


RESEARCH ARTICLE

WILEY

Sapwood and heartwood are not isolated compartments: Consequences for isotope ecohydrology

Ginevra Fabiani^{1,2,3}  | Daniele Penna²  | Adrià Barbeta⁴ | Julian Klaus⁵

¹Catchment and Eco-Hydrology Group, Luxembourg Institute of Science and Technology, Belvaux, Luxembourg

²Department of Agriculture, Food, Environment and Forestry, University of Florence, Florence, Italy

³Doctoral School in Science and Engineering (DSSE), University of Luxembourg, Belval, Luxembourg

⁴Department of Evolutionary Biology, Ecology and Environmental Sciences, University of Barcelona, Barcelona, Spain

⁵Department of Geography, University of Bonn, Bonn, Germany

Correspondence

Ginevra Fabiani, Catchment and Eco-Hydrology Group, Luxembourg Institute of Science and Technology, Belvaux, Luxembourg.
Email: ginevra.fabiani@list.lu

Funding information

Fonds National de la Recherche Luxembourg, Grant/Award Number: AFR/STEP-UP ID 12546983; COST Action WATSON, Grant/Award Number: CA19120

Abstract

In most tree species, xylem consists of two different functional parts: sapwood and heartwood. While sapwood, as the flowpath for sap, has received more attention in isotope studies assessing water sources accessed by trees (e.g. soil water from different depths, groundwater, stream water or a mixture of these), much remains unknown about heartwood and the possible water exchange between the two functional parts. We investigated four tree species (*Fagus sylvatica*, *Quercus petraea*, *Pseudotsuga menziesii* and *Picea abies*) characterised by different xylem anatomy and timing of physiological activity to evaluate the degree of differentiation in isotopic composition of water between sapwood and heartwood on a biweekly time scale. We found that the sapwood and heartwood of all species displayed a concurrent variation in their isotopic composition throughout the growing season and on a day-night scale suggesting that the two are not isolated compartments. While the two functional parts display a consistent difference in isotopic composition in conifers, they are characterised by more similar values in broadleaved species in broadleaved species, suggesting a higher degree of water exchange. Furthermore, we have also observed a progressive change in the isotopic composition in broadleaved species with sampling depth rather than functional parts of xylem. Our study highlights the value of accounting for radial isotopic variation, which might potentially lead to uncertainties concerning the origin of the extracted water for water uptake studies.

KEYWORDS

ecohydrology, heartwood, sapwood, stable isotopes, xylem anatomy

1 | INTRODUCTION

A widely applied method to investigate tree water uptake is the use of stable isotopes of hydrogen ($^2\text{H}/^1\text{H}$) and oxygen ($^{18}\text{O}/^{16}\text{O}$) in the water molecule. Xylem water isotopic composition is deemed to reflect the combined isotopic composition of water sources accessed by the tree, with the assumption that no isotopic fractionation occurs during root water uptake and water flow in plant xylem (White et al., 1985). However, this assumption was challenged when multiple studies observed that the isotopic composition of xylem water does not always fall within the range of the isotopic composition of the source waters (Penna et al., 2018). Some studies hypothesised that

physiological mechanisms at the soil-root interface (Poca et al., 2019), leaf-atmosphere scale (Cernusak et al., 2016) and along the flow of the sap within the xylem might cause a depletion in ^2H xylem water and consequently, a deviation from the uptake sources (de la Casa et al., 2022). Additionally, there is increasing evidence that water compartmentalisation between flowing and stored water can occur within the stem (Barbeta et al., 2022; Zhao et al., 2016) and that the daily withdrawal of water from storage cells might affect the xylem isotopic composition (Dubbert & Werner, 2018). Zhao et al. (2016) found that bulk stem water in poplar trees displayed a different isotopic composition compared with sap water. Relying on a new water extraction technique for tree stems able to selectively extract sap water from

xylem conduits, Barbeta et al. (2022) provided experimental evidences that a certain degree of compartmentalisation exists between water in nonconducting woody cells and sap, with the latter being a better proxy of source water than bulk stem water. Water compartmentalisation in plant tissue implies the occurrence of restricted 'communication' between water compartments (Yakir, 1992). This makes the identification of tree water sources challenging, since isotopic heterogeneity in the wood matrix may imply that bulk xylem water does not accurately reflect its source water but is rather an approximation of it (Barbeta et al., 2022; Beyer & Penna, 2021; von Freyberg et al., 2020).

In most tree species, xylem consists of two physiologically different parts: sapwood (SW) and heartwood (HW). SW is characterised by the presence of living cells, which act as water and nutrient storage, and nonliving cells that function as the route of sap flow (Spicer, 2005). As the tree ages, old SW is compartmentalised in the centre of the stem as HW (Spicer, 2005) and becomes disconnected from the root system, which also develops HW close to the stem base (Hillis, 1987). HW xylem provides the mechanical support to the tree in spite of not participating in the vital activities of the tree as result from the loss to conduct water due to pit aspiration, incrustation and vessel tyloses (Nakada et al., 2019; Siau, 1984). Thus, no flow occurs in the HW (Cermak et al., 1992). The volumetric ratio of HW to SW is not only species-specific (Hillis, 1987) but also varies depending on tree age, climatic and soil conditions and the height at which the analysed stem cross-section is located (Nawrot et al., 2008). The SW of conifers contains a larger amount of water than HW (Cermak et al., 1992) where moisture content is almost equal to the fibre saturation point (Nakada et al., 2019), whereas the HW of some broadleaved species contains more moisture than the SW (Gartner, 1995; Hillis, 1987; Peck, 1953; Treydte et al., 2021). The large majority of the water transport in the SW xylem occurs in the axial direction through a dense network of dead cells constituting water conduits; these are tracheids in conifers and vessels in broadleaves. Functional connectivity is also enabled by the axial and ray parenchyma, the only living cells present in the xylem that act both as a storage of water and nutrient (Morris et al., 2016). However, water does not flow exclusively as a bulk flow via water conduits, because water transport also occurs via diffusion as transfer of water vapour through the air in cell lumen (Siau, 1984).

Water is also present in SW living cells as symplastic water, between cells as capillary water and within cells wall as fibre water. Multiple studies have reported the relevance of the internal storage of SW as a water reserve for transpiration in several species, depending on wood density, SW area and also on wood anatomy (Köcher et al., 2013; Phillips et al., 2003). The stem water reservoir does not only buffer transpiration demand during soil water shortage (Köcher et al., 2013) but also contributes to daily water use, accounting for 5%–22% of the daily water use in broadleaved trees (Köcher et al., 2013) and for 20%–25% in conifers (Cermak et al., 2007; Phillips et al., 2003). By measuring water potential variations in the trunk, Betsch et al. (2011) proved that water is exchanged between the elastic tissues and the vertical transpiration flow within the tree. By

testing different degrees of storage scenarios, Knighton et al. (2020) shed light on the importance of considering internal mixing in relation to potential water sources when interpreting xylem isotopic composition. Despite there being evidence of a potential exchange between mobile and less mobile water within the tree stem, there is still little knowledge available about the magnitude of this isotopic exchange and how this uncertainty affects the identification of sources of tree water uptake (Berry et al., 2017).

Several attempts to investigate the variability of isotopic composition at the tree scale, the canopy scale (Goldsmith et al., 2019) and within leaf tissues (Cernusak et al., 2016; Goldsmith et al., 2019) were recently carried out, but few studies have focused on the isotopic composition of the wood matrix and wood functional parts (Treydte et al., 2021; White et al., 1985). Stems are difficult to study and in most cases require destructive sampling that can compromise the integrity of the plant (Holbrook, 1995). Several studies sampled the xylem water by coring the tree trunk (Goldsmith et al., 2012; Knighton et al., 2020; Matheny et al., 2017; Meißner et al., 2014, 2012; Muñoz-Villers et al., 2018). However, few studies specified whether the sampling encompassed only the SW (Gessler et al., 2021; Kahmen et al., 2021; Kühnhammer et al., 2021; Tetzlaff et al., 2021), which occupies the outer sheath of the stem that is the cross-sectional area deployed for water flow. The structural heterogeneity of stems has been often overlooked, leaving a crucial component of the regolith-soil-plant-atmosphere continuum almost neglected. Additionally, current knowledge on the isotopic difference and rate of water exchange between HW and SW is still scarce, which potentially leads to uncertainties as to the estimated origin of the extracted water. To date, a few studies addressed this topic by applying isotopically enriched water (James et al., 2003; Treydte et al., 2021) and found the occurrence of radial water transport. Despite the early warning by White et al. (1985) on the need to better understand the mixing processes between SW and HW, to our knowledge, no study has yet documented the temporal evolution of SW and HW isotopic composition among multiple species and functional groups. In order to address this gap, we sampled SW and HW from two broadleaved species, European beech (*Fagus sylvatica*) and sessile oak (*Quercus petraea*), and two evergreen conifers, Douglas fir (*Pseudotsuga menziesii*) and spruce (*Picea abies*), in a temperate forest in Luxembourg. The sampling covered one growing season (March–October) and took place on a biweekly basis in order to address the isotopic composition changes over time and relate this pattern to species-specific xylem properties. We included a night-sampling campaign to test whether flow conditions are linked to a change in xylem isotopic composition. The four forest species addressed are characterised by different xylem anatomy and timing of physiological activity, aspects that may regulate the water exchange between SW and HW.

More specifically, we propose the following hypotheses: (i) The isotopic composition of SW is more seasonally variable than that of HW, because the latter is generally presumed to be disconnected from water sources, and (ii) Conifers have a higher degree of water compartmentalisation between SW and HW compared with broadleaved species due to the lower water storage capacity of their HW.

2 | MATERIALS AND METHODS

2.1 | Sampling strategy

We conducted the study during the 2020 growing season (16 March to 19 October 2020) in a species-rich temperate forest in the Weierbach experimental catchment in Luxembourg (Hissler et al., 2021; Rodriguez & Klaus, 2019; Schoppach et al., 2021), where *Fagus sylvatica* L., *Quercus petraea* (Matt.) Liebl., *Pseudotsuga menziesii* (Mirb.) Franco and *Picea abies* (L.) Karst. are present. A mixed beech-oak-forest is covering the vast majority of the catchment, with some minor cover by spruce and Douglas fir in pure artificial stands. We selected healthy mature trees (diameter ranged between 35 and 55 cm) in order to exclude tree size-related effects. Biweekly wood cores were taken at breast height (approximately 1.3 m above ground) with a 5-mm diameter increment borer from the same three individual trees for each species. Tree cores were collected around the stem circumference avoiding sampling above or below previous cores to minimise possible disturbances due to the wounding of trees. Based on the wood core translucency given by the water content, we separated SW from HW (Kravka et al., 1999; Wang et al., 2019) in conifers and beech trees. Light is transmitted through the water-filled conduits lumen but reflects and scatters at the surface of embolised conduits, resulting in contrast in wood colour (Taneda et al., 2021) (Figure S1). For oak, the SW–HW boundary was determined visually based on the distinct colour change between the two (Figure S1). The sampled SW length for oak, spruce and Douglas fir was on average 3.28, 5.44 and 3.79 cm, respectively. Beech tends to develop HW only after decades (Gebauer et al., 2008; Zweifel & Sterck, 2018); thus, we sampled cores for beech with an average length of 7 cm which was split into two, representative for outer and inner SW. SW and HW cores were directly placed in vials after removing bark and phloem. All vials were directly capped and sealed with Parafilm® and kept frozen until extraction. In preliminary experiments, we did not observe a strong statistical difference between SW and HW in broadleaved species; therefore, we decided to test whether sampling depth would result in a stronger differentiation between the sampled xylem portions. To do this, we sampled deeper cores in broadleaved trees (beech and oak) during five sampling campaigns (18 June, 30 June, 16 August and 4 September). We sampled deeper core in beech trees in order to reach the actual HW during these sampling dates. To verify if the degree of compartmentalisation between SW and HW decreased during low flow conditions (predawn), we repeated the SW and HW sampling during the night between 4 and 5 September between 1 and 2 AM. We collected a total of 78 samples for each coniferous species and 91 samples for each broadleaved species.

2.2 | Laboratory analysis

We extracted xylem water through a cryogenic vacuum distillation line (Orlowski et al., 2016). After decapping the sampling vials, we

immediately inserted them into larger extraction vials which were submerged in a 100°C oil bath for 3 h and connected to a vacuum extraction line. The pressure was maintained at 0.03 hPa and was continuously monitored. We collected the evaporated water from U-shaped tubes, which were submerged in liquid nitrogen (−197°C). We continuously recorded the pressure to ensure that the lines remained leak-tight throughout the entire extraction.

We assessed the gravimetric water content and extraction yields for each xylem sample by weighting them before and after cryogenic water extraction. We monitored the extraction efficiency for 271 out of 338 samples (covid-lockdown prevented us to have access to labs and to monitor this parameter from the very beginning of the experiment) by oven drying the samples at 105°C for 24 h and weighting them afterwards. The extraction efficiency, defined as the amount of water extracted divided by the total water of each sample, was above 99.7%, value which has been argued to guarantee a negligible effect of residual water content on the isotopic ratios of stem samples (Zhao et al., 2022). For each sample, we assessed the wood moisture content defined as the ratio of the weight of extracted water (liquid mass) to the weight of dry wood (Berry & Roderick, 2005; Peck, 1953; Siau, 1984; Steppe et al., 2010; Steward, 1967) after cryogenic water extraction (Equation 1):

$$\text{moisture content (\%)} = \frac{(\text{fresh weight} - \text{dry weight})}{\text{dry weight}} \times 100 \quad (1)$$

Oven-dry density (g cm^{-3}) was also assessed on a subset of 253 samples as the ratio of the mass of dry weight wood to volume of dry wood (Equation 2):

$$\text{dry density} = \frac{\text{mass of dry wood}}{\text{volume of dry wood}} \quad (2)$$

The isotopic composition of the extracted water ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) was analysed using a Picarro cavity ring-down spectrometer (CRDS) (L2140-i, Picarro, USA) coupled with a Micro-Combustion Module™ (MCM) in order to remove organic compounds (ethanol, methanol and/or other biogenic volatile compounds). For each sample, aliquots of 1.8 μl were injected and analysed in 10 replications. The first five injections were always discarded, and the remaining five injections were averaged in order to account for the memory effect (Penna et al., 2012). Every 12 samples, two standards were used for calibration. Another sample with a known value was analysed in triplicate throughout the sequence as a quality control. To detect possible organic contamination, xylem samples were screened with ChemCorrect™ (Picarro Inc., Santa Clara, CA, USA) software, which attempts to identify contamination through fitting to a known library of spectral features. No organic contamination was found in any sample. For the sake of convenience, when mentioning SW and HW isotopic composition, we refer to the isotopic composition of the water extracted from these two functional parts.

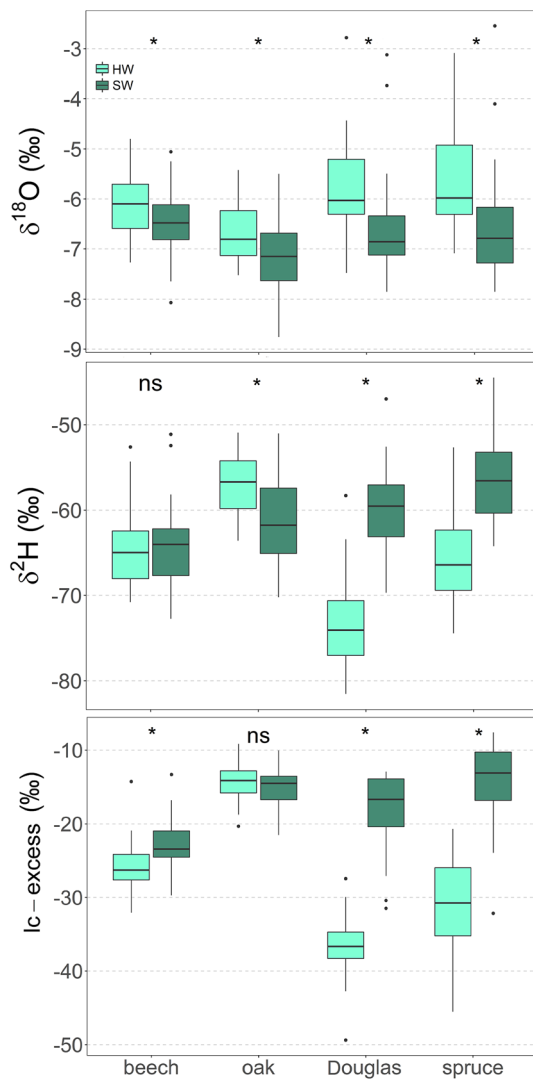


FIGURE 1 (up) $\delta^{18}\text{O}$, (middle) $\delta^2\text{H}$ and (bottom) lc-excess of xylem water for all sampling campaigns conducted in 2020. The centre line in the boxplot indicates the median, and the lower and upper extremes indicate the first and third quartiles, respectively. The whiskers indicate points within 1.5 times of the interquartile range above or below the median. Above each species, asterisks (symbols “*”, “ns” correspond to p -values ≤ 0.05 or > 0.05 , respectively) denote significant statistical differences between heartwood (HW) and sapwood (SW) (Mann–Whitney U test)

2.3 | Data analysis

We calculated the line-conditioned excess (lc-excess) as a function of the slope ($a = x$) and the intercept ($b = x \text{‰}$) of the local meteoric water line (LMWL) (Equation 3) (Landwehr & Coplen, 2004) for each sample:

$$lc\text{-excess}(\text{‰}) = \delta^2\text{H} - a \cdot \delta^{18}\text{O} - b \quad (3)$$

The lc-excess describes the deviation of the sample's $\delta^2\text{H}$ value from the LMWL in the dual-isotope space (Landwehr & Coplen, 2004),

which indicates nonequilibrium kinetic fractionation processes due to evaporation after precipitation. The LMWL at the study site was based on biweekly precipitation samples collected from 2011 to 2020 and is $\delta^2\text{H} = 7.5 \delta^{18}\text{O} + 7.4$ and consistent with the long-term regional precipitation behaviour at Trier (Klaus et al., 2015; Stumpp et al., 2014).

We analysed differences in xylem water isotopic composition ($\delta^2\text{H}$ and $\delta^{18}\text{O}$ and lc-excess) with the software R, version v. 4.0.5 (R Core Team, 2021). Statistical analysis between species, SW and HW were assessed with the nonparametric Mann–Whitney U test, which examines the differences in medians for two independent groups. Statistical differences between the two groups derived from the Mann–Whitney U test are indicated by an asterisk (p -values ≤ 0.05) (Figures 1, 5 and S3). We used $\alpha = 0.05$ to determine statistical significance for all statistical tests. We calculated the difference in tissue water (Δ moisture %), $\delta^2\text{H}$ ($\Delta \delta^2\text{H}$), and $\delta^{18}\text{O}$ ($\Delta \delta^{18}\text{O}$) between SW and HW. We applied a linear regression analysis to test for statistic relationship between Δ moisture and Δ in isotopic composition.

3 | RESULTS

3.1 | SW and HW isotopic composition in coniferous and broadleaved species

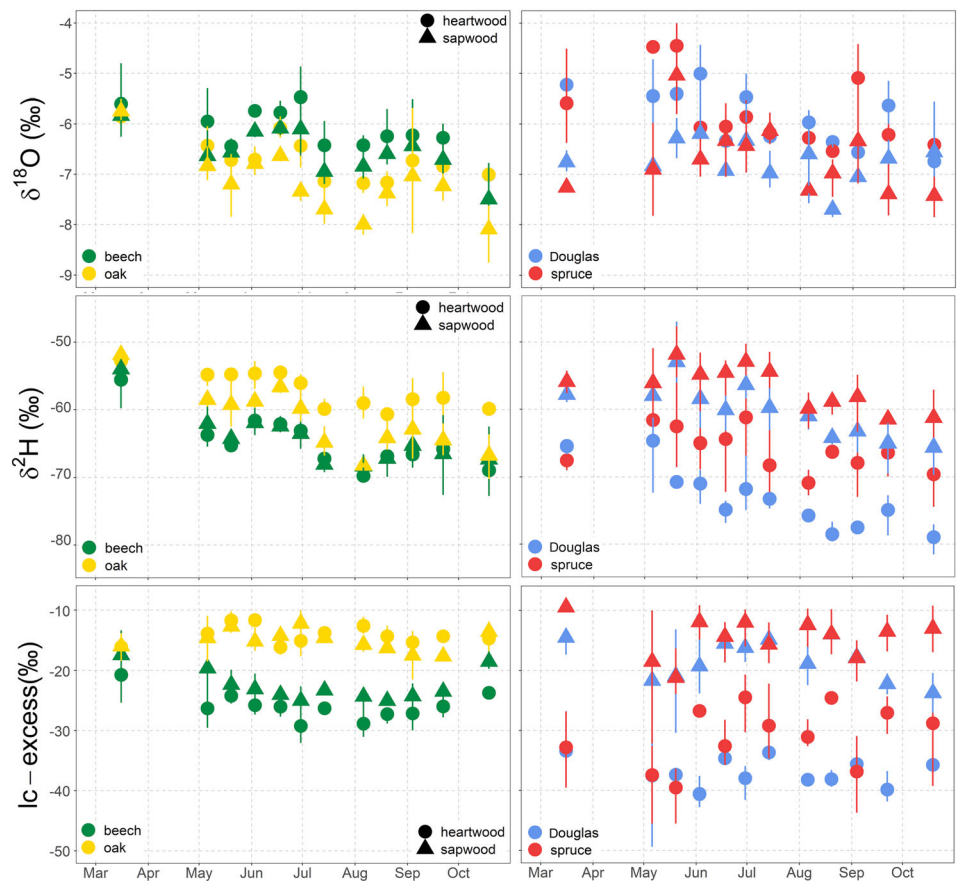
SW isotopic composition was significantly lighter in $\delta^{18}\text{O}$ ($p < 0.01$, Mann–Whitney U test) than HW for the four investigated species (Figure 1). On the contrary, $\delta^2\text{H}$ values were significantly heavier in SW than in HW in conifers but not in beech trees ($p > 0.05$, Mann–Whitney U test) (Figure 1). In oak trees, $\delta^2\text{H}$ values in SW were instead significantly lighter than HW. The studied conifers displayed a prominent difference between SW and HW in both isotopes compared with broadleaved species (Table 1). For both isotopes, the inter-quartile range and variability over the whole sampling season were highest for spruce followed by oak, Douglas fir and beech trees (Table 1, Figure 1). Before leaf-flush in beech and oak trees were, SW and HW displayed a similar isotopic composition in both isotopes but maintained a constant difference at later stages of the growing season (Figure 2). We observed a consistent difference in $\delta^2\text{H}$ throughout the study period in conifers (Figure 2). While we also observed a consistent difference between SW and HW in $\delta^{18}\text{O}$ for Douglas fir, the SW and HW of spruce tended to have similar values from June until the end of July (Figure 2). Relationships between the delta values of SW and HW for ^{18}O and ^2H were statistically significant ($p < 0.05$, shown in Figure S3) for the four investigated species. R^2 values were lower for conifers (0.25 and 0.33 for spruce and Douglas fir, respectively) than broadleaves (0.71 and 0.69 for beech and oak).

3.2 | Temporal variability of the isotopic composition of water from SW and HW

Xylem water from all four species consistently plotted below the LMWL in the dual-isotope space across all sampling campaigns

TABLE 1 Values of mean, standard deviation and interquartile range (IQR) for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ per species and then grouped according to their functional group

	Sapwood				Heartwood			
	$\delta^{18}\text{O}$	IQR $\delta^{18}\text{O}$	$\delta^2\text{H}$	IQR $\delta^2\text{H}$	$\delta^{18}\text{O}$	IQR $\delta^{18}\text{O}$	$\delta^2\text{H}$	IQR $\delta^2\text{H}$
Beech	-6.5 ± 0.6	0.68	-64.4 ± 4.7	5.17	-6.1 ± 0.5	0.83	-64.7 ± 4.1	4.62
Oak	-7.1 ± 0.7	0.97	-61.3 ± 4.9	5.78	-6.7 ± 0.6	1.1	-57.3 ± 3.3	6.39
Douglas fir	-6.5 ± 1.0	0.89	-60.0 ± 4.7	7.71	-5.8 ± 0.9	0.81	-73.8 ± 5.6	6.07
Spruce	-6.6 ± 1.0	1.02	-56.8 ± 4.7	7.18	-5.6 ± 1.0	1.26	-66.5 ± 5.9	7.97
Broadleaves	-6.7 ± 0.7	1.00	-63.0 ± 4.9	6.47	-6.4 ± 0.6	1.01	-60.3 ± 5.3	9.24
Conifers	-6.5 ± 1.0	0.97	-58.0 ± 5.0	6.96	-5.7 ± 0.9	1.16	-69.5 ± 6.3	8.52

FIGURE 2 Isotopic composition (upper and middle panels) and Ic-excess (bottom panel) of xylem water for each sampling campaign across the growing season. Data of each species were averaged. Solid dots represent the mean; the whiskers indicate the confidence interval (95%). For beech trees, sapwood (SW) and heartwood (HW) correspond to inner and outer SW.

(Figures 3 and S2). SW samples from beech trees plotted further away from the LMWL than SW samples from the other three species (Figures 3 and 1). SW samples of spruce plotted closer to the LMWL than any other species. The isotopic signature in SW and HW was highly variable throughout the growing season for the four species (Figures 2 and 3). Xylem water from the first sampling campaign (16 March) before leaves were flushed out from beech and oak was significantly higher in δ -values for both isotopes (Wilcoxon rank sum test, $p < 0.05$) compared with later sampling dates (Figure 2) and plotted in the upper right area in the dual-isotope space (Figure 3). In broadleaved species, both isotopes displayed a progressively lighter

isotopic composition over time (Figure 3). The isotopic composition of conifers displayed a different and less clear pattern than broadleaved species (Figure 3).

3.3 | Relationship between moisture content and xylem isotopic composition

Moisture content (%) (Figure S4) was significantly higher ($p < 0.05$) in SW than in HW for beech, Douglas fir and spruce but not for oak trees. We also observed a stronger temporal variability of moisture

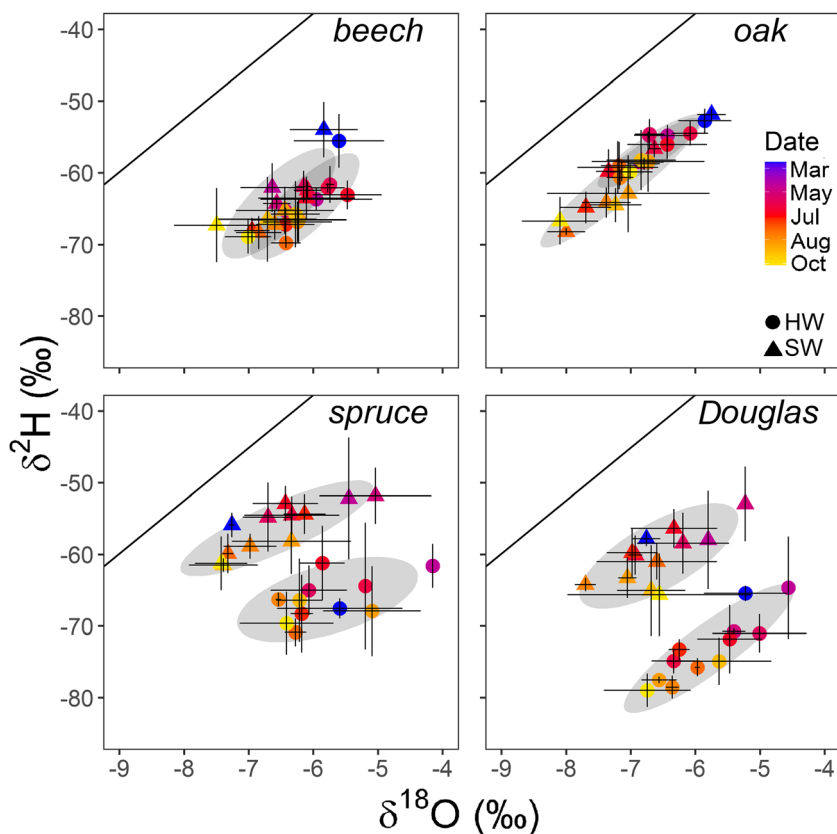


FIGURE 3 Dual-isotope plot of xylem water per species for all sampling campaigns conducted in 2020. Each point represents the average isotopic composition and whiskers indicate standard deviation. The black line indicates the local meteoric water line (LMWL). Ellipses are drawn on the basis of 80% confidence intervals. For beech trees, sapwood (SW) and heartwood (HW) correspond to inner and outer SW.

content in SW than in HW for all species (Figure S5). Correlations between Δ moisture and $\Delta\delta^{18}\text{O}$ were highly significant ($p < 0.001$) (Figure 4) in general but not for each species individually ($p > 0.05$). The relationship between Δ moisture and $\Delta\delta^{18}\text{O}$ was statistically significant, yet the R^2 value was low (Figure 4) showing that Δ moisture is not able to explain the variation in $\Delta\delta^{18}\text{O}$ alone. Conversely, correlations between Δ moisture and $\Delta\delta^2\text{H}$ were highly significant ($p < 0.001$) (Figure 4), not only when all species were considered but also for the individual species ($p < 0.05$). Conifers scored higher Δ -values for moisture content, $\delta^{18}\text{O}$ and $\delta^2\text{H}$ compared with broadleaved species (Table S1). Xylem density was higher in broadleaved species (0.60 and 0.57 g cm^{-3} in beech and oak, respectively) than in conifers (0.49 and 0.38 g cm^{-3} in Douglas fir and spruce trees, respectively).

3.4 | Day-night variability of isotopic composition and moisture content in SW and HW

Due to the limited number of samples (three samples per functional part per species), we could not perform any statistical tests, but we reported the data as mean and standard deviation of $\delta^{18}\text{O}$, $\delta^2\text{H}$ and moisture content in Table 2. HW isotopic composition displayed generally a lighter isotopic composition at night than during the day for all species, while a less consistent pattern was observed in SW. The moisture content (%) of SW tended to increase over night for Douglas fir, oak and beech trees, but it decreased in spruce trees. The moisture content of HW tended to increase overnight only for beech trees and

remained rather constant for the three other species. We did observe a strong differentiation but there were small changes in the SW-HW isotopic composition and moisture content between day- and night-time (Table 2).

3.5 | Xylem depth-related variability of isotopic composition in broadleaved species

For beech trees, xylem $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values were not significantly different between the different sampling depths (i.e. outer, middle, inner) and functional parts (i.e. SW and HW) (Figure 5). Despite the lack of statistical difference, $\delta^{18}\text{O}$ values tended to become progressively heavier with depth. In oak trees, both $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values became progressively heavier with depth and the outer and inner portion of the cores were significantly different (Wilcoxon test, $p < 0.05$) (Figure 5). While moisture content remained unvaried in beech, oak moisture content was significantly higher in the inner part than in the outer part (Figure S6).

4 | DISCUSSION

4.1 | SW and HW do isotopically exchange

We found that both SW and HW displayed a similar temporal variability in their isotopic composition (Figures 2, 3 and S3) suggesting that

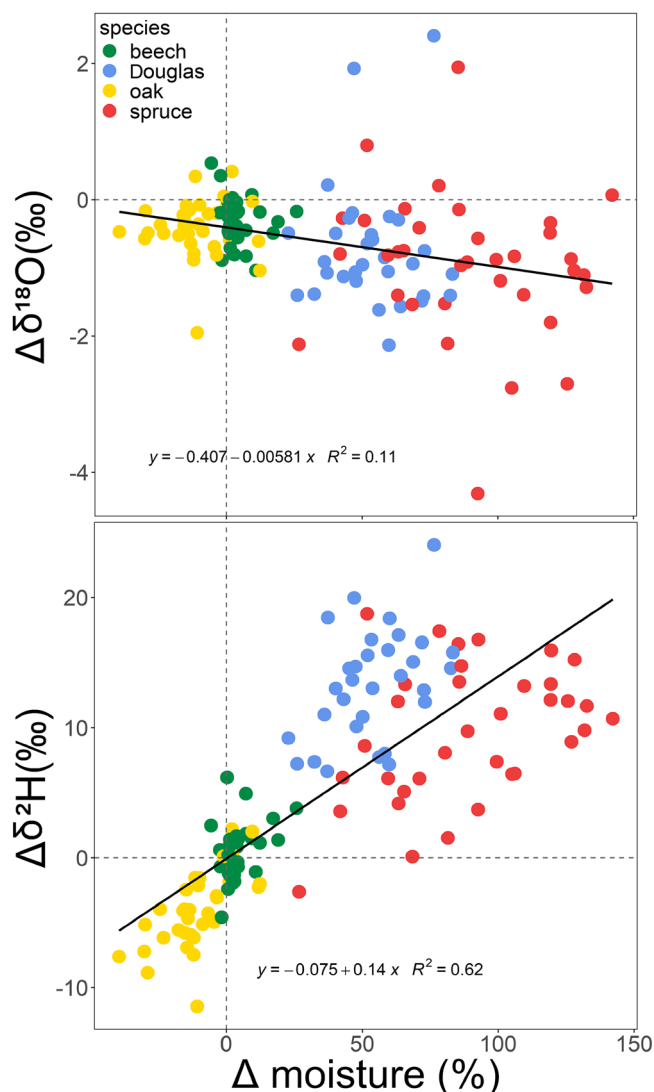


FIGURE 4 Relationship between $\Delta\text{moisture}$ and $\Delta\delta^{18}\text{O}$ (upper plot) and $\Delta\text{moisture}$ and $\Delta\delta^2\text{H}$ (bottom plot) for all species. Δ is the difference in isotopic composition (top panel) and moisture (bottom panel) between sapwood (SW) and heartwood (HW) of the same tree.

the two functional parts were interconnected. If an exchange between SW and HW had not occurred, HW isotopic composition would have displayed a rather constant pattern, if we assume that the isotopically dynamic sap flow is likely the only process able to underlie such isotopic variations. A certain degree of isotopic exchange between the two functional parts would require the radial transport of water in both directions, and this has been observed in previous studies (Gessler, 2021; James et al., 2003; Kitin et al., 2009). Contrary to our results, White et al. (1985) observed that $\delta^2\text{H}$ of HW showed rather uniform values over time, but the authors did not provide a clear interpretation due to the short measurement period in their study. In addition to the biweekly variation, we also observed that in some species, both SW and HW isotopic composition changed even on the day–night scale (Table 2), indicating that SW and HW water exchange can occur at the subdaily time scale.

We observed that SW and HW in broadleaved species displayed similar isotopic composition only when sap flow fluxes were very low or negligible for extended periods, that is, before bud break (Figures 2 and 3). We did not observe the same isotopic difference during night when transpiration ceases (Table 2), which suggests that the isotopic equilibrium between SW and HW would require more than approximately 6 h (time between sunset and sampling time) to be reached. We can envisage that the two functional parts tend to reach an isotopic equilibrium over the dormant season due to the inactivity of the SW and the consequential lack of temporal change in the SW isotopic composition by water uptake. Additionally, during the dormant season, $\delta^{18}\text{O}$ and $\delta^2\text{H}$ showed the heaviest values of the studied period (Figures 2 and 3). The occurrence of the heavy isotopic composition in xylem water during leafless periods was previously reported in other studies (Martín-Gómez et al., 2017; Oerter et al., 2019; Phillips & Ehleringer, 1995) and also observed in the study area in 2019 (Fabiani et al., 2021). This observation has been explained by the evaporation through the bark that occurs over the dormant season when sap flow is ceased (Martín-Gómez et al., 2017; Oerter et al., 2019; Phillips & Ehleringer, 1995). Therefore, the heavy isotopic composition of xylem water observed before leaf flush both in SW and in HW is another indication that SW and HW are not isolated compartments in broadleaved species. At the onset of the growing season, the xylem water of the broadleaved species became progressively lighter in both isotopes and at both functional parts (Figures 2 and 3), likely caused by the input of fresh, nonenriched soil water. However, as the growing season progressed, SW and HW of broadleaves began to display different isotopic composition, as the equilibration process between SW and HW seems to be slower than the rate of change in the isotopic composition in the SW (from root uptake).

In conifers, we observed a consistent difference over time between SW and HW even at the beginning of the growing season (Figures 2 and 3). This is likely due the lack of a complete cessation of transpiration in the dormant season for evergreen species (Chan & Bowling, 2017), which reduces the effect of fractionation through the bark. Overall, the differences between the isotopic composition of water in functional parts of conifers and broadleaved trees (Table 1) might not be exclusively linked to stem hydraulic processes (Martín-Gómez et al., 2017) and xylem anatomy (i.e. water storage capacity, parenchyma fraction, Gessler, 2021; Treydte et al., 2021) but could also be related to the phenology of the species.

Although water does not flow readily between growth rings and water movement is mainly promoted within a growth ring (Gartner, 1995), our observed seasonal variability in isotopic composition in SW and HW (Figures 2 and 3) suggests that isotopic exchange between the functional parts does occur to a certain degree. Consistently with our findings, James et al. (2003) showed through a labelling experiment in tropical species that substantial diffusion of deuterated water occurs from SW to HW through the rays and axial parenchyma. Treydte et al. (2021) injected deuterated water into *Eucalyptus* trees and identified the presence of this tracer even in the HW of the opposite side of the injection point. This was interpreted

TABLE 2 Mean and standard deviation for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ and moisture (%) per species ($n = 3$)

	Sapwood				Heartwood			
	$\delta^{18}\text{O}$ day	$\delta^{18}\text{O}$ night	$\delta^2\text{H}$ day	$\delta^2\text{H}$ night	$\delta^{18}\text{O}$ day	$\delta^{18}\text{O}$ night	$\delta^2\text{H}$ day	$\delta^2\text{H}$ night
Beech	-6.4±0.7	-6.4±0.6	-65.2±3.3	-64.2±4.3	-6.2±0.7	-6.1±0.3	-66.6±3.0	-64.9±1.7
Oak	-7.0±1.2	-7.5±0.4	-62.9±5.3	-64.8±0.6	-6.7±0.9	-7.0±0.4	-58.4±4.2	-60.6±1.9
Douglas fir	-7.0±0.1	-5.9±1.3	-63.0±2.8	-61.0±3.6	-6.5±0.3	-6.8±0.4	-77.5±0.4	-81.8±0.6
Spruce	-6.3±0.9	-7.1±0.5	-58.8±4.5	-62.1±2.2	-5.1±0.7	-6.5±0.5	-67.9±6.2	-72.8±9.9

	Sapwood		Heartwood	
	Moisture (%) day	Moisture (%) night	Moisture (%) day	Moisture (%) night
Beech	59.6±3.6	61.7±3.1	55.3±5.7	60.6±4.8
Oak	74.9±13.7	78.6±21.0	88.8±9.6	86.1±14.1
Douglas fir	81.5±14.3	90.8±3.9	33.3±0.6	33.4±1.1
Spruce	123.3±15.6	112.1±4.2	33.7±1.9	31.7±0.0

Note: In orange are the day-time sampling values; in blue are the night-time sampling values.

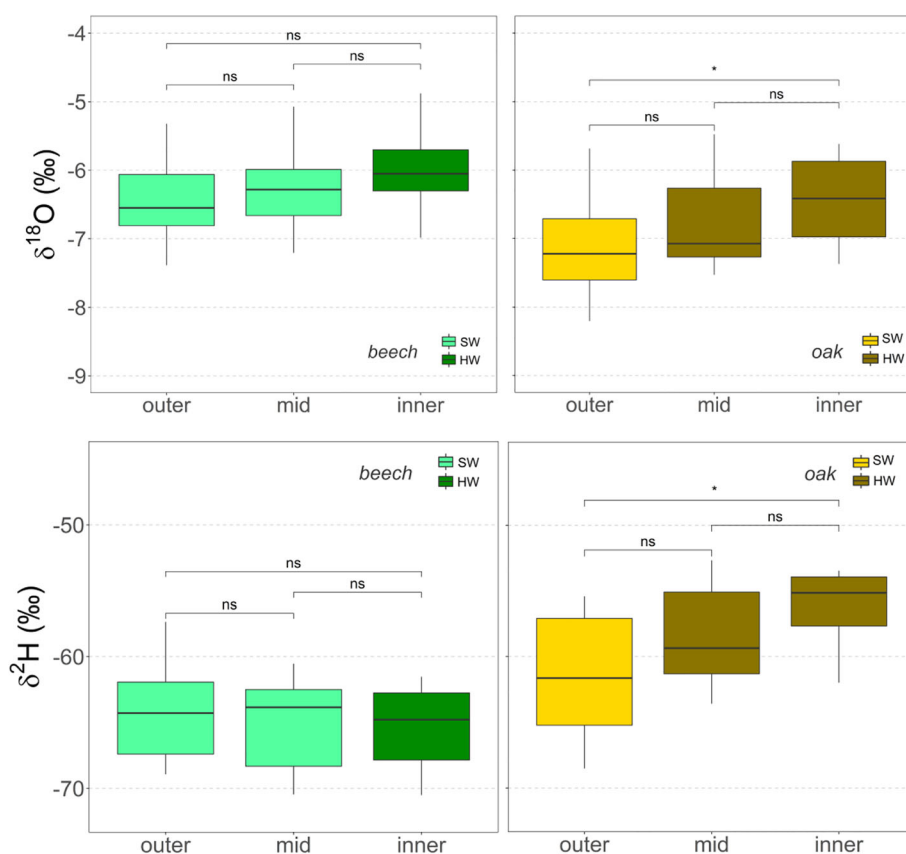


FIGURE 5 $\delta^{18}\text{O}$ (top panels), $\delta^2\text{H}$ (bottom panels) isotopic composition of beech (left plots) and oak (right plots) trees for the different sampling depths and functional parts (sapwood [SW] and heartwood [HW]). The centre line in the boxplot indicates the median, and the lower and upper extremes indicate the first and third quartiles, respectively. The whiskers indicate points within 1.5 times of the interquartile range above or below the median. Above each boxplot, asterisks (symbols ‘*’, ‘ns’ correspond to p -values ≤ 0.05 or > 0.05 , respectively) denote significant statistical differences between the different sampling depth (Wilcoxon test).

as evidence for the circumferential water movement as bulk flow through the SW and the subsequent equilibration with HW. Bulk flow might be allowed by the radial connectivity, which is granted by the intercellular spaces of the xylem participating in gas exchange and water conduction (Kitin et al., 2009; Zhang et al., 2004) and by parenchymatic rays (Pfautsch, 2016; Pfautsch et al., 2015). Parenchyma provides storage for water during water shortages and grants functional connectivity to water transport both in an axial direction by

axial parenchyma (Chen et al., 2020) and radial direction between the inner and outer xylem by ray parenchyma (Steward, 1967). The axial and ray parenchyma run perpendicularly to each other, originating a living network interspaced among fibres and water conduits (Morris et al., 2016). The proportionality of different parenchymatic rays and xylem elements varies among coniferous (3%–12%) (Panshin and de Zeeuw, 1980) and broadleaved species (5%–33%) (Pfautsch et al., 2015; Siau, 1984), and their water contribution to the total

transpiration flux might be related to the tree water status and how easily water is withdrawn from storage (Tyree & Yang, 1990). Conifers are also characterised by the presence of heterogeneous rays, where parenchymatic cells and tracheids run radially (Siau, 1984) and facilitate water transport in a radial direction. Heterogeneous rays are lacking in broadleaved species, but the connectivity among adjacent xylem vessels is granted by intervessel pit pairs that allow water to spread from one vessel to the next at points of vessel to vessel contact (Tyree & Zimmermann, 2002). Intervessel pits size and density vary among species and determine their effectiveness in decreasing resistance to lateral flow and the tangential spread of water (Orians et al., 2004). Although HW is presumed to be characterised by tyloses formation and the complete dieback of living cells (parenchymatic cells), some of the cells may actually survive and pits (which allow water to pass between xylem conduits) may maintain their functionality (Frey-Wyssling & Bosshard, 1959). An additional driving force for the internal radial movement from SW to HW is the water potential gradient between the two (Nakada et al., 2019). Nakada et al. (2019) argued that water moves radially in the vapour phase rather than in the liquid phase. This argument is also consistent with the lighter isotopic composition observed in $\delta^2\text{H}$ of the HW (Figure 1), as the light isotopes are more likely to be transported in higher fractions through the vapour phase. However, we observed the opposite for $\delta^{18}\text{O}$ (Figure 1) leaving the transport path subject to further research.

Our hypothesis of a different degree of compartmentalisation (i.e. isotopic exchange) between broadleaved species and conifers is also supported by the linear relationship observed between $\Delta\delta^{18}\text{O}$ and $\Delta\delta^2\text{H}$ with $\Delta\text{moisture}$ between the two functional parts (Figure 4 and Table S1). When the $\Delta\text{moisture}$ was low (Figure 4), the two functional parts displayed a closer isotopic composition compared with high $\Delta\text{moisture}$. This indicates a higher degree of exchange. Seasonal variations in moisture content in wood are confined largely to SW (Figure 4), confirming that species-specific moisture content of wood in living trees is governed by their anatomy (Steward, 1967). The wood of conifers, which is characterised by a lower density than broadleaved species (see Section 3), is able to absorb more water than broadleaved species (Figure S4). Despite this, HW has limited storage capacity (Figure S4) due to the physiological changes that xylem has encountered during SW-HW conversion (Tyree & Zimmermann, 2002). The seasonal variations in moisture content are less pronounced in the SW-HW of coniferous species than broadleaved species, probably reflecting the hydraulic separation between the conducting portion of the stem and the HW (Holbrook, 1995). An exceptional case among broadleaved species is that of oak (Tomczak et al., 2018), where HW moisture content is comparable and sometimes even exceeds SW moisture content (Figure 4, S4, Table 2). However, the contribution of HW water to the transpiration for this species remains uncertain. It may be that the day–night scale fluctuations of moisture content in the HW of broadleaves indicate the occurrence of water logging and withdrawal of water on the day-time scale. In conifers, we observed a change in the isotopic composition of HW independent of a variation in moisture

content, potentially indicating that HW likely does not contribute significantly to tree transpiration despite the occurrence of an isotopic exchange.

From a methodological point of view, we must bear in mind that the cryogenic water extraction does not only extract water flowing through xylem conduits but rather a mixture of symplastic water, capillary water and fibre water (Barbeta et al., 2022). Thus, we acknowledge that our study incorporates multiple degrees of isotopic heterogeneity occurring not only between different functional parts (i.e. SW and HW) but also at a finer scale between different xylem elements within the same portion of functional xylem. Xylem is composed of conducting elements (i.e. vessels in broadleaved species and tracheids in conifers), parenchyma and fibres (Chen et al., 2020), which contribute to different extent to the total hydraulic functioning of the tree. Water in the intercellular space and xylem conduits is isotopically in-equilibrium (Barbeta et al., 2022), while water in parenchymatic cells and within cell walls has been assumed to be more depleted in hydrogen (Barbeta et al., 2022; Zhao et al., 2016). The most recent literature has focused on whether xylem depletion in hydrogen originates from the isotopic heterogeneity between xylem elements (Barbeta et al., 2022; Zhao et al., 2016) or rather whether it results from the technological limitations of the extraction technique (Chen et al., 2021). It has been argued that cryogenic water extraction has systematically biased $\delta^2\text{H}$ estimates due to the hydrogen exchange of water with organic compounds during distillation (Chen et al., 2021). However, the work of Barbeta et al. (2022) showed that an extraction artefact alone cannot quantitatively explain the depletion in deuterium, and thus, other mechanisms would be behind the relative depletion of water in nonconducting woody cells. Among possible mechanisms, chemical bonding and capillary (i.e. surface) forces could have an effect on the hydrogen (Berry & Roderick, 2005) (and not oxygen) especially when moisture content is low, such as in conifer HW (Figure S4). More research is clearly needed in order to quantify the influence of organics and capillary forces on deuterium exchange during the xylem extraction process and define the implications for tree water uptake studies. Still, both studies agree on the reliability of $\delta^{18}\text{O}$ as a tracer (Barbeta et al., 2022; Chen et al., 2021), pointing to the need to build interpretations on both isotopes.

4.2 | The radial variability in isotopic signal

We observed a progressive transition in isotopic composition with increasing radial depth toward the stem centre suggesting the occurrence of an isotopic gradient or the presence of a transition zone between SW and HW (Figure 5). This might be an indication of high xylem connectivity, granted by intervessel pitting that allows water movement between adjacent vessels (Orians et al., 2004). The increasingly heavier isotopic composition with sampling depth observed in oak trees (Figure 5) may result from the radial variability in xylem sap flux as SW does not conduct water uniformly with depth. The importance of SW as source water for leaf transpiration increases with depth (Cermak et al., 2007), and most of the water flow is largely

confined in the outer SW (Steward, 1967). In ring-porous species such as oak trees, the axial flow is restricted to either the last or last few annual rings (Cermak et al., 1992; Granier et al., 1994; Kozłowski & Winget, 2015; Matheny et al., 2017) where the width of the late-wood vessel creates sufficient transport capacity to supply the entire crown (Gartner, 1995). In oak species, it has been estimated that 80% of flow occurred in the outer portion of the SW, while only some xylem transport occurs in the inner rings. Diffuse porous species such as beech trees rely on multiple growth rings to sustain transpiration (Matheny et al., 2017), which could potentially explain the lack of statistical difference observed in isotopic composition between different sampling depths in the xylem of beech (Figure 5).

The progressive decrease in water flow with depth is explained by two co-occurring phenomena: (i) leaf petioles attached to the xylem that withdraw water from the outer ring (Gartner & Meinzer, 2005) and (ii) the higher flow resistance experienced by the water flow in the inner part of the SW (Ford et al., 2004). As the light isotopes are more likely to be transported in higher fractions through the vapour phase and leave through the bark, we speculate that water stored in older SW might have developed heavier isotopic composition over time. Furthermore, the outer sheath of xylem may be considered the more faithful proxy of the water taken up as it contains a higher percentage of the water which flows to the leaves. As outer xylem sustains the upper and outer crown and inner xylem sustains lower and older branches (Pfautsch, 2016), we expect that different vegetative parts will be supplied by water with a different isotopic composition that matches that of the corresponding xylem depth supplier.

4.3 | Implications of isotope patterns in xylem for ecohydrological studies

Our results add to the recent literature showing how the xylem structure (Barbeta et al., 2022; Zhao et al., 2016) and tree physiology (Martín-gómez et al., 2016) affect xylem water isotopic composition. Although the anatomical structure of xylem provides slow rates of radial movement for water in comparison with the axial transport (Kitin et al., 2009), we showed that this exchange might take place even on the day-time scale for some species. In light of this, we can think of different xylem compartments as potential end-members that complicate the identification of tree water use. This is in agreement with Seeger and Weiler (2021) who highlighted that xylem water incorporates the transient or intermediate sources such as roots, trunks, branches and leaves. It is still unclear to what extent nonuniform isotope signatures might also impact measurements with gas-permeable in situ probes (e.g. Volkman et al., 2016), since they sample a mixture of recent water uptake flowing in vessels and water stored in fibres, parenchymatic cells and intercellular spaces, possibly affecting the interpretation of the measurements. Furthermore, Seeger and Weiler (2021) raised concerns that the measurements with in situ probes might be influenced by the isotopic signature of immobile water stored in the HW impairing the characterisation tree

water uptake. We suggest that the analysis of the tracer breakthrough curve with in situ probes (Kahmen et al., 2021; Seeger & Weiler, 2021) could provide valuable information about the xylem hydraulic capacitance driving the exchange of water between storage compartments and the transpiration stream. As hydraulic capacitance depends on wood density (Köcher et al., 2013), we argue that the breakthrough curve arrival time would be species-specific. Future studies should aim to discriminate between the total bulk of xylem water and conduits water in order to understand how the xylem anatomy might affect the isotopic measurements and ultimately, to better understand tree hydraulic functioning.

5 | SUMMARY AND CONCLUSIONS

Our results provide previously missing evidence of different degrees of water exchange between SW and HW for multiple forest species and functional groups. We found that:

1. Both SW and HW display a variable isotopic composition throughout the growing season and on a day-night scale, but conifers display a higher isotopic compartmentalisation reflected by the consistent isotopic difference between SW and HW compared with broadleaved species as a result of the difference in xylem anatomy and phenology.
2. In broadleaved species, the radial variation in isotopic composition is driven by sampling depth rather than xylem functional parts

With the present work, we hope to draw attention to the need for ecohydrological studies to consider the multidirectionality of water transport in wood and the heterogeneity of the isotopic composition in xylem. Future work should seek to link species-specific xylem hydraulic capacitance with water transport processes to better understand the processes underlying the isotopic exchange between storage compartments and the transpiration stream.

ACKNOWLEDGEMENTS

This work was supported by the Fonds National de la Recherche Luxembourg (AFR/STEP-UP ID 12546983) and by the COST Action “WATSON” CA19120 (www.cost.eu). We thank Enrico Bonanno and Adnan Moussa for their help during the field work. We also thank Adnan Moussa for the soil isotopic data. We thank François Barnich, Enrico Bonanno, Samuele Ceolin, Adnan Moussa, Laurent Pfister and Remy Schoppach for their fruitful input and discussions. We thank Lindsey Auguin for the language editing help. We also thank the Editor and two anonymous reviewers for their comments that greatly helped to improve the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could appear to influence the work reported in this paper. Julian Klaus is an Associated Editor of Ecohydrology at the time of the review process.

AUTHOR CONTRIBUTIONS

Ginevra Fabiani: conceptualisation, investigation, formal analysis, visualisation and writing—original draft. **Daniele Penna:** supervision and writing—review and editing. **Adrià Barbeta:** supervision and writing—review and editing. **Julian Klaus:** conceptualisation, funding acquisition, supervision, validation, writing—review and editing and project administration.

DATA AVAILABILITY STATEMENT

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

ORCID

Ginevra Fabiani  <https://orcid.org/0000-0002-1977-6285>

Daniele Penna  <https://orcid.org/0000-0001-6915-0697>

REFERENCES

- Barbeta, A., Burlett, R., Martín-Gómez, P., Fréjaville, B., Devert, N., Wingate, L., Domec, J., & Ogée, J. (2022). Evidence for distinct isotopic compositions of sap and tissue water in tree stems: Consequences for plant water source identification. *The New Phytologist*, 233, 1121–1132. <https://doi.org/10.1111/nph.17857>
- Berry, S. L., & Roderick, M. L. (2005). Plant–water relations and the fibre saturation point. *The New Phytologist*, 168, 25–37. <https://doi.org/10.1111/j.1469-8137.2005.01528.x>
- Berry, Z. C., Evaristo, J., Moore, G., Poca, M., Steppe, K., Verrot, L., Asbjornsen, H., Borma, L. S., Bretfeld, M., Herve-Fernandez, P., Seyfried, M., Schwendenmann, L., Sinacore, K., De Wispelaere, L., & McDonnell, J. (2017). The two water worlds hypothesis: Addressing multiple working hypotheses and proposing a way forward. *Ecohydrology*, 1–10, 229–236. [https://doi.org/10.1002/\(SICI\)1096-9063\(199703\)49:3<229::AID-PS525>3.0.CO;2-T](https://doi.org/10.1002/(SICI)1096-9063(199703)49:3<229::AID-PS525>3.0.CO;2-T)
- Betsch, P., Bonal, D., Breda, N., Montpied, P., Peiffer, M., Tuzet, A., & Granier, A. (2011). Drought effects on water relations in beech: The contribution of exchangeable water reservoirs. *Agricultural and Forest Meteorology*, 151, 531–543. <https://doi.org/10.1016/j.agrformet.2010.12.008>
- Beyer, M., & Penna, D. (2021). On the Spatio-Temporal Under-Representation of Isotopic Data in Ecohydrological Studies. *Frontiers in Water*, 3, 1–9. <https://doi.org/10.3389/frwa.2021.643013>
- Cermak, J., Cienciala, E., Kucera, J., & Hallgren, J.-E. (1992). Radial velocity profiles of water flow in trunks of Norway spruce and oak and the response of spruce to severing. *Tree Physiology*, 10, 367–380. <https://doi.org/10.1093/treephys/10.4.367>
- Cermak, J., Kucera, J., Bauerle, W. L., Phillips, N., & Hinckley, T. M. (2007). Tree water storage and its diurnal dynamics related to sap flow and changes in stem volume in old-growth Douglas-fir trees. *Tree Physiology*, 27, 181–198. <https://doi.org/10.1093/treephys/27.2.181>
- Cernusak, L. A., Barbour, M. M., Arndt, S. K., Cheesman, A. W., English, N. B., Feild, T. S., Helliiker, B. R., Holloway-Phillips, M. M., Holtum, J. A. M., Kahmen, A., Mcinerney, F. A., Munksgaard, N. C., Simonin, K. A., Song, X., Stuart-Williams, H., West, J. B., & Farquhar, G. D. (2016). Stable isotopes in leaf water of terrestrial plants. *Plant, Cell & Environment*, 39, 1087–1102. <https://doi.org/10.1111/pce.12703>
- Chan, A. M., & Bowling, D. R. (2017). Assessing the thermal dissipation sap flux density method for monitoring cold season water transport in seasonal snow-covered forests. *Tree Physiology*, 37, 984–995. <https://doi.org/10.1093/treephys/tpx049>
- Chen, Y., Helliiker, B. R., Tang, X., Li, F., Zhou, Y., & Song, X. (2021). Stem water cryogenic extraction biases estimation in deuterium isotope composition of plant source water. *Proceedings of the National Academy of Sciences of the United States of America*, 117, 33345–33350. <https://doi.org/10.1073/PNAS.2014422117>
- Chen, Z., Zhu, S., Zhang, Y., Luan, J., Li, S., Sun, P., Wan, X., & Liu, S. (2020). Tradeoff between storage capacity and embolism resistance in the xylem of temperate broadleaf tree species. *Tree Physiology*, 40, 1029–1042. <https://doi.org/10.1093/treephys/tpaa046>
- de la Casa, J., Barbeta, A., Rodríguez-Uña, A., Wingate, L., Ogée, J., & Gimeno, T. E. (2022). Isotopic offsets between bulk plant water and its sources are larger in cool and wet environments. *Hydrology and Earth System Sciences*, 26, 4125–4146. <https://doi.org/10.5194/hess-26-4125-2022>
- Dubbert, M., & Werner, C. (2018). Water fluxes mediated by vegetation: Emerging isotopic insights at the soil and atmosphere interfaces. *The New Phytologist*, 221, 1754–1763. <https://doi.org/10.1111/nph.15547>
- Fabiani, G., Schoppach, R., Penna, D., & Klaus, J. (2021). Transpiration patterns and water use strategies of beech and oak trees along a hillslope. *Ecohydrology*, 15, e2382. <https://doi.org/10.1002/eco.2382>
- Ford, C. R., Goranson, C. E., Mitchell, R. J., Will, R. E., & Teskey, R. O. (2004). Diurnal and seasonal variability in the radial distribution of sap flow: Predicting total stem flow in Pinus taeda trees. *Tree Physiology*, 24, 951–960. <https://doi.org/10.1093/treephys/24.9.951>
- Frey-Wyssling, A., & Bosshard, H. (1959). Cytology of the ray cells in sapwood, and heartwood. *Holzforschung*, 13, 129–137. <https://doi.org/10.1515/hfsg.1959.13.5.129>
- Gartner, B. L. (1995). *Plant stems - physiology and functional morphology*. Elsevier.
- Gartner, B. L., & Meinzer, F. C. (2005). Structure-function relationships in sapwood water transport and storage. In: *Vascular Transport in Plants* (pp. 307–331).
- Gebauer, T., Horna, V., & Leuschner, C. (2008). Variability in radial sap flux density patterns and sapwood area among seven co-occurring temperate broad-leaved tree species. *Tree Physiology*, 28, 1821–1830. <https://doi.org/10.1093/treephys/28.12.1821>
- Gessler, A. (2021). Water transport in trees—The importance of radial and circumferential transport. *Tree Physiology*, 41, 2245–2247. <https://doi.org/10.1093/treephys/tpab131>
- Gessler, A., Bächli, L., Rouholahnejad Freund, E., Treydte, K., Schaub, M., Haeni, M., Weiler, M., Seeger, S., Marshall, J., Hug, C., Zweifel, R., Hagedorn, F., Rigling, A., Saurer, M., & Meusburger, K. (2021). Drought reduces water uptake in beech from the drying topsoil, but no compensatory uptake occurs from deeper soil layers. *The New Phytologist*, 233, 194–206. <https://doi.org/10.1111/nph.17767>
- Goldsmith, G. R., Allen, S. T., Braun, S., Engbersen, N., González-Quijano, C. R., Kirchner, J. W., & Siegwolf, R. T. W. (2019). Spatial variation in throughfall, soil, and plant water isotopes in a temperate forest. *Ecohydrology*, 12, 1–11. <https://doi.org/10.1002/eco.2059>
- Goldsmith, G. R., Muñoz-Villers, L. E., Holwerda, F., McDonnell, J. J., Asbjornsen, H., & Dawson, T. E. (2012). Stable isotopes reveal linkages among ecohydrological processes in a seasonally dry tropical montane cloud forest. *Ecohydrology*, 5, 779–790. <https://doi.org/10.1002/eco.268>
- Granier, A., Anfodillo, T., Sabatti, M., Cochard, H., Dreyer, E., Tomasi, M., Valentini, R., & Breda, N. (1994). Axial and radial water flow in the trunks of oak trees: A quantitative and qualitative analysis. *Tree Physiology*, 14, 1383–1396. <https://doi.org/10.1093/treephys/14.12.1383>
- Hillis, W. E. (1987). *Heartwood and tree exudates, springer series in wood science*. Springer Berlin Heidelberg. <https://doi.org/10.1007/978-3-642-72534-0>
- Hissler, C., Martínez-Carreras, N., Barnich, F., Gourdol, L., Iffly, J. F., Juilleret, J., Klaus, J., & Pfister, L. (2021). The Weierbach experimental catchment in Luxembourg: A decade of critical zone monitoring in a temperate forest - from hydrological investigations to ecohydrological

- perspectives. *Hydrological Processes*, 35, 1–7. <https://doi.org/10.1002/hyp.14140>
- Holbrook, M. N. (1995). Stem Water Storage. In *Plant Stem* (pp. 151–174). Academic Press. <https://doi.org/10.1016/b978-012276460-8/50009-6>
- James, S. A., Meinzer, F. C., Goldstein, G., Woodruff, D., Jones, T., Restom, T., Mejia, M., Clearwater, M., & Campanello, P. (2003). Axial and radial water transport and internal water storage in tropical forest canopy trees. *Oecologia*, 134, 37–45. <https://doi.org/10.1007/s00442-002-1080-8>
- Kahmen, A., Buser, T., Hoch, G., Grun, G., & Dietrich, L. (2021). Dynamic 2H irrigation pulse labelling reveals rapid infiltration and mixing of precipitation in the soil and species-specific water uptake depths of trees in a temperate forest. *Ecohydrology*, 14, 1–15. <https://doi.org/10.1002/eco.2322>
- Kitin, P., Fujii, T., Abe, H., & Takata, K. (2009). Anatomical features that facilitate radial flow across growth rings and from xylem to cambium in *Cryptomeria japonica*. *Annals of Botany*, 103, 1145–1157. <https://doi.org/10.1093/aob/mcp050>
- Klaus, J., Chun, K. P., & Stumpp, C. (2015). Temporal trends in $\delta^{18}\text{O}$ composition of precipitation in Germany: Insights from time series modelling and trend analysis. *Hydrological Processes*, 29, 2668–2680. <https://doi.org/10.1002/hyp.10395>
- Knighton, J., Kuppel, S., Smith, A., Soulsby, C., Sprenger, M., & Tetzlaff, D. (2020). Using isotopes to incorporate tree water storage and mixing dynamics into a distributed Ecohydrologic modelling framework. *Ecohydrology*, 13, e2201. <https://doi.org/10.1002/eco.2201>
- Köcher, P., Horna, V., & Leuschner, C. (2013). Stem water storage in five coexisting temperate broad-leaved tree species: Significance, temporal dynamics and dependence on tree functional traits. *Tree Physiology*, 33, 817–832. <https://doi.org/10.1093/treephys/tpt055>
- Kozłowski, T. T., & Winget, C. H. (2015). Patterns of water movement in Forest trees. *Botanical Gazette*, 124, 301–311. <https://doi.org/10.2307/2472915>
- Kravka, M., Krejzar, T., & Čermák, J. (1999). Water content in stem wood of large pine and spruce trees in natural forests in Central Sweden. *Agricultural and Forest Meteorology*, 98–99, 555–562. [https://doi.org/10.1016/S0168-1923\(99\)00123-9](https://doi.org/10.1016/S0168-1923(99)00123-9)
- Kühnhammer, K., Dahlmann, A., Iraheta, A., Gerchow, M., Birkel, C., Marshall, J. D., & Beyer, M. (2021). Continuous in situ measurements of water stable isotopes in soils, tree trunk and root xylem: Field approval. *Rapid Communications in Mass Spectrometry*, 36, e9232. <https://doi.org/10.1002/rcm.9232>
- Landwehr, J. M., & Coplen, T. B. (2004). Line-conditioned excess: A new method for characterizing stable hydrogen and oxygen isotope ratios in hydrologic systems. In: *Isotopes in Environmental Studies* (pp. 132–135).
- Martín-gómez, P., Serrano, L., & Ferrio, J. P. (2016). Short-term dynamics of evaporative enrichment of xylem water in woody stems: Implications for ecohydrology. *Tree Physiology*, 37, 511–522. <https://doi.org/10.1093/treephys/tpw115>
- 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, 511–522. <https://doi.org/10.1093/treephys/tpw115>
- Matheny, A. M., Fiorella, R. P., Bohrer, G., Poulsen, C. J., Morin, T. H., Wunderlich, A., Vogel, C. S., & Curtis, P. S. (2017). Contrasting strategies of hydraulic control in two codominant temperate tree species. *Ecohydrology*, 10, 1–16. <https://doi.org/10.1002/eco.1815>
- Meißner, M., Köhler, M., Schwendenmann, L., & Hölscher, D. (2012). Partitioning of soil water among canopy trees during a soil desiccation period in a temperate mixed forest. *Biogeosciences*, 9, 3465–3474. <https://doi.org/10.5194/bg-9-3465-2012>
- Meißner, M., Köhler, M., Schwendenmann, L., Hölscher, D., & Dyckmans, J. (2014). Soil water uptake by trees using water stable isotopes ($\delta^2\text{H}$ and $\delta^{18}\text{O}$)—a method test regarding soil moisture, texture and carbonate. *Plant and Soil*, 376, 327–335. <https://doi.org/10.1007/s11104-013-1970-z>
- Morris, H., Brodersen, C., Schwarze, F. W. M. R., & Jansen, S. (2016). The Parenchyma of Secondary Xylem and Its Critical Role in Tree Defense against Fungal Decay in Relation to the CODIT Model. *Frontiers in Plant Science*, 7, 1–18. <https://doi.org/10.3389/fpls.2016.01665>
- Muñoz-Villers, L. E., Holwerda, F., Alvarado-Barrientos, M. S., Geissert, D. R., & Dawson, T. E. (2018). Reduced dry season transpiration is coupled with shallow soil water use in tropical montane forest trees. *Oecologia*, 188, 303–317. <https://doi.org/10.1007/s00442-018-4209-0>
- Nakada, R., Okada, N., Nakai, T., Kuroda, K., & Nagai, S. (2019). Water potential gradient between sapwood and heartwood as a driving force in water accumulation in wetwood in conifers. *Wood Science and Technology*, 53, 407–424. <https://doi.org/10.1007/s00226-019-01081-4>
- Nawrot, M., Pazdrowski, W., & Szymański, M. (2008). Dynamics of heartwood formation and axial and radial distribution of sapwood and heartwood in stems of European larch (*Larix decidua* mill.). *Journal of Forest Science*, 54, 409–417. <https://doi.org/10.17221/30/2008-jfs>
- Oerter, E. J., Siebert, G., Bowling, D. R., & Bowen, G. (2019). Soil water vapour isotopes identify missing water source for streamside trees. *Ecohydrology*, 12, e2083. <https://doi.org/10.1002/eco.2083>
- Orians, C. M., Van Vuuren, M. M. I., Harris, N. L., Babst, B. A., & Ellmore, G. S. (2004). Differential sectoriality in long-distance transport in temperate tree species: Evidence from dye flow, 15N transport, and vessel element pitting. *Trees*, 18, 501–509. <https://doi.org/10.1007/s00468-004-0326-y>
- Orłowski, N., Breuer, L., & McDonnell, J. J. (2016). Critical issues with cryogenic extraction of soil water for stable isotope analysis. *Ecohydrology*, 9, 3–10. <https://doi.org/10.1002/eco.1722>
- Panshin, A. J., & de Zeeuw, C. (1980). *Textbook of wood technology: Structure, identification, properties, and uses of the commercial woods of the United States* (4th ed., 722 pp). McGraw-Hill.
- Peck, E. C. (1953). The sap of moisture in wood. U. S Dept. Agr. For. Serv. For. Prod. Lab. Rpt. No.
- Penna, D., Hopp, L., Scandellari, F., Allen, S. T., Benetton, P., Beyer, M., Geris, J., Klaus, J., Marshall, J. D., Schwendenmann, L., Volkmann, T. H. M., von Freyberg, J., Amin, A., Ceperley, N., Engel, M., Frentress, J., Giambastiani, Y., McDonnell, J. J., Zuecco, G., ... Kirchner, J. W. (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. <https://doi.org/10.5194/bg-15-6399-2018>
- Penna, D., Stenni, B., Šanda, M., Wrede, S., Bogaard, T. A., Michelini, M., Fischer, B. M. C., Gobbi, A., Mantese, N., Zuecco, G., Borga, M., Bonazza, M., Sobotková, M., Čejková, B., & Wassenaar, L. I. (2012). Technical note: Evaluation of between-sample memory effects in the analysis of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of water samples measured by laser spectrometers. *Hydrology and Earth System Sciences*, 16, 3925–3933. <https://doi.org/10.5194/hess-16-3925-2012>
- Pfautsch, S. (2016). Hydraulic anatomy and function of trees—Basics and critical developments. *Current Forestry Reports*, 2, 236–248. <https://doi.org/10.1007/s40725-016-0046-8>
- Pfautsch, S., Hölttä, T., & Mencuccini, M. (2015). Hydraulic functioning of tree stems - fusing ray anatomy, radial transfer and capacitance. *Tree Physiology*, 35, 706–722. <https://doi.org/10.1093/treephys/tpv058>
- Phillips, N. G., Ryan, M. G., Bond, B. J., McDowell, N. G., Hinckley, T. M., & Čermák, J. (2003). Reliance on stored water increases with tree size in three species in the Pacific northwest. *Tree Physiology*, 23, 237–245. <https://doi.org/10.1093/treephys/23.4.237>
- Phillips, S. L., & Ehleringer, J. R. (1995). Limited uptake of summer precipitation by bigtooth maple (*Acer grandidentatum* Nutt) and Gambel's

- oak (*Quercus gambelii* Nutt). *Trees*, 9, 214–219. <https://doi.org/10.1007/BF00195275>
- Poca, M., Coomans, O., Urcelay, C., Zeballos, S. R., 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. <https://doi.org/10.1007/s11104-019-04139-1>
- R Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Rodriguez, N. B., & Klaus, J. (2019). Catchment travel times from composite StorAge selection functions representing the superposition of streamflow generation processes. *Water Resources Research*, 55, 9292–9314. <https://doi.org/10.1029/2019WR024973>
- Schoppach, R., Chun, K. P., He, Q., Fabiani, G., & Klaus, J. (2021). Species-specific control of DBH and landscape characteristics on tree-to-tree variability of sap velocity. *Agricultural and Forest Meteorology*, 307, 108533. <https://doi.org/10.1016/j.agrformet.2021.108533>
- Seeger, S., & Weiler, M. (2021). Temporal dynamics of tree xylem water isotopes: In-situ monitoring and modelling. *Biogeosciences Discussions*, 18, 4603–4627. <https://doi.org/10.5194/bg-2021-35>
- Siau, J. F. (1984). *Transport processes in wood, springer series in wood science*. Springer Berlin Heidelberg. <https://doi.org/10.1007/978-3-642-69213-0>
- Spicer, R. (2005). *Senescence in secondary Xylem: Heartwood Formation as an Active Developmental Program, Vascular Transport in Plants*. Elsevier Inc. <https://doi.org/10.1016/B978-012088457-5/50024-1>
- Steppe, K., De Pauw, D. J. W., Doody, T. M., & Teskey, R. O. (2010). A comparison of sap flux density using thermal dissipation, heat pulse velocity and heat field deformation methods. *Agricultural and Forest Meteorology*, 150, 1046–1056. <https://doi.org/10.1016/j.agrformet.2010.04.004>
- Steward, C. M. (1967). Moisture content of living trees. *Nature*, 216, 615–616. <https://doi.org/10.1038/216615a0>
- Stumpp, C., Klaus, J., & Stichler, W. (2014). Analysis of long-term stable isotopic composition in German precipitation. *Journal of Hydrology*, 517, 351–361. <https://doi.org/10.1016/j.jhydrol.2014.05.034>
- Taneda, H., Yazaki, K., Hiramatsu, T., Shimizu, B., Sugiura, D., & Miyazawa, Y. (2021). A simple method to observe water distribution in tracheid-bearing wood of subalpine conifer. *Trees*, 35, 697–707. <https://doi.org/10.1007/s00468-020-02070-y>
- Tetzlaff, D., Buttle, J., Carey, S. K., Kohn, M. J., Laudon, H., McNamara, J. P., Smith, A., Sprenger, M., & Soulsby, C. (2021). Stable isotopes of water reveal differences in plant – Soil water relationships across northern environments. *Hydrological Processes*, 35, 1–19. <https://doi.org/10.1002/hyp.14023>
- Tomczak, A., Tomczak, K., Rutkowski, N. S. K., Wenda, M., & Jelonek, T. (2018). The gradient of wood moisture within-stem of sessile oak (*Quercus Petraea* [matt.] Liebl.) in summer. *Wood Research*, 63, 809–820.
- Treydte, K., Lehmann, M. M., Wyczesany, T., & Pfautsch, S. (2021). Radial and axial water movement in adult trees recorded by stable isotope tracing. *Tree Physiology*, 1–14, 2248–2261. <https://doi.org/10.1093/treephys/tpab080>
- Tyree, M., & Zimmermann, M. H. (2002). *Xylem structure and the ascent of sap* (2nd ed.). Springer. <https://doi.org/10.1007/978-3-662-04931-0>
- Tyree, M. T., & Yang, S. (1990). Water-storage capacity of Thuja, Tsuga and Acer stems measured by dehydration isotherms - the contribution of capillary water and cavitation. *Planta*, 182, 420–426. <https://doi.org/10.1007/BF02411394>
- Volkman, T. H. M., 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. <https://doi.org/10.1111/nph.13868>
- von Freyberg, J., Allen, S. T., Grossiord, C., & Dawson, T. E. (2020). Plant and root-zone water isotopes are difficult to measure, explain, and predict: Some practical recommendations for determining plant water sources. *Methods in Ecology and Evolution*, 11, 1352–1367. <https://doi.org/10.1111/j.2041-210x.2010.00016.x>
- Wang, H., Tetzlaff, D., & Soulsby, C. (2019). Hysteretic response of sap flow in Scots pine (*Pinus sylvestris*) to meteorological forcing in a humid low-energy headwater catchment. *Ecohydrology*, 12, e2125. <https://doi.org/10.1002/eco.2125>
- White, J. W. C., Cook, E. R., Lawrence, J. R., & Wallace, S. B. (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. [https://doi.org/10.1016/0016-7037\(85\)90207-8](https://doi.org/10.1016/0016-7037(85)90207-8)
- Yakir, D. (1992). Water compartmentation in plant tissue: Isotopic evidence. In *Water and Life* (pp. 205–222). Springer. https://doi.org/10.1007/978-3-642-76682-4_13
- Zhang, C., Fujita, M., & Takabe, K. (2004). Extracellular diffusion pathway for heartwood substances in *Albizia julibrissin* Durazz. *Holzforchung*, 58, 495–500. <https://doi.org/10.1515/HF.2004.075>
- Zhao, L., Wang, L., Cernusak, L. A., 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. <https://doi.org/10.1111/pce.12753>
- Zhao, P., Sprenger, M., Barzegar, R., Tang, X., & Adamowski, J. (2022). Similar isotopic biases of plant stems bulk water from different water sources by cryogenic vacuum distillation demonstrated through rehydration experiments. *Geophysical Research Letters*, 49, e2021GL096474. <https://doi.org/10.1029/2021GL096474>
- Zweifel, R., & Sterck, F. (2018). A conceptual tree model explaining legacy effects on stem growth. *Frontiers in Forests and Global Change*, 1, 1–9. <https://doi.org/10.3389/ffgc.2018.00009>

SUPPORTING INFORMATION

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

How to cite this article: Fabiani, G., Penna, D., Barbata, A., & Klaus, J. (2022). Sapwood and heartwood are not isolated compartments: Consequences for isotope ecohydrology. *Ecohydrology*, e2478. <https://doi.org/10.1002/eco.2478>