ($U_{\text{bark-gas}}$) as:

$$U_{\text{bark-gas}} = {}^2C_{\text{tracer}} g_{\text{bark}} W_{sat} S_{\text{bark}}. \quad (8)$$

In our setup, the air space underneath the bandage should have been saturated with water vapour that has an isotopic composition equal to that of the tracer injected ($^2C_{\text{tracer}}$, eight orders of magnitude greater than natural abundance). The remaining terms in Equation (8) describe the: conductance to water diffusion through the bark ($g_{\text{bark}}$, 1 mmol $m^{-2}$ $s^{-1}$), calculated from measured $g_{\text{bark}}$ for $CO_2$ in *Pinus monticola* (Cernusak et al., 2001); saturation vapour pressure for a given air temperature ($W_{\text{sat}}$, in mol $mol^{-1}$) and bark surface area under the bandage ($S_{\text{bark}}$, in $m^2$).

### 2.6 | Statistical analyses

We used linear mixed models (LMMs) to test for differences in the isotopic composition ($\delta^2H$ and $\delta^{18}O$) of xylem, bark and leaf water among control, upstream and downstream segments, within species and study sites. In our LMM, we used $\delta^2H$ and $\delta^{18}O$ of the extracted plant water as our response variables, campaign (only for *P. sylvestris*), segment type (control, upstream and downstream) and their interaction as fixed factors and tree as a random factor, since multiple bandages were applied to each tree and the same trees were sampled in consecutive campaigns at each site. We ran separate LMMs for each species and study site because we were not interested in the processes underlying differences in water isotopic composition between sites or species, e.g., climate, varying isotopic composition of precipitation with latitude or contrasting vertical root distribution (Dawson & Simonin, 2011). We also used LMMs to test for the relationships of *d*-excess (calculated from measurements of online water vapour isotopic composition) with transpiration rate, taking into account the random tree-to-tree variability. Finally, we assessed whether isotopically enriched water (injected into the bandage) had been taken up through the bark with *t*-tests. To do so, we calculated the difference in isotopic composition (for both $\delta^2H$ and $\delta^{18}O$) between pairs of upstream and downstream segments from the same branch and then used *t*-tests to assess differences within campaigns and species. All analyses were performed in R v. 3.6.3 (R Development Core Team R, 2019) using packages 'multcomp' (Hothorn et al., 2008) and 'nlme' (Pinheiro et al., 2009).

## 3 | RESULTS

### 3.1 | Xylem water isotopic composition from different branch segments, campaigns and species

Results of the LMMs showed that there were no significant differences in $\delta^{18}O$ of xylem water among branch segment types (Table 1, Figure 2a,b). The $\delta^{18}O$ of xylem water from control branch segments (no bandage), did not differ from that of segments upstream or downstream from the bandages, neither in *P. sylvestris* (Figure 2a) nor *F. sylvatica* (Figure 2b). For $\delta^2H$ of xylem water, we found that there were no significant differences between control segments and those upstream from the bandage (Table 1, Figure 2c,d). The $\delta^2H$ of xylem water from segments downstream from the bandage was always enriched with respect to that of upstream or control segments, for both *P. sylvestris* (Figure 2c) and *F. sylvatica* (Figure 2d), in both study sites and in all campaigns (Table S1). The results of the LMMs were consistent with those of the *t*-tests: xylem $\delta^{18}O$ of paired upstream and downstream segments was not significantly different (Table 2), whereas this difference was always significant for $\delta^2H$, in both study species, sites and in all campaigns (Table 2).

Besides the differences among branch segments for $\delta^2H$, we found that there were significant differences in the $\delta^{18}O$ and $\delta^2H$ of xylem water between campaigns within sites, for *P. sylvestris* (Table S1). At the boreal forest, $\delta^{18}O$ (all segments) and $\delta^2H$ (control and upstream segments) of xylem water were more enriched in autumn than in summer and at the temperate forest, xylem water was more enriched in summer than in winter (Figure 2). These latter differences were likely caused by contrasting isotopic compositions of soil and precipitation water across seasons. The campaign × segment type interaction was significant for $\delta^2H$ of xylem water in *P. sylvestris* at the temperate forest in Northern Spain (Table S1): the $\delta^2H$-enrichment in downstream segments, with respect to control and upstream segments, was larger in summer than in winter (Figure 2c).

### 3.2 | Bark and leaf water isotopic composition from different branch segments, campaigns and species

There were no significant differences among branch segments (control, upstream and downstream) in $\delta^{18}O$ of water extracted from either leaves or bark (Tables 1 and S1), with the exception of $\delta^{18}O$ from leaf water measured at the boreal forest in Sweden in the autumn campaign (leaf water from upstream and downstream segments was more enriched in $\delta^{18}O$ than that of control segments, likely due to slight differences in the sampling time, Table 1). There were some differences in leaf and bark (including cambium and phloem) water $\delta^{18}O$ between campaigns and within sites (for *P. sylvestris*), in agreement with the results for xylem water (Tables 1 and S1). For *P. sylvestris*, there were no differences in bark water $\delta^2H$ between control and upstream segments at the temperate forest in Northern Spain (unfortunately no bark samples from control segments collected at the boreal forest in Sweden were suitable for analyses of water isotopic composition). In contrast, bark water extracted from downstream segments was always enriched in $\delta^2H$ with respect to water from upstream segments, in both sites and all campaigns. For *F. sylvatica*, we did not find significant differences in bark water $\delta^2H$ among segments, although the large variability of measurements from downstream segments (mean ± se: 52.6 ± 37.9‰), may have obscured differences with respect to control (−44‰, $n = 1$) and upstream (−42.4 ± 0.6) segments. Leaf water from downstream segments was more enriched in $\delta^2H$ in *P. sylvestris* at the boreal forest in Sweden in the autumn campaign and at the temperate forest in Spain in the winter campaign (Tables 1 and S1). For the rest of our measurements, we did not find significant differences in leaf water $\delta^2H$ among segments, although, the small final number of measurements and the large variability, particularly for measurements from downstream segments, could have masked some trends, since mean leaf water $\delta^2H$ from downstream segments was consistently more enriched than that of upstream and control segments (Tables 1 and S1).

### 3.3 | Water isotopic composition of transpiration and estimated branch water uptake rate

In the summer-2018 campaign, transpiration rate per unit of leaf area ($E_{leaf}$) and online measurements of water vapour isotopic

**TABLE 1** Mean (SE) isotopic composition ($\delta^2H$ and $\delta^{18}O$ in ‰) of water extracted from xylem, bark or leaves from branches without bandages (control) and from branches upstream and downstream from the bandages for the two study sites (boreal forest in Sweden and temperate forest in Spain), four sampling campaigns and for the temperate forest for *Pinus sylvestris* and *Fagus sylvatica*

| | | | | | Branch segment | | |
|---|---|---|---|---|---|---|---|
| Species | Site | Campaign | Plant species | Tissue | Control | Upstream | Downstream |
| $\delta^2H$ | Boreal | Summer-18 | *P. sylvestris* | Xylem | | −100.1 (1.1) | **74.1 (50.5)** |
| | | | | Bark | | −103.7 (1.1) | **30.1 (112.4)** |
| | | | | Leaf | | −56.2 | 43.0 (31.8) |
| | | Autumn-18 | *P. sylvestris* | Xylem | −72.4 (1.1) | −73.3 (1.7) | **130.2 (59.1)** |
| | | | | Bark | | −69.5 (2.5) | **199 (74.3)** |
| | | | | Leaf | −70.5 (1) | −16 (3.9) | **148 (55.9)** |
| | Temperate | Winter-19 | *P. sylvestris* | Xylem | −49.3 (6.5) | −49.1 (1.7) | **51.2 (23.1)** |
| | | | | Bark | −48.2 (9.2) | −30.5 (4.9) | **−1.4 (15.7)** |
| | | | | Leaf | | −2.5 | 92.7 |
| | | Summer-19 | *P. sylvestris* | Xylem | −39.0 (5.6) | −39.2 (2.7) | **154.3 (32.9)** |
| | | | | Bark | −28.5 | −36.7 (3) | **171.1 (35.2)** |
| | | | | Leaf | 17.4 | 10 (2.6) | **72.4 (12.7)** |
| | | | *F. sylvatica* | Xylem | −47.4 (1.3) | −46.8 (1.7) | **78.8 (32.7)** |
| | | | | Bark | −44.0 | −42.4 (0.6) | 52.6 (37.9) |
| | | | | Leaf | 16.5 | 15.6 (0.7) | 77.4 (38.1) |
| $\delta^{18}O$ | Boreal | Summer-18 | *P. sylvestris* | Xylem | | −12.1 (0.2) | −11.6 (0.6) |
| | | | | Bark | | −11.7 (0.2) | −11.0 (0.5) |
| | | | | Leaf | | 3 | 2.2 (0.1) |
| | | Autumn-18 | *P. sylvestris* | Xylem | −9.2 | −8.9 (0.2) | −9.3 (0.2) |
| | | | | Bark | | −10.0 (0.2) | −9.8 (0.5) |
| | | | | Leaf | **−6.5 (0.5)** | 5.3 (0.5) | 5.0 (0.4) |
| | Temperate | Winter-19 | *P. sylvestris* | Xylem | −9.3 (0.5) | −9.1 (0.3) | −8.7 (0.2) |
| | | | | Bark | −9.3 (0.1) | −8.9 (0.3) | −8.7 (0.6) |
| | | | | Leaf | | 3.5 | 1.4 |
| | | Summer-19 | *P. sylvestris* | Xylem | −5.5 (0.5) | −6.1 (0.3) | −6.3 (0.4) |
| | | | | Bark | −5.9 | −5.4 (0.4) | −5.5 (0.1) |
| | | | | Leaf | 1.7 | 4.2 (0.8) | 2.4 (2.3) |
| | | | *F. sylvatica* | Xylem | −5.2 (0.2) | −5.0 (0.2) | −5.0 (0.2) |
| | | | | Bark | −3.8 | −3.6 (0.4) | −3.5 (0.4) |
| | | | | Leaf | 18.2 | 17.2 (0.1) | 16.5 (1.1) |

*Note*: Numbers in bold indicate significant differences among branch segments (control, upstream and downstream). No control branches were collected in the summer-2018 campaign. Other missing values are due to insufficient water volume obtained following cryogenic water extraction.

composition were monitored for two hours (13-15H on 24 July 2018) in two cuvettes (Figure S1). Four days before the onset of these measurements, bandages soaked in $\delta^2H$-enriched water had been applied to the corresponding branches. During the summer-2018 campaign, we measured similar mean $E_{leaf}$ for the two branches: $1.44 \pm 0.07$ and $1.17 \pm 0.09$ mmol $m^{-2}$ $s^{-1}$ (±se, $n = 5$ measurements per cuvette). The *d*-excess of the air coming out of the two cuvettes (364 ± 5.9 and 49.5 ± 0.5‰) was larger than that of incoming air (30.4 ± 0.4‰), but there was a large difference between cuvettes. In this campaign, we did not find any significant relationship between *d*-excess and $E_{leaf}$. During the summer-2018 campaign, our estimates of oxygen isotopic composition of transpiration ($\delta^{18}O_E$) indicated that none of our measurements met the conditions to be considered under steady-state

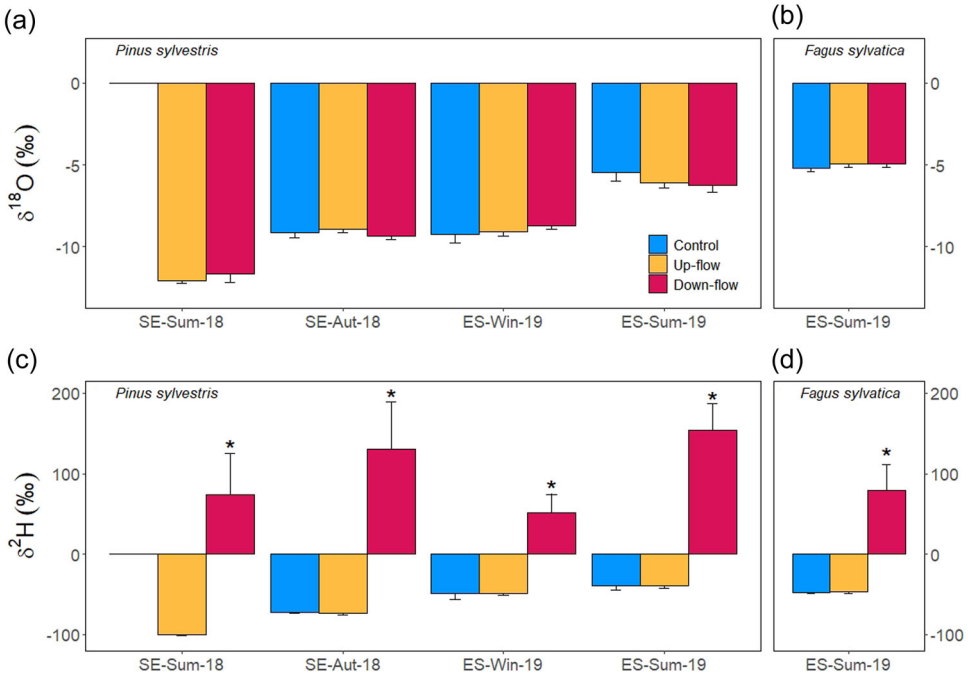


**FIGURE 2** Mean (+SE, $n = 5–17$) isotopic composition ($\delta^{18}O$, a and b, and $\delta^{2}H$, c and d) of xylem water for the two sites (boreal forest in Northern Sweden, SE, and temperate forest in Northern Spain, ES), four campaigns (Summer-18, Autumn-18, Winter-19 and Summer-19) and two study species (*Pinus sylvestris* and *Fagus sylvatica*), for branches without bandages soaked in $\delta^{2}H$-enriched water (control) and for branch segments upstream and downstream from the bandages. Asterisks (*) indicate significant differences in $\delta^{2}H$ for upstream segments with respect to control and downstream segments. [Color figure can be viewed at wileyonlinelibrary.com]

| Site | Campaign | Plant species | $\delta^{2}H$ | | $\delta^{18}O$ | | N |
|---|---|---|---|---|---|---|---|
| | | | *t* | *p* | *t* | *p* | |
| Boreal | Summer-18 | *P. sylvestris* | 3.5 | **0.013** | 1.1 | 0.32 | 7 |
| | Autumn-18 | *P. sylvestris* | 3.4 | **0.009** | −1.6 | 0.15 | 9 |
| Temperate | Winter-19 | *P. sylvestris* | 4.3 | **<0.001** | 1.4 | 0.19 | 16 |
| | Summer-19 | *P. sylvestris* | 5.7 | **<0.001** | −0.5 | 0.63 | 17 |
| | Summer-19 | *F. sylvatica* | 4 | **0.001** | −0.1 | 0.92 | 16 |

*Note*: Significant differences ($p < 0.05$) between upstream and downstream are indicated in **bold**.

**TABLE 2** Results (*t*, *p* and sample size, *N*) for the comparison of xylem water isotopic composition ($\delta^{2}H$ and $\delta^{18}O$) between pairs of upstream and downstream segments from branches with bandages soaked in $\delta^{2}H$-enriched water, for the two study sites (boreal forest in Northern Sweden and temperate forest in Northern Spain), four sampling campaigns and for the temperate forest for *Pinus sylvestris* and *Fagus sylvatica*

(Figure S1), hence no estimates of bark water uptake rate were calculated.

In the autumn-2018 campaign, $E_{leaf}$, $\delta^{2}H_E$ and $\delta^{18}O_E$ were monitored for eight days (4–11 September 2018) in four cuvettes (Figure S2). Bandages were applied on the corresponding branches on 3 September 2018. On the 9 September 2018, the site received significant rainfall, hence data collected on this date were excluded. In the autumn-2018 campaign, mean $E_{leaf}$ was lower than that measured in the summer campaign: $0.79 \pm 0.11$ mmol $m^{-2}$ $s^{-1}$ ($n = 4$ cuvettes, with 3–7 measurements per cuvette and day between 10 and 16 H, Figure S2). Calculated *d*-excess for the air coming out of the cuvette was significantly and positively correlated with $E_{leaf}$ ($p < 0.001$, Figure 3) and this correlation varied over time, mainly because *d*-excess was not significantly correlated with $E_{leaf}$ on the first 24 h after the application of the bandage. We found that *d*-excess peaked 48–72 h after the application of the bandage and dropped on the last two measurement days, which were cooler, cloudier and preceded by a rainy day (Table S2). The relationship between *d*-excess and $E_{leaf}$ also varied across cuvettes (Figure 3).

In the autumn-2018 campaign, on average, we recorded eight measurements of gas-exchange per day and per cuvette under steady state, according to our criteria ($|\delta^{18}O_E - \delta^{18}O_x| \leq | 2\sigma_{18O-x}|$). We used instantaneous measurements of $E_{leaf}$ and $\delta^{2}H_E$ under steady state to estimate the rate of liquid water (tracer) uptake through the bark ($U_{bark}$, Figure 4), when $\delta^{2}H_E \geq \delta^{2}H_{xylem}$. Overall mean estimated $U_{bark}$ was $1.03 \pm 0.2$ µmol $s^{-1}$ ($n = 7$ days), but there were significant differences among cuvettes ($F = 41.8$, $p < 0.001$) and dates ($F = 5.4$, $p < 0.001$), the latter related to climatic conditions driving $E_{leaf}$ (Figure 4). For one of the cuvettes

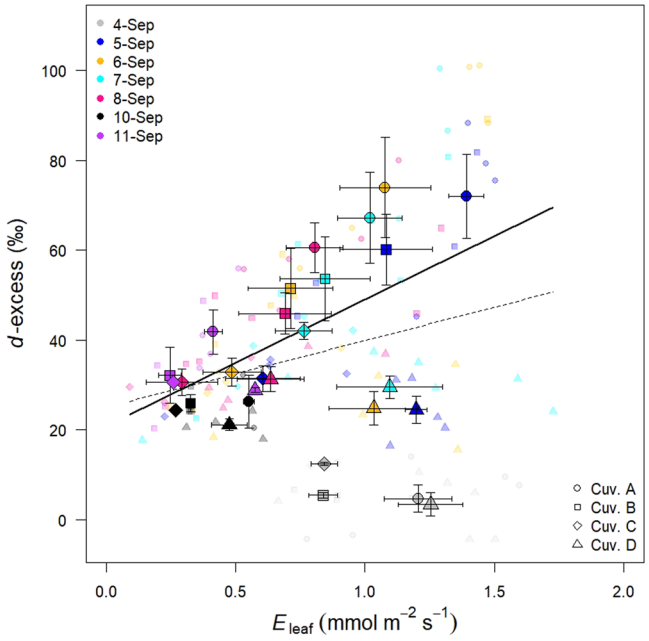


**FIGURE 3** Deuterium-excess of the air coming out of the cuvette (*d*-excess) and midday transpiration rates per unit of leaf area ($E_{leaf}$) measured on *Pinus sylvestris* branches at the boreal forest in Northern Sweden in the autumn-2018 campaign. Symbol shapes depict the four cuvettes (A, B, C and D) and colours measurement dates. Smaller symbols are individual measurements and larger symbols with error bars are the mean (±se) for each day and cuvette (branch). The dashed line is the fitted linear relationship with all measurement days included and the continuous line is the fitted line for measurements collected more than two days after the application of the bandage soaked in $\delta^2$H-enriched water (5–8 and 10–11 September, the bandages were applied on 3-September and isotopically enriched water was reinjected on the 10-September after a rainy day). [Color figure can be viewed at wileyonlinelibrary.com]

(D), we did not detect significant uptake any of the measurement days (Figure 4).

Mean and maximum daily estimates of vapour-phase diffusion flow through the bark ($U_{bark\text{-}gas}$) were: 0.74 ± 0.03 and 0.86 ± 0.03 nmol $s^{-1}$, respectively. Hence, we found that $U_{bark\text{-}gas}$ was a negligible flux, a thousand times smaller than mean estimates of liquid uptake ($U_{bark}$) through the bark, under the same conditions.

## 4 | DISCUSSION

Here we used a novel method to detect and quantify the uptake of water through bark in intact upper-canopy branches of Scots pine (*P. sylvestris*) and European beech (*F. sylvatica*). We applied bandages soaked in $\delta^2$H-labelled water and we found that the isotopic composition of xylem water of segments downstream from the bandage was enriched with respect to that of their corresponding upstream segments. We argue that this observation was driven by an uptake of $\delta^2$H-labelled water through the bark. Furthermore, we found that the isotopic composition of leaf water, as well as that of transpired water vapour, were significantly enriched in $\delta^2$H in branches where we had applied bandages soaked in $\delta^2$H-labelled water. In addition, we found that this enrichment was proportional to transpiration rate. These results indicate that the $\delta^2$H-labelled water was exchanged between the bandage and the xylem through the bark, as evidenced by the significant enrichment in $\delta^2$H of the transpiration stream. These findings were consistent across seasons; study sites, a boreal and a temperate forest; and tree species: Scots pine (an evergreen conifer) and European beech (a broadleaved deciduous angiosperm). Our study suggests that bark water uptake might be more ubiquitous and physiologically meaningful than previously acknowledged and builds on the literature documenting the importance of bark water uptake for embolism repair.

Our experiment with labelled water in intact branches was conceptually similar to Katz et al. (1989)'s seminal work where fluorescent dye was used to track the water pathway from the bark into the xylem, in cut branches. Studies before ours had also used isotopically labelled water to detect bark water uptake, but only in cut branches or under artificial conditions (Earles et al., 2016; Mayr et al., 2014). Our results from intact branches and under natural conditions agree with previous works where water uptake through the bark was linked to embolism repair (Earles et al., 2016; Mayr et al., 2014) and corticular photosynthesis (Liu et al., 2019). Here, in contrast to some of these previous studies, we did not find a significant increase in xylem water content in downstream segments with respect to their upstream counterparts (Table S3). Nonetheless, it should be noted that in our setup under natural conditions, bark water uptake occurred while transpiration was active, which would quickly counterbalance any potential mass gain. In addition to transpiration, bark water uptake could have also been partially counterbalanced by xylem evaporative losses through the bark, particularly in the summer campaigns (Martín-Gómez et al., 2017; Wolfe, 2020). Still, it could be argued that the observed isotopic enrichment of xylem water downstream from the bandages could have resulted from mere isotopic exchange without actual mass-flow, as shown for well-hydrated leaves in the laboratory (Goldsmith et al., 2017). However, our results suggest that this process was unlikely to be the sole mechanism driving the observed enrichment. We argue so mainly because the estimated rate of water vapour exchange through the bark was a thousand times smaller than that of liquid water uptake, in pine. In fact, although water uptake is not among the primary functions of bark, we found that our estimated rates of water uptake though the bark in pine were of comparable magnitude to some estimates of water flux through roots (Varney & Canny, 1993). In addition, we found that *d*-excess of transpired water vapour increased with transpiration rate (Figure 3) and that the enrichment of downstream xylem water was greater than that of the bark, in most cases (Table 1). Alternatively, it could be argued that the observed isotopic enrichment of transpired water and of bulk xylem water was not directly related to transpiration and instead it resulted from overnight isotopic equilibration between the wet bark and the xylem. However, diffusion rates are much slower than those of sap-flow (Nobel, 1983) and we still detected significant

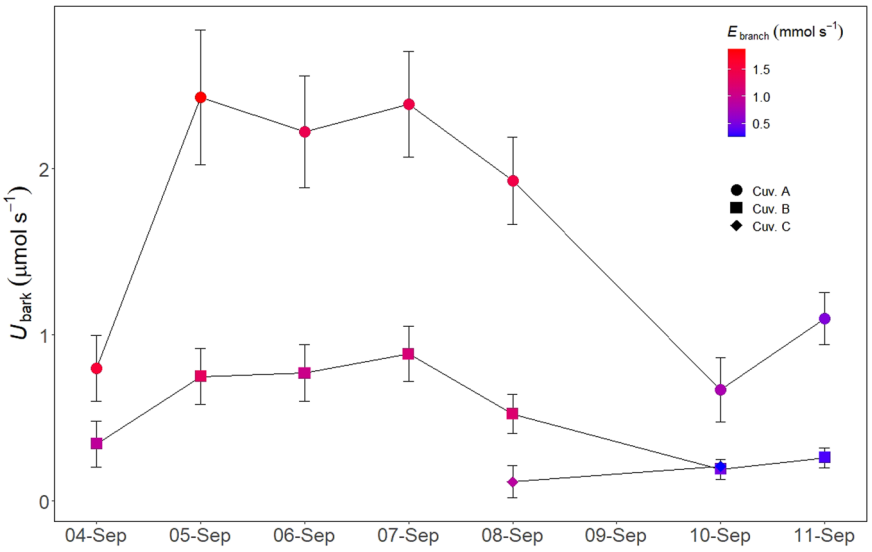


**FIGURE 4** Estimated rate of water uptake through the bark per branch ($U_{bark}$), over the autumn-2018 campaign, for three cuvettes for which we detected water uptake (A–C) installed on intact upper-canopy branches of *Pinus sylvestris* at the boreal forest in Northern Sweden. Points are the daily means (±SE, *n* varies depending on the number of measurements under steady state recorded per day and cuvette) for each cuvette. Symbol fill colours depict mean daily transpiration rates per branch ($E_{branch}$), for each cuvette. [Color figure can be viewed at wileyonlinelibrary.com]

enrichment of bulk water in leaves located 0.5–0.75 m downstream from the bandage application and collected the morning after the application (in the winter campaign this was approximately 3 h after sunrise). If the enrichment had resulted only from overnight equilibration, our observations would have required that sap-flow velocity should have been close to 20–25 cm h$^{-1}$, which is an unrealistic estimate based on measurements on the same species from similar sites (Poyatos et al., 2005). Taken together our results suggest that liquid uptake of isotopically enriched water through the bark and redistribution via xylem transport, related to transpiration, was the most plausible mechanism underlying enrichment of transpired water vapour, xylem and leaf water downstream from the application of our isotopic tracer.

In this study, we also aimed to assess how bark water uptake varied with species identity, ecosystem type (boreal vs. temperate forest) and seasonality. Regarding the effect of species identity, we found that $\delta^2$H-enrichment in segments downstream from the bandage was larger in Scots pine (*P. sylvestris*) than in European beech (*F. sylvatica*), indicating higher water uptake through the bark in pine than in beech. Wood and bark traits could underlie this difference: pine has porous and thick bark that can store large amounts of water, whereas beech bark is thinner (stores less water) and more impermeable (Ilek & Kucza, 2014). In addition, beech wood has wider vessels that under favourable conditions transport water efficiently from the soil to the upper canopy, whereas the narrow tracheid system responsible for water transport in pine is less efficient (Bar et al., 2018). Hence, we would expect upper-canopy and terminal branches of pine to benefit more from the uptake of water through the bark than those of beech, where large vessel conduits would supply the transpiration demand more efficiently. Nonetheless, besides vessel diameter, other wood anatomical traits such as contact pits or the proportion of parenchyma tissue could be influencing radial water redistribution within the woody matrix (Treydte et al., 2021) and this latter proportion has been shown to be larger in angiosperms than in gymnosperms (Morris et al., 2016). Indeed, our results showed that bark water uptake also occurred in upper-canopy branches of beech at our study site during a clear and warm summer day. Regarding differences among seasons and between forest types, our results showed that relative enrichment was larger in the temperate than in the boreal forest and in summer than in winter. At the temperate forest, isotopic enrichment of xylem water was significantly greater in the summer, suggesting greater bark water uptake, when transpiration demand was higher and presumably leaf water potentials reached their minimum. This result would indicate that bark water uptake increases with xylem tension, consistent with the results from previous experiments (Katz et al., 1989; Mayr et al., 2014). At the boreal forest, we did not find greater isotopic enrichment in downstream segments in summer than in autumn, but the number of days during which the bandages remained in place was not comparable between campaigns (14 days in the autumn campaign vs. 1–5 days in the summer campaign). Still, at the boreal forest online measurements of the water isotopic composition of transpiration showed that *d*-excess increased with transpiration, consistent with the premise that bark water uptake increased with xylem tension. Nonetheless, we cannot discard that the significant and positive correlation found between *d*-excess and $E_{leaf}$ could have also been partially caused by changes in the meteorological conditions along the experiment (Table S2) affecting both transpiration and the rate of *d*-enrichment.

In our experiments, we detected a significant $\delta^2H$-enrichment of leaf water downstream from the bandage application, although this enrichment varied greatly among branches, with leaves from some branches clearly showing enrichment, whilst being completely absent in others. We hypothesize that heterogeneities in water isotopic composition among vessels within the woody matrix of our branches might explain this variability. This is because within each branch not all vessels supply water to all leaf fascicles (Loepfe et al., 2007; Nygren & Pallardy, 2008) and water taken up through the bark might have only been incorporated to the outermost vessels of certain branches (Katz et al., 1989). Overall, the significant enrichment observed in xylem, bark and leaf waters downstream from the application of the bandages consistently supports our expectation that water taken up through the bark would exchange with the xylem water pools and eventually it could contribute to supplement the transpiration stream, similar to what has been observed for foliar water uptake (e.g., Binks et al., 2019; Breshears et al., 2008; Coopman et al., 2021; Welp et al., 2008).

Our experiment was conducted using intact branches in situ in adult tall trees. However, we acknowledge that our experimental setup did not reproduce realistic environmental conditions. We conducted our experiments over mostly sunny and cloudless days, while the bark surface underneath the bandages remained wet. Under natural conditions, wet bark is usually associated with impairment of transpiration, for example during rainy periods, cloud immersion or dew formation (Alvarado-Barrientos et al., 2014; Aparecido et al., 2016). Thus, our estimated rates of water uptake through the bark should be interpreted as maximum potential rates, whereas the realised rate of water uptake through bark is likely to be lower. Despite its relatively small magnitude, bark water uptake could still be a flux with a significant role at the organ (branch) level, particularly during periods when the bark is wet, trees are tall, and the transpiration demand is high.

In hydrological studies, water intercepted by canopy leaves and branches is usually assumed to be either returned to the atmosphere in the form of evaporation or redirected to the root zone either in the form of stemflow or throughfall (Crockford & Richardson, 2000; Gash, 1979). Furthermore, in some of these hydrological studies, it is not rare to encounter disagreements between methodologies used to estimate water use partitioning across ecosystem compartments (canopy evaporation following interception, soil evaporation, understorey and overstorey transpiration), particularly during rainy periods (Gimeno et al., 2018; Soubie et al., 2016). Recently, Binks et al. (2019) found that incorporating foliar water uptake improved estimates of the actual contribution of tree canopy transpiration to the water balance. Similarly, incorporating the contribution of water taken up through the bark could help close the water balance in hydrological studies, particularly in forests where scattered precipitation events that wet the canopy alternate with periods of high evaporative demand (Dietrich & Kahmen, 2019). Besides, neglecting the contributions of bark and/or foliar water uptake could also lead to an overestimation of the risk of hydraulic failure (Earles et al., 2016; Mayr et al., 2014), and eventually of the risk of mortality, as these alternative water uptake pathways (through the leaves and/or the bark) could contribute to partially alleviate drought stress (Breshears et al., 2008). Obtaining estimates of bark and/or foliar water uptake might not be straightforward for most study sites, but coupling complementary approaches to eddy covariance and/or sapflux measurements, such as online measurements of $\delta^2H$ and $\delta^{18}O$ or other atmospheric tracers (e.g., COS), could help identify missing sources contributing to water uptake (Aron et al., 2020; Wehr et al., 2017; Yakir & Sternberg, 2000).

## 5 | CONCLUSIONS

Here, we used a novel methodological approach to monitor water uptake through the bark in intact upper-canopy branches across seasons, in two forest types, and on two tree species. We applied bandages soaked in isotopically labelled water and detected this label in all tissues downstream from the segment of application, with consistent results observed in both a boreal and a temperate forest and in a conifer and an angiosperm. The rate of label uptake was positively related to transpiration and we found that the presence of labelled water in xylem water could not be explained by mere isotopic exchange without mass flow. We conclude that labelled water taken up through the bark was likely being incorporated into the transpiration stream across the xylem all the way into the leaves and through the stomata. Our results support the idea that water absorption through the bark could complement soil water uptake, particularly in upper-canopy branches, and contribute to feeding the transpiration stream.

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### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## REFERENCES


Alvarado-Barrientos, M.S., Holwerda, F., Asbjornsen, H., Dawson, T.E. & Bruijnzeel, L.A. (2014) Suppression of transpiration due to cloud immersion in a seasonally dry Mexican weeping pine plantation. *Agricultural & Forest Meteorology*, 186, 12–25. https://doi.org/10.1016/j.agrformet.2013.11.002

Ambrose, A.R., Sillett, S.C. & Dawson, T.E. (2009) Effects of tree height on branch hydraulics, leaf structure and gas exchange in California redwoods. *Plant, Cell & Environment*, 32(7), 743–757. https://doi.org/10.1111/j.1365-3040.2009.01950.x

Aparecido, L.M.T., Miller, G.R., Cahill, A.T. & Moore, G.W. (2016) Comparison of tree transpiration under wet and dry canopy conditions in a Costa Rican premontane tropical forest. *Hydrological Processes*, 30(26), 5000–5011. https://doi.org/10.1002/hyp.10960

Aron, P.G., Poulsen, C.J., Fiorella, R.P., Matheny, A.M. & Veverica, T.J. (2020) An isotopic approach to partition evapotranspiration in a mixed deciduous forest. *Ecohydrology*, 13(6). https://doi.org/10.1002/eco.2229

Bar, A., Nardini, A. & Mayr, S. (2018) Post-fire effects in xylem hydraulics of *Picea abies*, *Pinus sylvestris* and *Fagus sylvatica*. *New Phytologist*, 217(4), 1484–1493. https://doi.org/10.1111/nph.14916

Barbeta, A., Burlett, R., Martín-Gómez, P., Fréjaville, B., Devert, N., Wingate, L. et al. (2022) Evidence for distinct isotopic compositions of sap and tissue water in tree stems: consequences for plant water source identification. *New Phytologist*, 233(3), 1121–1132. https://doi.org/10.1111/nph.17857

Barbeta, A., Camarero, J.J., Sangüesa-Barreda, G., Muffler, L. & Peñuelas, J. (2019) Contrasting effects of fog frequency on the radial growth of two tree species in a Mediterranean-temperate ecotone. *Agricultural & Forest Meteorology*, 264, 297–308. https://doi.org/10.1016/j.agrformet.2018.10.020

Berry, Z.C., Emery, N.C., Gotsch, S.G. & Goldsmith, G.R. (2019) Foliar water uptake: processes, pathways, and integration into plant water budgets. *Plant, Cell & Environment*, 42(2), 410–423. https://doi.org/10.1111/pce.13439

Binks, O., Mencuccini, M., Rowland, L., da Costa, A., de Carvalho, C., Bittencourt, P. et al. (2019) Foliar water uptake in Amazonian trees: evidence and consequences. *Global Change Biology*, 25(8), 2678–2690. Available from: https://doi.org/10.1111/gcb.14666

Breshears, D.D., McDowell, N.G., Goddard, K.L., Dayem, K.E., Martens, S.N., Meyer, C.W. et al. (2008) Foliar absorption of intercepted rainfall improves woody plant water status most during drought. *Ecology*, 89(1), 41–47. Available from: https://doi.org/10.1890/07-0437.1

Burgess, S.S.O. & Dawson, T.E. (2004) The contribution of fog to the water relations of *Sequoia sempervirens* (D. Don): foliar uptake and prevention of dehydration. *Plant, Cell & Environment*, 27(8), 1023–1034. Available from: https://doi.org/10.1111/j.1365-3040.2004.01207.x

Cabon, A., Martínez-Vilalta, J., de Aragón, J.M., Poyatos, R. & De Cáceres, M. (2018) Applying the eco-hydrological equilibrium hypothesis to model root distribution in water-limited forests. *Ecohydrology*, 11(7), 16. Available from: https://doi.org/10.1002/eco.2015

Cermak, J., Riguzzi, F. & Ceulemans, R. (1998) Scaling up from the individual tree to the stand level in Scots pine. I. needle distribution, overall crown and root geometry. *Annales des Sciences Forestieres*, 55(1-2), 63–88. Available from: https://doi.org/10.1051/forest:19980105

Cernusak, L.A., Marshall, J.D., Comstock, J.P. & Balster, N.J. (2001) Carbon isotope discrimination in photosynthetic bark. *Oecologia*, 128(1), 24–35. Available from: https://doi.org/10.1007/s004420100629

Coopman, R.E., Nguyen, H.T., Mencuccini, M., Oliveira, R.S., Sack, L., Lovelock, C.E. et al. (2021) Harvesting water from unsaturated atmospheres: deliquescence of salt secreted onto leaf surfaces drives reverse sap flow in a dominant arid climate mangrove, *Avicennia marina*. *New Phytologist*, 231(4), 1401–1414. Available from: https://doi.org/10.1111/nph.17461

Crockford, R.H. & Richardson, D.P. (2000) Partitioning of rainfall into throughfall, stemflow and interception: effect of forest type, ground cover and climate. *Hydrological Processes*, 14(16-17), 2903–2920. Available from: https://doi.org/10.1002/1099-1085(200011/12)14:16/17%3C2903::aid-hyp126%3E3.0.co;2-6

Dansgaard, W. (1964) Stable isotopes in precipitation. *Tellus*, 16(4), 436–468.

Dawson, T.E. & Simonin, K.A. (2011) The roles of stable isotopes in forest hydrology and biogeochemistry. In: Levia, D.F., CarlyleMoses, D. & Tanaka, T. (Eds.) *Forest hydrology and biogeochemistry: synthesis of past research and future directions*, 216. Dordrecht: Springer. pp. 137–161.

Dietrich, L. & Kahmen, A. (2019) Water relations of drought-stressed temperate trees benefit from short drought-intermitting rainfall events. *Agricultural & Forest Meteorology*, 265, 70–77. Available from: https://doi.org/10.1016/j.agrformet.2018.11.012

Earles, J.M., Sperling, O., Silva, L.C.R., McElrone, A.J., Brodersen, C.R., North, M.P. et al. (2016) Bark water uptake promotes localized hydraulic recovery in coastal redwood crown. *Plant, Cell & Environment*, 39(2), 320–328. Available from: https://doi.org/10.1111/pce.12612

Eller, C.B., Lima, A.L. & Oliveira, R.S. (2013) Foliar uptake of fog water and transport belowground alleviates drought effects in the cloud forest tree species, *Drimys brasiliensis* (Winteraceae). *New Phytologist*, 199(1), 151–162. Available from: https://doi.org/10.1111/nph.12248

Ellison, D., Morris, C.E., Locatelli, B., Sheil, D., Cohen, J., Murdiyarso, D. et al. (2017) Trees, forests and water: cool insights for a hot world. *Global Environmental Change-Human and Policy Dimensions*, 43, 51–61. Available from: https://doi.org/10.1016/j.gloenvcha.2017.01.002

Fernández-Marín, B., Esteban, R., Míguez, F., Artetxe, U., Castañeda, V., Pintó-Marijuan, M. et al. (2015) Ecophysiological roles of abaxial anthocyanins in a perennial understorey herb from temperate deciduous forests. *Annals of Botany Plants*, 7, 12. Available from: https://doi.org/10.1093/aobpla/plv042

Gash, J.H.C. (1979) An analytical model of rainfall interception by forests. *Quarterly Journal of the Royal Meteorological Society*, 105(443), 43–55. Available from: https://doi.org/10.1002/qj.49710544304

Gimeno, T.E., McVicar, T.R., O'Grady, A.P., Tissue, D.T. & Ellsworth, D.S. (2018) Elevated $CO_2$ did not affect the hydrological balance of a mature native Eucalyptus woodland. *Global Change Biology*, 24(7), 3010–3024. Available from: https://doi.org/10.1111/gcb.14139

Goldsmith, G.R., Lehmann, M.M., Cernusak, L.A., Arend, M. & Siegwolf, R.T.W. (2017) Inferring foliar water uptake using stable isotopes of water. *Oecologia*, 184(4), 763–766. Available from: https://doi.org/10.1007/s00442-017-3917-1

Goldsmith, G.R., Matzke, N.J. & Dawson, T.E. (2013) The incidence and implications of clouds for cloud forest plant water relations. *Ecology Letters*, 16(3), 307–314. Available from: https://doi.org/10.1111/ele.12039

Hasselquist, N.J., Metcalfe, D.B. & Hogberg, P. (2012) Contrasting effects of low and high nitrogen additions on soil $CO_2$ flux components and ectomycorrhizal fungal sporocarp production in a boreal forest. *Global Change Biology*, 18(12), 3596–3605. Available from: https://doi.org/10.1111/gcb.12001

Hill, A.J., Dawson, T.E., Dody, A. & Rachmilevitch, S. (2021) Dew water-uptake pathways in Negev desert plants: a study using stable isotope tracers. *Oecologia*, 196(2), 353–361. Available from: https://doi.org/10.1007/s00442-021-04940-9

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, 50(3), 346–363. Available from: https://doi.org/10.1002/bimj.200810425

Ilek, A. & Kucza, J. (2014) Hydrological properties of bark of selected forest tree species. part I: the coefficient of development of the interception surface of bark. *Trees-Structure and Function*, 28(3), 831–839. Available from: https://doi.org/10.1007/s00468-014-0995-0

Jasechko, S., Sharp, Z.D., Gibson, J.J., Birks, S.J., Yi, Y. & Fawcett, P.J. (2013) Terrestrial water fluxes dominated by transpiration. *Nature*, 496(7445), 347–350. Available from: https://doi.org/10.1038/nature11983

Johnson, D.M., McCulloh, K.A., Woodruff, D.R. & Meinzer, F.C. (2012) Hydraulic safety margins and embolism reversal in stems and leaves: why are conifers and angiosperms so different. *Plant Science*, 195, 48–53. Available from: https://doi.org/10.1016/j.plantsci.2012.06.010

Jones, S.P., Ogée, J., Sauze, J., Wohl, S., Saavedra, N., Fernández-Prado, N. et al. (2017) Non-destructive estimates of soil carbonic anhydrase activity and associated soil water oxygen isotope composition. *Hydrology and Earth System Sciences*, 21(12), 6363–6377. Available from: https://doi.org/10.5194/hess-21-6363-2017

Katz, C., Oren, R., Schulze, E.D. & Milburn, J.A. (1989) Uptake of water and solutes through twigs of *Picea abies* (L.) Karst. *Trees-Structure and Function*, 3(1), 33–37.

Leen, J.B., Berman, E.S.F., 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(4), 044305. Available from: https://doi.org/10.1063/1.4704843

Liu, J., Gu, L., Yu, Y., Huang, P., Wu, Z., Zhang, Q. et al. (2019) Corticular photosynthesis drives bark water uptake to refill embolized vessels in dehydrated branches of *Salix matsudana*. *Plant, Cell & Environment*, 42(9), 2584–2596. Available from: https://doi.org/10.1111/pce.13578

Loepfe, L., Martinez-Vilalta, J., Pinol, J. & Mencuccini, M. (2007) The relevance of xylem network structure for plant hydraulic efficiency and safety. *Journal of Theoretical Biology*, 247(4), 788–803. Available from: https://doi.org/10.1016/j.jtbi.2007.03.036

Martín-Gómez, P., Barbeta, A., Voltas, J., Peñuelas, J., Dennis, K., Palacio, S. et al. (2015) Isotope-ratio infrared spectroscopy: a reliable tool for the investigation of plant-water sources? *New Phytologist*, 207(3), 914–927. Available from: https://doi.org/10.1111/nph.13376

Martín-Gómez, P., Serrano, L. & Ferrio, J.P. (2017) Short-term dynamics of evaporative enrichment of xylem water in woody stems: implications for ecohydrology. *Tree Physiology*, 37(4), 511–522. Available from: https://doi.org/10.1093/treephys/tpw115

Mayr, S., Schmid, P., Laur, J., Rosner, S., Charra-Vaskou, K., Damon, B. et al. (2014) Uptake of water via branches helps timberline conifers refill embolized xylem in late winter. *Plant Physiology*, 164(4), 1731–1740. Available from: https://doi.org/10.1104/pp.114.236646

Morris, H., Plavcová, L., Cvecko, P., Fichtler, E., Gillingham, M.A.F., Martínez-Cabrera, H.I. et al. (2016) A global analysis of parenchyma tissue fractions in secondary xylem of seed plants. *New Phytologist*, 209(4), 153–1565. Available from: https://doi.org/10.1111/nph.13737

Munne-Bosch, S., Nogues, S. & Alegre, L. (1999) Diurnal variations of photosynthesis and dew absorption by leaves in two evergreen shrubs growing in Mediterranean field conditions. *New Phytologist*, 144(1), 109–119. Available from: https://doi.org/10.1046/j.1469-8137.1999.00490.x

Nelson, J.A., Pérez-Priego, O., Zhou, S., Poyatos, R., Zhang, Y., Blanken, P.D. et al. (2020) Ecosystem transpiration and evaporation: insights from three water flux partitioning methods across FLUXNET sites. *Global Change Biology*, 26(12), 6916–6930. Available from: https://doi.org/10.1111/gcb.15314

Nobel, P.S. (1983) In: Freeman, W.H. (Ed.) *Biophysical plant physiology and ecology*. New York (NY), USA: W.H. Freeman & Co Ltd.

Nygren, P. & Pallardy, S.G. (2008) Applying a universal scaling model to vascular allometry in a single-stemmed, monopodially branching deciduous tree (Atim's model). *Tree Physiology*, 28(1), 1–10.

Olson, M.E., Soriano, D., Rosell, J.A., Anfodillo, T., Donoghue, M.J., Edwards, E.J. et al. (2018) Plant height and hydraulic vulnerability to drought and cold. *Proceedings of the National Academy of Sciences of the United States of America*, 115(29), 7551–7556. Available from: https://doi.org/10.1073/pnas.1721728115

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 Sensor Systems*, 2(2), 179–193. Available at https://doi.org/10.5194/jsss-2-179-2013

Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D., R Development Core Team, R. (2009). nlme: Linear and Nonlinear Mixed Effects Models.

Poyatos, R., Llorens, P. & Gallart, F. (2005) Transpiration of montane *Pinus sylvestris* L. and *Quercus pubescens* Willd. forest stands measured with sap flow sensors in NE Spain. *Hydrology and Earth System Sciences*, 9(5), 493–505. Available from: https://doi.org/10.5194/hess-9-493-2005

R Development Core Team, R. (2019). R: A Language and Environment for Statistical Computing.

Rosell, J.A., Gleason, S., Mendez-Alonzo, R., Chang, Y. & Westoby, M. (2014) Bark functional ecology: evidence for tradeoffs, functional coordination, and environment producing bark diversity. *New Phytologist*, 201(2), 486–497. Available from: https://doi.org/10.1111/nph.12541

Schlesinger, W.H. & Jasechko, S. (2014) Transpiration in the global water cycle. *Agricultural & Forest Meteorology*, 189, 115–117. Available from: https://doi.org/10.1016/j.agrformet.2014.01.011

Schultz, N.M., Griffis, T.J., Lee, X.H. & Baker, J.M. (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(21), 3360–3368. Available from: https://doi.org/10.1002/rcm.5236

Sellers, P.J., Dickinson, R.E., Randall, D.A., Betts, A.K., Hall, F.G., Berry, J.A. et al. (1997) Modelling the exchanges of energy, water, and carbon between continents and the atmosphere. *Science*, 275(5299), 502–509. Available from: https://doi.org/10.1126/science.275.5299.502

Seneviratne, S.I., Corti, T., Davin, E.L., Hirschi, M., Jaeger, E.B., Lehner, I. et al. (2010) Investigating soil moisture-climate interactions in a changing climate: a review. *Earth-Science Reviews*, 99(3-4), 125–161. Available from: https://doi.org/10.1016/j.earscirev.2010.02.004

Soubie, R., Heinesch, B., Granier, A., Aubinet, M. & Vincke, C. (2016) Evapotranspiration assessment of a mixed temperate forest by four methods: eddy covariance, soil water budget, analytical and model. *Agricultural & Forest Meteorology*, 228, 191–204. Available from: https://doi.org/10.1016/j.agrformet.2016.07.001

Stangl, Z.R., Tarvainen, L., Wallin, G., Ubierna, N., Rantfors, M. & Marshall, J.D. (2019) Diurnal variation in mesophyll conductance and its influence on modelled water-use efficiency in a mature boreal *Pinus sylvestris* stand. *Photosynthesis Research*, 141(1), 53–63. Available from: https://doi.org/10.1007/s11120-019-00645-6

Treydte, K., Lehmann, M., Wyczesany, T. & Pfautsch, S. (2021) Radial and axial water movement in adult trees recorded by stable isotope tracing. *Tree Physiology*, 41, 2248–2261. Available from: https://doi.org/10.1093/treephys/tpab080

Ukkola, A.M., Prentice, I.C., Keenan, T.F., van Dijk, A.I.J.M., Viney, N.R., Myneni, R.B. et al. (2016) Reduced streamflow in water-stressed climates consistent with $CO_2$ effects on vegetation. *Nature Climate Change*, 6(1), 75–78. Available from: https://doi.org/10.1038/nclimate2831

Varney, G.T. & Canny, M.J. (1993) Rates of water-uptake into the mature root system of maize plants. *New Phytologist*, 123(4), 775–786. Available from: https://doi.org/10.1111/j.1469-8137.1993.tb03789.x

Wallin, G., Linder, S., Lindroth, A., Rantfors, M., Flemberg, S. & Grelle, A. (2001) Carbon dioxide exchange in Norway spruce at the shoot, tree and ecosystem scale. *Tree Physiology*, 21(12-13), 969–976. Available from: https://doi.org/10.1093/treephys/21.12-13.969

Wehr, R., Commane, R., Munger, J.W., McManus, J.B., Nelson, D.D., Zahniser, M.S. et al. (2017) Dynamics of canopy stomatal conductance, transpiration, and evaporation in a temperate deciduous forest, validated by carbonyl sulfide uptake. *Biogeosciences*, 14(2), 389–401. Available from: https://doi.org/10.5194/bg-14-389-2017

Welp, L.R., Lee, X., Kim, K., Griffis, T.J., Billmark, K.A. & Baker, J.M. (2008) $\delta^{18}O$ of water vapour, evapotranspiration and the sites of leaf water evaporation in a soybean canopy. *Plant, Cell & Environment*, 31(9), 1214–1228. Available from: https://doi.org/10.1111/j.1365-3040.2008.01826.x

Wolfe, B.T. (2020) Bark water vapour conductance is associated with drought performance in tropical trees. *Biology Letters*, 16(8), 20200263. Available from: https://doi.org/10.1098/rsbl.2020.0263

Yakir, D. & Sternberg, L.D.L. (2000) The use of stable isotopes to study ecosystem gas exchange. *Oecologia*, 123(3), 297–311. Available from: https://doi.org/10.1007/s004420051016

Yang, S., Sterck, F.J., Sass-Klaassen, U., Cornelissen, J.H.C., van Logtestijn, R.S.P., Hefting, M. et al. (2022) Stem trait spectra underpin multiple functions of temperate tree species. *Frontiers in Plant Science*, 13, Available from: https://doi.org/10.3389/fpls.2022.769551

Yang, Y., Donohue, R.J. & McVicar, T.R. (2016) Global estimation of effective plant rooting depth: implications for hydrological modeling. *Water Resources Research*, 52(10), 8260–8276. Available from: https://doi.org/10.1002/2016WR019392

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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