Tree- and stand-scale variability of xylem water stable isotope signatures in mature beech, oak, and spruce

Fabian Bernhard[1], Marius G. Floriancic[2], Kerstin Treydte[1], Arthur Gessler[1], James Kirchner[1], and Katrin Meusburger[1]

[1]Eidgenossische Forschungsanstalt fur Wald Schnee und Landschaft WSL
[2]Eidgenossische Technische Zurich Hochschule Departement Umweltsystemwissenschaften

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Fabian Bernhard[1,2] | Marius G. Floriancic[2,3] | Kerstin Treydte[1] | Arthur Gessler[1,2] | James W. Kirchner[1,2] | Katrin Meusburger[1]

[1]Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf, Switzerland

[2]Department of Environmental Systems Science, ETH Zürich, Zürich, Switzerland

[3]Department of Civil, Environmental and Geomatic Engineering, ETH Zürich, Zürich, Switzerland

Correspondence
Fabian Bernhard, Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf, Switzerland.
Email: fbernhard@ethz.ch; fabian.bernhard@wsl.ch

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Abstract

In ecohydrology, water isotopologues are used to assess potential sources of root water uptake by comparing xylem water signatures with source water signatures. Such comparisons are affected by the variability and uncertainty of the isotope signatures of plant water and water sources. The tree-scale and stand-scale variabilities of the isotope signatures in stem xylem water are often unknown but are important for sampling design and uncertainty estimation in assessing the sources of tree water uptake. Here, we quantified tree-scale and stand-scale variabilities of xylem water isotope signatures in beech, oak and spruce trees in a mature forest on the Swiss plateau. For stem xylem water, sub-daily replicates and replicates in different cardinal directions showed no systematic differences, but we found systematic differences with sampling height. The observed variability of isotope signatures at different heights along the stem suggests that water residence times within trees need to be considered, along with their effects on the isotope signatures in different compartments (stem, branches, leaves). Further, concerning the hydrogen signatures, we found height- and species-specific offsets (SW-excess δ^2H). Stem xylem water's tree-scale variability was similar in magnitude to its stand-scale variability and smaller than the variabilities in branch xylem and bulk soil water around each tree. Xylem water from stem cores close to the ground, therefore, can give a more precise estimate of the isotopic signal of the most recent root water uptake and facilitate more accurate source water attribution.

KEYWORDS
cryogenic vacuum extraction, measurement error, plant waters, root water uptake, stable water isotopes, stem xylem, uncertainty quantification, Waldlabor Zürich

1 | INTRODUCTION

Isotopologues of the water molecule are useful tools for estimating water partitioning and assessing water fluxes in the critical zone. Among their many other applications, hydrogen (δ^2H) and oxygen ($\delta^{18}O$) isotope signatures are widely used to study changes in root water uptake (RWU) of plants in laboratory settings and of forest trees in mature stands (e.g., Barbeta et al., 2020; Brinkmann et al., 2018). The method can be applied to infer water sources of individual trees as well as of entire tree stands. An implicit assumption is

that the sampled xylem water represents the entire water taken up by the root system of a tree or tree stand during a certain period of interest. Widely used methods for xylem water sampling in trees include the collection of branches or leaves followed by water extraction (Benettin et al., 2021; Brinkmann et al., 2019; Treydte et al., 2014) or in-situ vapour equilibration probes installed in stems, branches or roots (Gessler et al., 2022; Marshall et al., 2020; Oerter & Bowen, 2017; Volkmann et al., 2016). Tree stems can also be sampled with increment borers (De Deurwaerder et al., 2020; Zhao et al., 2016) or with drills (Sohel et al., 2023; Vega-Grau et al., 2021), which has the advantage of easy access even in larger trees and does not require selecting branches with variable exposure to the sun. In addition, sampling at the stem is relatively time-efficient. However, core sampling is destructive, so repeated sampling requires varying the orientation of the cores, thereby probing different xylem conduits and thus water pathways.

Stem xylem water typically contains a mixture from different (older and newer) sources that can be stored in the stem tissues. Various compartments of trees are hydraulically connected along the transpiration stream (i.e., water flowing through roots, stem, branches, twigs, leaves) but also across tissue boundaries, e.g., through a hydraulic connection between xylem and phloem tissues (Treydte et al., 2021). The physiology of the hydraulic network depends on the tree species; such differences in the hydraulic network can affect the degree of circumferential mixing (Treydte et al., 2021; Zanne et al., 2006) and alter sampling biases in various ways.

Water movement through the hydraulic pathways of trees is not instantaneous but instead occurs through xylem conduits with finite sap-flow velocities controlled by their flow resistances and pressure gradients (Sperry, 1995). Thus, lag times exist across the different compartments of a tree. Hence any shift in source water causes a transient response. Shifts in root water uptake and/or sap-flow velocities prior to sampling could lead to a spatial distribution of multiple source water pools – taken up at different times or from different soil layers – that are simultaneously present inside an individual tree. The topology of the root system and lack of mixing across root and xylem-flow conduits could propagate spatial variability in the sourced soil waters into the tree xylem and lead to isotopic variations with sampling height and cardinal direction. Thus, differences in the sampling strategy – e.g. between stem xylem sampling and branch xylem sampling (Amin et al., 2021) – could alter the mixture of source water pools measured, exhibit different sampling biases, and result in different spatiotemporal representativeness. Isotopic signatures of available soil water pools typically vary with depth down to more than 1 m (Allen et al., 2022; Sprenger et al., 2019). They can also vary laterally due to spatially correlated interception (Goldsmith et al., 2019) and spatial differences in root water uptake or evaporative fractionation. When an individual tree is sampled, spatial and temporal variation in the measured isotope signature can lead to uncertainties. Potential sources of variation, and thus of uncertainties in the tree's xylem water isotope signature, include the time, height and cardinal direction of sampling, as well as the compartments of the tree (i.e. stem, branch) being sampled. Within tree stands, i.e., across multiple individual trees growing close to each other, isotope signatures may vary at scales beyond individual trees, reflecting (for example) spatial heterogeneities in source waters or differences in individual trees' root systems. These variations could be distinct between species or even between individual trees of the same species in a single stand.

Besides the variations in isotope signatures due to spatiotemporal factors, methodological artefacts cannot be ruled out and should always be considered when interpreting observations. Cryogenically extracted plant waters have exhibited inconsistencies between the oxygen and hydrogen isotopologues when compared with soil waters. These offsets appeared larger for δ^2H than for $\delta^{18}O$ (Barbeta et al., 2020; Chen et al., 2020; de la Casa et al., 2022; Diao et al., 2022; Zhao, 2021). Because of this, our study focused on $\delta^{18}O$. Nevertheless, δ^2H observations are also presented, providing further evidence of inconsistencies between the two isotopologues when comparing extracted bulk soil and xylem waters.

Previous studies have assessed variations in xylem water signatures using naturally occurring isotopes or artificial application of isotopic tracers. Their findings are summarized by the potential sources of uncertainty as follows:

1.1 | Sampling time

Sub-daily variations of xylem waters in stems, (suberized) branches or roots have been observed previously without identifying regular patterns of diel isotopic variability (De Deurwaerder et al., 2020; Magh et al., 2020; Nehemy et al., 2022; Zhao et al., 2016). Regular sub-daily patterns have been observed in unsuberized branches or leaf waters (Cernusak et al., 2005; Dawson & Ehleringer, 1993). Sub-daily isotopic variability tended to be lower in stems than in root and branch xylem (Zhao et al., 2016), and lower in branch xylem than in branch phloem (Nehemy et al., 2022). Spatial variability within the plant could potentially explain the irregular patterns of sub-daily variability in plant water signatures in stems, (suberized) branches or roots in many of these studies: given the destructive nature of the sampling, different branches or roots needed to be sampled throughout a day. Such a potential explanation would align with the observations of lower sub-daily variability in stem samples from Zhao et al. (2016) – which were sampled in a less destructive manner by means of a syringe – relative to their root and branch samples.

Regular sub-daily patterns of plant water isotope signatures have been related to evaporative enrichment: the magnitude of sub-daily variation was linked to the proximity to leaves or unsuberized branches, where xylem water is assumed to be more easily lost to evaporation than in fully suberized parts of the tree (Dawson & Ehleringer, 1993). Dawson and Ehleringer (1993) observed large differences between mid-day and pre-dawn δ^2H signatures in branch xylem waters from non-suberized branches, while fully suberized branches showed no differences. Martín-Gómez et al. (2017) observed regular sub-daily variations also in *suberized* branches. Despite being suberized, these branches were still relatively close to the leaves. Consistent with these observations, Cernusak et al. (2005)

showed that sub-daily variations of leaf waters had a systematic sinusoidal shape, with strongest enrichment in the afternoon and largest differences in $\delta^{18}O$ between 08h00 and 14h00.

1.2 | Sampling height

In a potted willow tree, Nehemy et al. (2021) found no significant differences in $\delta^{18}O$ and $\delta^{2}H$ in branch xylem sampled at the bottom and the top of multiple main stems. Similarly, Zhao et al. (2016) found no significant isotopic differences between root xylem waters sampled near ground level and stem xylem waters sampled at 1 m and 2 m height in larger trees in a riparian forest. They also reported good agreement of stem and root xylem water with the xylem waters from first and third-order branches. Treydte et al. (2021) also found homogeneous stem xylem water $\delta^{18}O$ and $\delta^{2}H$ signatures across different sampling heights in two eucalyptus tree species; stem phloem waters, by contrast, varied with sampling height. Interestingly, both Zhao et al. (2016) and Treydte et al. (2021) found larger variabilities in stem xylem signatures very close to the ground (i.e., <1 m). Treydte et al. (2021) attributed this variability to an artefact of their sampling. In another study, De Deurwaerder et al. (2020) observed large variations in six different tree species over vertical distances of ~15 m. De Deurwaerder et al. (2020) suggest that the large variation could emerge from sub-daily shifts in root water uptake and species-specific transport rates, but their study was limited to one tree per species. Cernusak et al. (2005) showed that xylem water $\delta^{18}O$ signatures exhibited a slight tendency towards greater enrichment at higher positions along the stems and in the branches. Their observations are consistent with earlier results for isotope signatures of branches by Dawson and Ehleringer (1993) across multiple tree species. Vega-Grau et al. (2021) sampled xylem waters in stems and branches from multiple heights in two tree species, finding "possibly the largest yet observed" variations within individual trees, but they did not detect clear patterns with sampling height. Across these previous studies, isotope signatures have been observed to be both heterogeneous and homogeneous with sampling height. Variations in isotope signatures with height along the stem could originate from mixing or displacement of water stored in the stem with waters taken up by roots over a period prior to xylem sampling, with time lags and hence the duration of this period depending on the tree size and sap-flow velocity (Seeger & Weiler, 2021; Mennekes et al., 2021; Nehemy et al., 2022). Thus, we would expect isotope signatures at different heights along the stem to be relatively homogeneous (as observed in some of the previous studies), if isotope signatures of water taken up by roots are relatively stable during the period preceding the xylem sampling or if residence times within the trees are short. Conversely, heterogeneous signatures along tree height might develop if isotope signatures of root water uptake vary on similar time scales as the water residence times in trees. Strong shifts in root water uptake signatures can be induced by applying tracers. Several recent studies found transport lags of multiple days after tracer application, using continuous in-situ measurements of the xylem water isotope signatures (Magh et al., 2020; Marshall et al., 2020; Seeger & Weiler, 2021). Knighton et al. (2020) achieved improved agreement between modelled and observed xylem water isotopes by modelling stem water storage and mixing.

1.3 | Sampling orientation

Previous studies found no consistent variations in the isotopic signals of branch or stem xylem waters in repeated sampling at different cardinal directions. Observed differences ranged up to 0.9‰ (3.5‰) for $\delta^{18}O$ ($\delta^{2}H$) in stems and branches (Nehemy et al., 2021; Treydte et al., 2021). Goldsmith et al. (2019) quantified intra-crown standard deviations in branch xylem to be 0.3‰ (1.8‰) for $\delta^{18}O$ ($\delta^{2}H$) in three beech (*Fagus sylvatica*) trees and 0.8‰ (3.6‰) in three spruce (*Picea abies*) trees. They suggested that "sectorality" of xylem conduits is a potential reason, i.e., distinct flow paths with little circumferential mixing. Tracer-based studies showed different degrees of circumferential mixing in different species (Treydte et al., 2021; Volkmann et al., 2016; Zanne et al., 2006). An unequally distributed irrigation tracer was detected in all sampled directions but remained unequally distributed in the stem xylem of maple trees (*Acer campestre*) at 1.2 m above the ground (Volkmann et al., 2016). Treydte et al. (2021) demonstrated strong circumferential mixing by applying a tracer directly into the stem xylem on the north side of three trees, and afterwards detecting the tracer in the stem xylem on the south side of the stem. Furthermore, Treydte et al. (2021) found a large contrast in the magnitude of the circumferential mixing between species, with stronger mixing in *Eucalyptus sideroxylon* compared with *Eucalyptus tereticornis*, reflecting differences in the wood anatomical properties of the xylem of these two species (e.g. number of parenchyma rays).

1.4 | Sampled compartment

On its journey through a tree, water passes through a number of compartments (e.g., roots, stem, branches, leaves, phloem). Previous studies indicated that the variability in stem xylem waters (sampled at/below breast height) is lower than in waters of other sampling compartments (i.e., roots, branches and leaves) (Amin et al., 2021; Cernusak et al., 2005; Vega-Grau et al., 2021; Zhao et al., 2016). Further, xylem waters sampled close to the bottom of the stem are probably less affected by evaporative processes or by potential exchange between the xylem and the phloem or between the xylem and heartwood. Zhao et al. (2016) found no difference in isotope signatures between stem xylem and either first- or third-order branches. For stand averages, Vega-Grau et al. (2021) and Sohel et al. (2023) found isotopic differences between compartments along the transpiration stream when sampling roots, stems, branches and twigs across two tree species in the same stand (Vega-Grau et al., 2021) or when sampling stems and branches across four species (Sohel et al., 2023) in the same stand. In both of these studies, the direction of the differences (i.e. enrichment vs depletion) between two compartments

varied across the sampled species. Amin et al. (2021), however, did not find significant differences between isotope signatures from waters extracted from cores and branches in a potted 2-m olive tree that had been irrigated with labelled water over two months. Other studies found that water sampled from broadleaves or needles is isotopically enriched relative to xylem water (Cernusak et al., 2005; Treydte et al., 2014) due to evaporation effects that are only partially compensated with advection of xylem water that has not been enriched (Farquhar & Lloyd, 1993; Treydte et al., 2014). While Cernusak et al. (2005) observed that phloem waters are also enriched compared with xylem waters, others found that phloem waters were more depleted (Nehemy et al., 2022; Treydte et al., 2021). Exchanges between xylem and phloem waters and storage effects (Nehemy et al., 2022; Treydte et al., 2021) can result in both positive and negative deviations depending on the transient behaviour, and thus can explain inconsistent observations across studies. Similarly, exchanges between xylem and *heartwood* have been observed previously (Fabiani et al., 2022).

1.5 | Stand-scale

On the stand scale, stem xylem showed smaller variability than branch xylem in previous studies: Stand-scale standard deviations in stem xylem were lower than 0.6‰ (1.0‰) for $\delta^{18}O$ ($\delta^{2}H$) (Cernusak et al., 2005; Zhao et al., 2016). Vega-Grau et al. (2021) found comparatively larger standard deviations of up to 1.1‰ (3.4‰) in stem xylem sampled at breast height across their forest stand. Zhao et al. (2016) found 3 to 5 times higher standard deviations in branch xylem than in stem xylem of their trees. Multiple studies carried out in pure or mixed stands on beech, spruce, poplar or larch trees, have measured similar stand-scale standard deviations in branch xylem ranging from 0.3 to 1.6‰ for $\delta^{18}O$ (1.7 to 8.1‰ for $\delta^{2}H$) (Brinkmann et al., 2019; Goldsmith et al., 2019; Treydte et al., 2014; Vega-Grau et al., 2021; Zhao et al., 2016).

Water transit times or fractionation effects along the transpiration stream might induce variations of isotope signatures in waters from different compartments within the same tree (i.e., different heights along the stem, branches). Across a tree stand, multiple trees can show variations of isotope signatures. Quantifying uncertainties in isotope signatures in xylem water at the tree- and stand-scale helps to clarify how well measurements from single tree compartments represent the whole tree or an entire stand. This is important for the design of future studies as well as the interpretation of previously sampled isotope signatures. Spatiotemporal variability of stable isotope signatures of stem xylem water extracted from increment cores has not yet been systematically assessed in forests. For example, at the scale of an individual tree, it is currently unknown whether circumferential mixing between xylem conduits is strong enough at lower heights along the stem for a sample to be representative of whole-tree root water uptake. The influence of sampling height along the stem has not yet been systematically assessed. Isotope signatures of water sampled from different heights along the stem have not been compared with those of water extracted from other compartments of the same tree, such as branch xylem or stem phloem.

Thus, the main aim of this study was to quantify the uncertainty of xylem water isotope signatures in individual stem increment core samples and assess to what extent these stem samples are representative of the average xylem water signatures of individual trees or an entire tree stand.

Specifically, within this study, we aimed to assess:

- to what extent sampling time, sampling height or sampling orientation of stem increment cores affect the measured isotope signatures and their uncertainties in representing the source water taken up before sampling,
- to what extent isotope signatures from different tree compartments differ, i.e., whether xylem waters extracted from stem increment cores are similar to xylem waters extracted from branches,
- how large stand-scale variabilities of isotope signatures are, i.e., whether xylem waters from a single stem increment core extracted from the lower part of the stem can be representative for multiple individual trees of the same species within a stand across distances <100 m, and
- how uncertainties related to xylem water isotope signatures (methodological artefacts and spatial or temporal variabilities) affect comparisons with soil water signatures and thus isotope-derived root water uptake patterns of individual trees and stands.

The study was designed as a snapshot sampling campaign to assess the tree-scale and stand-scale variability of plant water isotope signatures in tree species common to Swiss forests. These were European beech (*F. sylvatica*), Norway spruce (*P. abies*) and common oak (*Quercus robur*). We quantified the tree-scale and stand-scale isotopic variability in xylem waters from branches, and xylem and phloem waters from stem cores. We compared the isotope signatures of single trees and multiple trees at one mixed stand at different heights along the stem and in different cardinal directions around the stem. We assessed to which extent xylem waters of different compartments of individual trees differed, and inferred which sampled compartments can be most representative of recent root water uptake.

2 | MATERIALS & METHODS

2.1 | Field site

The study site is located within "Waldlabor Zurich" (www.waldlabor.ch), a typical mixed forest on the Swiss Plateau, located at the edge of the city of Zurich. The forest plot is situated at an altitude of around 525 m asl, at a latitude of ~47.41°N and longitude of ~8.49°E, with a mean annual temperature of 9.3°C and mean annual precipitation of 1,134 mm. The 1.2 ha (12,000 m^2) experimental site (Figure 1) is gently sloping (<8% slope, N-exposed aspect of 17°) and hosts the following trees with breast height diameters exceeding 16 cm: 115 *P. abies*, 58 *Fraxinus excelsior*, 51 *Acer pseudoplatanus*,

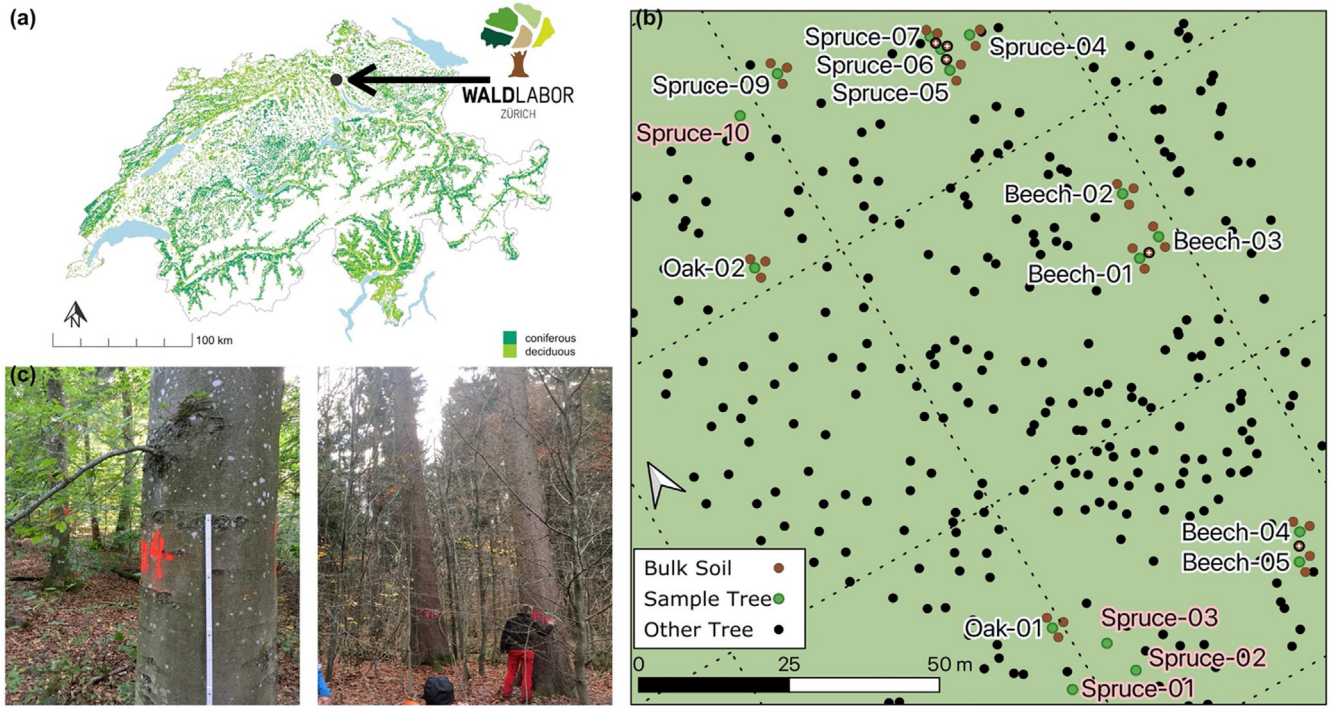

FIGURE 1 Location of the experimental site "Waldlabor Zurich" within Switzerland **(a)**, a map of the location of the individual trees (green dots) and bulk soil sampling locations (brown dots) **(b)** and pictures of the sampling site **(c)**. Trees labelled in red in **(b)** were sampled a week earlier and were not climbed. Five bulk soil samples marked with white crosses in **(b)** were attributed to more than one tree.

38 *F. sylvatica*, 2 *Q. robur* and 14 individual trees of other species. The dominant soil type, assessed from a nearby soil pit, is calcic luvisol covering moraine deposits from the last glacial maximum. No factors preventing root growth (i.e., bedrock, large boulders, waterlogging, unfavourable acidity) were found down to 130 cm. The root density of the soil was medium in layers down to 50 cm and low below 50 cm, estimated based on counting fine roots (<2 mm diameter) visible in the soil profiles (medium root density is defined by 11–50 roots per 0.01 m^2 and low root density is defined by <10 roots). Some roots were present until the bottom of the profile at 130 cm depth. The three major tree species – Norway spruce (*P. abies*), European beech (*F. sylvatica*) and Common oak (*Q. robur*) – were selected for sampling. We sampled five spruce, five beech and two oak trees along the stem up into the canopy, accessed by tree climbing. Three additional spruce trees were sampled at breast height only. The selected trees had breast height diameters ranging from 26 to 83 cm for spruce, 33 to 51 cm for beech and 93 to 113 cm for oak. Supplementary Figure S4 shows a graphical overview of the sampling heights, tree heights and tree diameters for each individual tree, as well as their approximate canopy size.

2.2 | Tree and soil sampling

Xylem and soil samples were taken from oak and beech trees and their surrounding soils, in northern (N), eastern (E) and southern (S) directions, over two dry and meteorologically similar days on September 9th and 10th, 2020. Spruce sampling started on November 23rd in dry conditions with breast height sampling of spruce trees numbered 01, 02 and 03 (samples in N, E and S directions) and spruce trees 04, 05, 06, 07 and 09 (single sample in western [W] direction). Stem xylem, branch xylem, stem phloem and soil samples from spruce trees and their surrounding soils (N, E and S directions) were collected on December 1st, 2020. See supplementary Figure S4 for dates and sampling heights. On the morning of December 1st, before spruce sampling, snowfall occurred. During sampling, however, meteorological conditions were dry. To estimate sap-flow decreases in September and December relative to peak summer rates, sap flow was measured throughout 2021 and 2022 in beech and spruce trees located approximately 700 m away from our study site.

Stem xylem and phloem samples were taken with increment borers (5.15 mm diameter; Haglöf, Långsele, Sweden). Cores of 5 cm length were extracted from the main stem, or from a major stem at upper heights. Each core was split into two parts at the cambium, and phloem and bark were stored and extracted separately from sapwood (xylem) and heartwood. Samples were taken at four heights, the first height (H1) being approximately 1.3 m above ground for beech trees and around 0.2 m to 0.5 m above ground for oak and spruce trees, and the three upper heights (H2, H3 and H4) approximately corresponding to 10, 20 and 30 m above ground. The H1 sampling positions for oak and spruce were lower to comply with a request by the forest owner to maintain the resale value of the timber. H1 was sampled twice per day at around 9 AM and 3 PM for beech and oak

species to test for daily fluctuations in the isotope signature of the xylem water. For each sampling height, one core was taken from the stem in three cardinal directions (N, E and S), and split into a phloem and xylem part. For spruce, we additionally took branch samples: 2 to 3 m long branches were cut from heights H2, H3 and H4 in three cardinal directions. The branch samples consisted of a needle-free part of the branch of approximately 1 cm diameter, where we removed bark and phloem (see Supplementary Figure S4 for sampling heights). Thus, we obtained multiple samples from a single tree (9 AM vs 3 PM, directions N vs E vs S, heights H1 to H4 and different compartments) to assess the tree-scale variability of plant waters. Further, we repeated the sampling at multiple trees to assess the stand-scale variability for the spruce, beech and oak stands.

Bulk soil was sampled in depths of 0–10 cm (only in the December campaign), 30–40 cm, 60–70 cm, 90–100 cm and 120–130 cm, using a "Pürckhauer" soil auger. To obtain the deep sample, a 100 cm deep hole was drilled, into which the soil auger was pushed for sampling. Bulk soil was sampled in three cardinal directions around each sampled tree at approximately 2 m distance from the stem (Figure 1). All xylem, phloem and bulk soil samples were stored in 12 ml air-tight vials (borosilicate glass exetainers – model 938Y – with chlorobutyl septa screwcaps, LabCo, Lampeter, Wales) and refrigerated at 2°C until water extraction.

2.3 | Cryogenic extraction and isotope analysis

Waters from bulk soil, phloem and xylem samples were cryogenically extracted using the method and device described in Diao et al. (2022). For each extraction batch, 20 vials were immersed in a water bath at 80°C while pressure in the extraction line was kept below 5 Pa. Evaporated water was collected in a U-shaped glass tube trap immersed in liquid nitrogen. Samples were extracted for 2 hours, after which ambient pressure was established with nitrogen gas. The glass tubes containing the frozen extract were detached, closed with rubber plugs, and left for thawing at room temperature for approximately 30–60 minutes. The liquid water was filtered with 0.45 µm nylon filters (BGB Analytik, Boeckten, Switzerland) and transferred into 0.33- or 1.5-ml glass vials (BGB Analytik, Boeckten, Switzerland). All bulk soil and xylem water samples were weighed before and after extraction to determine water content, accounting for individual weight differences of the vials and lids. For a subset of 61 samples, extraction efficiency was assessed by oven drying the extracted samples (for 24 h at 105°C) and reweighing them. For 54 out of 61 samples, >98% of water was extracted, and for the remaining 7 samples, between 96% and 98% of water was extracted. No samples were discarded based on the extraction efficiency, as the extraction efficiency was assessed for a subset only. Some phloem samples of different cardinal directions but from the same tree, height and sampling campaign were combined after extraction for a composite analysis to have a sufficient amount of water (this was done for samples in North/East/South (N/E/S) directions of spruces 04 (H4), 05 (H1, H2), 06 (H1), 07 (H2, H4) and 09 (H3)).

Stable water isotope ratios were analysed with a Picarro cavity ring-down spectrometer (Picarro L2140i, Picarro Inc., Santa Clara, USA) equipped with a micro-combustion module (MCM - Picarro Inc., Santa Clara, USA) to avoid measurement artefacts from organic compounds in the samples. The average of the last three measurements out of six injections was used to reduce injection memory effects. Measurement precision, determined as the standard deviation of these three measurements, was on average 0.02‰ for $\delta^{18}O$ and 0.06‰ for $\delta^{2}H$. Measurement accuracy was determined by regular measurement of a lab standard (B2193, Elemental Microanalysis, Okehampton, England) and resulted in a root mean squared error (RMSE) of 0.13‰ for $\delta^{18}O$ and 0.95‰ for $\delta^{2}H$. Forty samples were additionally analysed on a thermal combustion/elemental analyser (TC/EA) coupled to a DELTA^{PLUS}XP isotopic ratio mass spectrometer (IRMS - Finnigan MAT, Bremen, Germany) to confirm the validity of the $\delta^{18}O$ and $\delta^{2}H$ measurements from the cavity ring-down spectrometer. Both measurements yield comparable values for $\delta^{18}O$ and $\delta^{2}H$ (Supplementary Figure S1). Evaporation through the pierced septa between multiple analyses was observed and corrected based on the Craig-Gordon model, taking account of both the sample amount and the storage duration since piercing (see Appendix and Supplementary Figures S2 and S3 for further details).

Because of the presumed larger bias in $\delta^{2}H$ signatures of plant waters compared with $\delta^{18}O$ signatures, we show $\delta^{18}O$ results in the manuscript. Unless stated otherwise, the results for $\delta^{2}H$ yield similar findings and can be found in the supplementary material.

2.4 | Gradients in isotope signatures of precipitation throughfall and soil water

Precipitation throughfall water was sampled on an event basis (on average every 4.5 days) from March 2020 to March 2021 from a close-by field site (approximately 700 m from the site). We fitted amplitudes and phase shifts of sinusoidal functions – constrained to a yearly period – with ordinary least squares to the $\delta^{18}O$ and $\delta^{2}H$ isotope signatures of the throughfall water. Based on these functions, we calculated the temporal gradients used to illustrate the propagation of uncertainty of the stable isotope signatures in plant water for temporal source water attribution.

Soil water isotope signatures were used to calculate spatial gradients with depth. We computed the difference between average isotope signatures across all soil samples at depths 0–10, 30–40 and 60–70 cm for the September and December sampling campaigns to estimate depth gradients.

The maximum gradients in time or depth can give a lower limit of the uncertainty in source water attribution. In a mixing model, the standard error of the fractional contribution of two end-members (with isotope signatures δ_a and δ_b) to a common mixture (δ_m) is inversely proportional to the difference between the endmembers (Allen & Kirchner, 2022). Hence, larger differences between the end-members relative to the uncertainties in the isotope signatures lead to more robust inferences in source-water attribution. We used the

maximum spatial gradient observed in bulk soil water and the maximum temporal gradient observed in throughfall water to illustrate how the quantified uncertainty in stem xylem water isotope signatures propagates into an uncertainty in the relative contributions of the potential source waters. We derived lower bounds of separation in space or time of two potential endmembers that can be distinguished with a given degree of certainty. For simplicity, we neglected additional uncertainty due to errors in source-water measurements (i.e., on δ_a and δ_b) and any systematic biases. With these assumptions the standard error of the fractional contribution becomes $SE(f_a) \approx \frac{1}{|\delta_a - \delta_b|} SE(\delta_m)$, (adapted from Allen and Kirchner (2022)).

2.5 | Statistical data evaluation

Differences in isotope signatures between groups – i.e., when comparing (i) samples from the morning (~9 AM) and the afternoon (~3 PM), (ii) samples from different sampling heights (H1 versus H4) and (iii) samples from different cardinal directions (N to E, N to S, E to S) – are tested with two-sided paired t-tests, and additionally paired Wilcoxon rank sum tests as a non-parametric alternative, on data stratified for independence. For example, the AM/PM test was performed separately for the N, E and S directions to ensure independence. As a null hypothesis, these tests assume that the mean or median difference between the pairs of samples is zero. In case of multiple comparisons (N to E, E to S), we assessed the sensitivity of the results, i.e., the potential adjustment of *p*-values, by calculating results with the least conservative (no adjustment of *p*-values) and most conservative (Bonferroni correction) methods. The normal distribution of samples required for the paired t-tests was tested and confirmed with a Shapiro normality test. Differences between isotope signatures from the various tree compartments (stem xylem vs stem phloem and stem xylem vs branch xylem) were tested across all individual trees, heights and orientations using Wilcoxon rank sum tests. A few bulk soil samples were within 2 m distance of more than one tree and were therefore used for statistics on multiple trees (indicated in Figure 1). Differences in isotope signatures across individual trees (spruce, beech and oak species) were tested with a non-parametric Kruskal-Wallis test because it does not assume homogeneous variance within the groups. A single stem xylem sample with a $\delta^{18}O$ value of −5.53‰ (stem xylem of beech-04 at H2 in the southern direction from the September campaign) was removed as an outlier by visual comparison of its $\delta^{18}O$ value to the other beech stem xylem values. Regression lines in dual isotope space were fitted with ordinary least squares onto bulk soil water samples and xylem samples separately for each tree species. The soil water line was used to compute soil water excess δ^2H (SW-excess δ^2H) of each stem xylem sample as the difference in δ^2H between the observed stem xylem value and the soil water line evaluated at the observed $\delta^{18}O$ value of the stem xylem (Barbeta et al., 2019; de la Casa et al., 2022).

3 | RESULTS

3.1 | Stem xylem water isotope signatures at different times of the day

For oak (n = 3 × 2) and beech (n = 3 × 5), pairwise comparison of stem xylem $\delta^{18}O$ values between morning (AM) and afternoon (PM) showed no systematic differences. Instead, pairwise differences were scattered randomly around zero (Figures 2 and S5). The largest measured absolute differences between AM and PM were 1.0‰ in $\delta^{18}O$ and 5.2‰ in δ^2H, respectively. Of all AM-PM sample pairs, 80% had an absolute difference smaller than 0.6‰ for $\delta^{18}O$ and 2.5‰ for δ^2H. The absolute difference was within the propagated standard error of the differences between the two measurements $\left(=\text{RMSE} \times \sqrt{2}\right)$ in 29% of AM-PM sample pairs for $\delta^{18}O$, and in 52% of the sample pairs for δ^2H. Two-sided paired t-tests indicated no significant differences ($p > 0.05$) for $\delta^{18}O$ and δ^2H between AM and PM samples, suggesting no systematic sub-daily fluctuations in the isotope signatures. Non-parametric paired Wilcoxon signed-rank tests confirmed these results. The results for δ^2H can be found in Supplementary Figure S5.

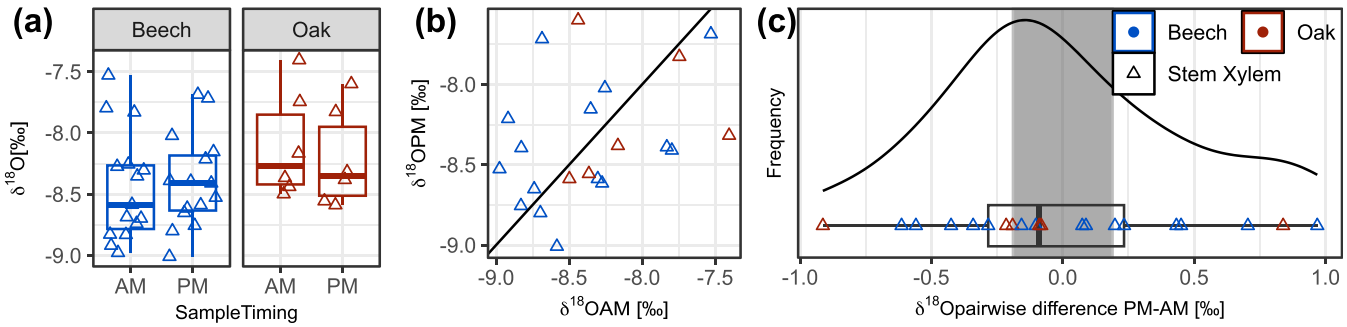

FIGURE 2 Differences in $\delta^{18}O$ isotope signatures between samples taken at the same tree at ~9 AM and ~3 PM of the same day for beech and oak species as indicated by boxplots **(a)**, a scatter plot **(b)** and as occurrence frequency of pairwise differences ($\Delta\delta_{PM-AM} = \delta_{PM} - \delta_{AM}$) **(c)**. The grey shading in **(c)** indicates the propagated standard error of the difference of two measurements. Sub-daily fluctuations in the isotopic composition are small and appear to be non-systematic.

3.2 | Stem xylem water isotope signatures in different heights and cardinal directions

Stem xylem $\delta^{18}O$ varied with sampling height (Figure 3). The isotopic differences between different heights, however, varied across the individual trees and across the sampling dates (Figure S7). During the September sampling campaign, no trees exhibited large differences in xylem $\delta^{18}O$ with height (except for individual trees beech 02 and beech 03 between H1 and H2-H4). In the December sampling campaign at the spruce stand, all trees tended to have more enriched $\delta^{18}O$ values higher up, but the enrichment was strongest in two specific trees (spruces 05 and 09). For $\delta^{2}H$, a systematic trend to more depleted values higher up was discernable in September in beech and oak (Figures S6a and S7), while in December, $\delta^{2}H$ height profiles varied even more strongly than for $\delta^{18}O$ across individual spruce trees (Figure S7), causing large stand-scale variability at heights H2-H4 (Figure S6a).

To assess the magnitude of the height effect, AM/PM-averaged $\delta^{18}O$ values were compared between H1 and H4 (Figure 3 c, e). Systematic influences were visible in lumped and pairwise comparisons for beech and spruce trees. Pairwise differences between H1 and H4 deviated systematically from zero depending on the sampling month: beech and oak sampled in September showed more enriched values at H1 compared with H4, whereas spruce, sampled in December, showed more depleted values at H1 compared with H4 (but differences were also quite variable across the five individual trees). Results for $\delta^{2}H$ were similar, but in oak, $\delta^{2}H$ was more clearly enriched than $\delta^{18}O$ between H1 and H4 (Figure S6e). Paired t-tests indicated significant differences in stem xylem signatures between H1 and H4 for spruce and beech in $\delta^{18}O$ ($p < 0.006$) and for all three species in $\delta^{2}H$

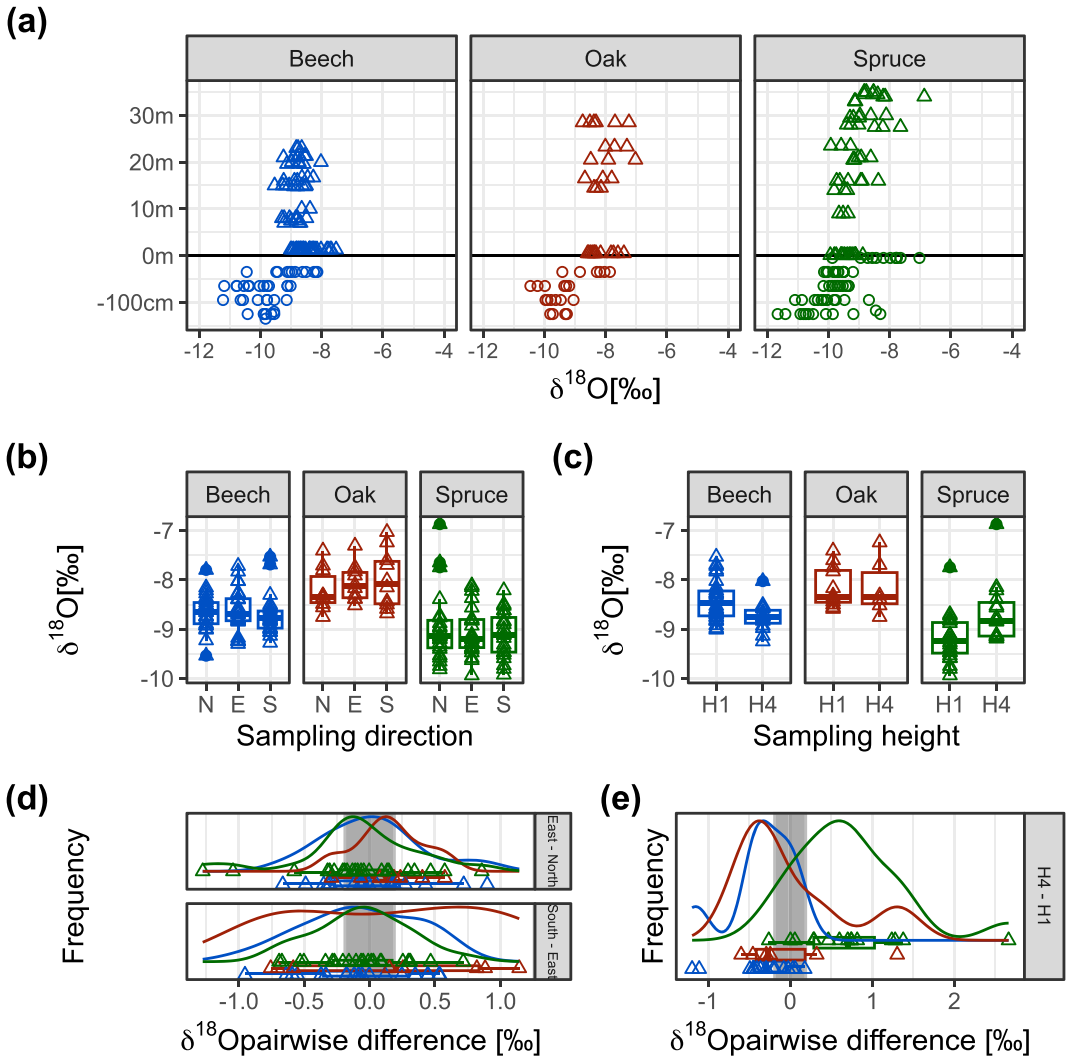

FIGURE 3 Stem xylem water $\delta^{18}O$ isotope signatures of the north (N), east (E) and south (S) sample replicates at different heights (triangles) and the bulk soil water $\delta^{18}O$ isotope signatures at different depths (circles) **(a).** Lumped **(b & c)** and pairwise comparison **(d & e)** of stem xylem water $\delta^{18}O$ signatures at different cardinal directions **(b & d)** and different heights **(c & e)**. Differences were computed: $\Delta\delta_{E-N} = \delta_E - \delta_N$, $\Delta\delta_{S-E} = \delta_S - \delta_E$ and $\Delta\delta_{H4-H1} = \delta_{H4} - \delta_{H1}$. The grey shading in **(d & e)** indicates the propagated standard error of the difference of two measurements. Isotope signatures showed no systematic differences among cardinal directions but consistent offsets between H1 and H4.

($p < 0.05$) (Table S2). However, no significant difference in $\delta^{18}O$ was detected between H1 and H4 in oak. In separate t-tests for the three directions N, S and E, two tests using $\delta^{18}O$ and three tests using $\delta^{2}H$ showed statistically significant differences between H1 and H4. With adjustment of the *p*-value for multiple comparisons, however, the differences between H1 and H4 for the different cardinal directions became non-significant (Table S2). Non-parametric paired Wilcoxon signed-rank tests between H1 and H4 were significant for $\delta^{18}O$ in beech and spruce, and for $\delta^{2}H$ in beech and oak (Table S3).

The orientation of sampling along the cardinal directions did not influence the obtained xylem signatures (Figures 3 b, d and S6 b, d). The overall variation in stem xylem water isotope signatures among different cardinal directions at each height was small for all three species and of similar magnitude for the different sampling heights (Table 1). The lowest variabilities were typically observed at heights H1 or H2. Tree-scale standard deviations in stem xylem $\delta^{18}O$ among the different cardinal directions at each height in individual trees varied for H1 to H4 from 0.2 to 0.3‰ for beech, 0.3 to 0.6‰ for oak and 0.2 to 0.4‰ for spruce. For $\delta^{2}H$, the same standard deviations ranged from 1.3 to 1.8‰ for beech, 1.1 to 2.2‰ for oak and 1.3 to 2.2‰ for spruce. Paired t-tests indicated no significant differences for $\delta^{18}O$ and $\delta^{2}H$, respectively, between N and E and between E and S, performed independently for heights H1, H2, H3 and H4 and each tree species (see stratified differences in Supplementary Figure S8). Even at the lowest sampling height H1 (1 m for beech and below 0.5 m for spruce and oak), the samples of stem xylem water in all three species were much less isotopically variable than the samples of bulk soil water (Tables 1 and 2; variability of bulk soil waters is presented in section 3.5).

3.3 | Isotope signatures of water in different tree compartments: stem phloem, stem xylem and branch xylem

Stem phloem water was significantly enriched in δ18O (Figure 4) and δ2H (Figure S9) compared with stem xylem water ($p < 0.002$ by non-parametric paired Wilcoxon signed-rank test combining all trees, heights and orientations; exceptions to this general pattern were observed at heights H3 and H4 in spruce 06, and height H4 of spruce 05). Likewise, the xylem water sampled from branches was enriched compared with the stem xylem for three of five spruce trees ($p < 0.0001$ combining all five trees, all heights and all orientations). Also, tree-scale standard deviations were higher by a factor of roughly 2 to 3 in stem phloem (up to 0.9‰ and 4.9‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively) and higher by a factor of roughly 2 to 4 in branch xylem (up to 1.2‰ and 4.8‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively) than in stem xylem (0.2–0.3‰ and 1.3–2.2‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively; Table S4). Similar ratios in isotopic variability (of stem phloem and branch xylem relative to stem xylem) were also observed at the stand scale. Standard deviations observed at the stand scale were larger overall than those observed at the tree scale, due to differences in height trends between individual trees. For instance, the stem phloem signatures of spruce 06, and branch xylem signatures of spruces 07 and 09, deviated from the stand-scale trend (Figures 4b and S9b).

TABLE 1 Averages and standard deviations (SD) of stem xylem water isotope signatures for each sampling height (H1 through H4) and tree species in ‰, shown either as stand-scale standard deviation or average of individual tree standard deviations (for N, E, S cardinal directions). Bold text indicates the height with the lowest variability. For spruce in November, only spruces 04 to 09 were used to compute the statistics. The lines denoted "all trees" are based on all spruces, including the ones not climbed (marked red in Figure 1). Sample sizes in terms of total number of cores and number of trees are reported for $\delta^{18}O$ only; the same sample sizes apply to $\delta^{2}H$.

		Stand mean (‰)				Within-tree SD* (‰)				Within-stand SD** (‰)				Sample size (n of samples/trees)			
		H1	H2	H3	H4	H1	H2	H3	H4	H1	H2	H3	H4	H1	H2	H3	H4
$\delta^{18}O$:																	
Beech	Sep	−8.4	−8.9	−8.8	−8.7	0.3	**0.2**	0.3	0.2	**0.3**	**0.3**	0.4	0.3	15/5	14/5	15/5	15/5
Oak	Sep	−8.2	−8.2	−7.7	−8.2	0.3	**0.3**	0.5	0.6	**0.3**	**0.3**	0.5	0.6	6/2	6/2	6/2	6/2
Spruce	Nov	−9.5	-	-	-	-	-	-	-	0.2	-	-	-	5/5	-	-	-
Spruce	Nov, all trees	−9.1	-	-	-	0.4	-	-	-	0.6	-	-	-	15/9	-	-	-
Spruce	Dec	−9.4	−9.3	−8.9	−8.7	**0.2**	0.2	0.3	0.2	**0.3**	0.4	0.6	0.6	15/5	15/5	15/5	15/5
$\delta^{2}H$:																	
Beech	Sep	−75.4	−76.1	−77.5	−77.9	1.5	**1.3**	1.8	1.5	2.2	2.0	2.5	**1.5**	"	"	"	"
Oak	Sep	−64.4	−67.0	−67.6	−70.5	1.4	**1.1**	1.9	2.2	**1.4**	1.7	2.2	2.5	"	"	"	"
Spruce	Nov	−74.5	-	-	-	-	-	-	-	1.9	-	-	-	"	"	"	"
Spruce	Nov, all trees	−72.9	-	-	-	2.1	-	-	-	2.5	-	-	-	"	"	"	"
Spruce	Dec	−73.3	−71.6	−73.2	−71.0	1.8	1.8	2.2	**1.3**	**2.2**	3.1	5.4	3.0	"	"	"	"

*stand average of individual standard deviations (SD).

**standard deviation (SD) of all stand-wide measurements combined.

TABLE 2 Averages and standard deviations (SD) in ‰ of isotope signatures in bulk soil waters across the forest stand. Bold text indicates the depths with the lowest variability. Sample sizes (total numbers of soil samples) are reported for $\delta^{18}O$, the same sample sizes apply to $\delta^{2}H$. Samples that were located close to multiple individual trees (as shown in Figure 1) were included in computing the standard deviations around all of those trees. Total sample numbers are in those cases larger by +2 or +3 respectively, as indicated by "repeated" in the reported sample sizes.

		Stand mean (‰)					Around-tree SD* (‰)				
Depth (cm):		**120–130**	**90–100**	**60–70**	**30–40**	**0–10**	**120–130**	**90–100**	**60–70**	**30–40**	**0–10**
$\delta^{18}O$:											
Beech	Sep	−9.9	−10.0	−10.1	−8.9	-	**0.1**	0.6	0.5	0.6	-
Oak	Sep	−9.5	−9.6	−9.7	−8.4	-	**0.3**	**0.3**	0.6	0.6	-
Spruce	Dec	−10.2	−10.1	−9.7	−9.7	−8.2	0.9	0.6	0.3	**0.2**	0.8
$\delta^{2}H$:											
Beech	Sep	−65.6	−66.0	−67.2	−61.7	-	**0.7**	4.5	4.1	3.9	-
Oak	Sep	−63.0	−62.9	−65.8	−60.7	-	**1.2**	1.9	4.4	4.4	-
Spruce	Dec	−72.0	−68.2	−66.4	−69.8	−65.4	4.7	3.6	**1.0**	1.6	4.7

*stand average of standard deviations (SD) around individual trees with repeated samples.
**standard deviation (SD) of all stand-wide measurements combined.

TABLE 2 (Continued)

	Within-stand SD** (‰)					Sample size (n of samples/repeated/trees)				
Depth (cm):	**120–130**	**90–100**	**60–70**	**30–40**	**0–10**	**120–130**	**90–100**	**60–70**	**30–40**	**0–10**
$\delta^{18}O$:										
Beech	**0.3**	0.7	0.7	0.7	-	6/8/3	8/10/3	13/15/5	12/14/5	-
Oak	**0.3**	**0.3**	0.5	0.6	-	5/5/2	6/6/2	6/6/2	6/6/2	-
Spruce	1.1	0.6	**0.3**	**0.3**	0.7	12/15/5	12/15/5	12/15/5	12/15/5	12/15/5
$\delta^{2}H$:										
Beech	**1.1**	4.9	5.2	5.0	-	"	"	"	"	"
Oak	**1.2**	2.1	4.1	4.5	-	"	"	"	"	"
Spruce	6.1	4.1	**1.2**	1.9	4.6	"	"	"	"	"

*stand average of standard deviations (SD) around individual trees with repeated samples.
**standard deviation (SD) of all stand-wide measurements combined.

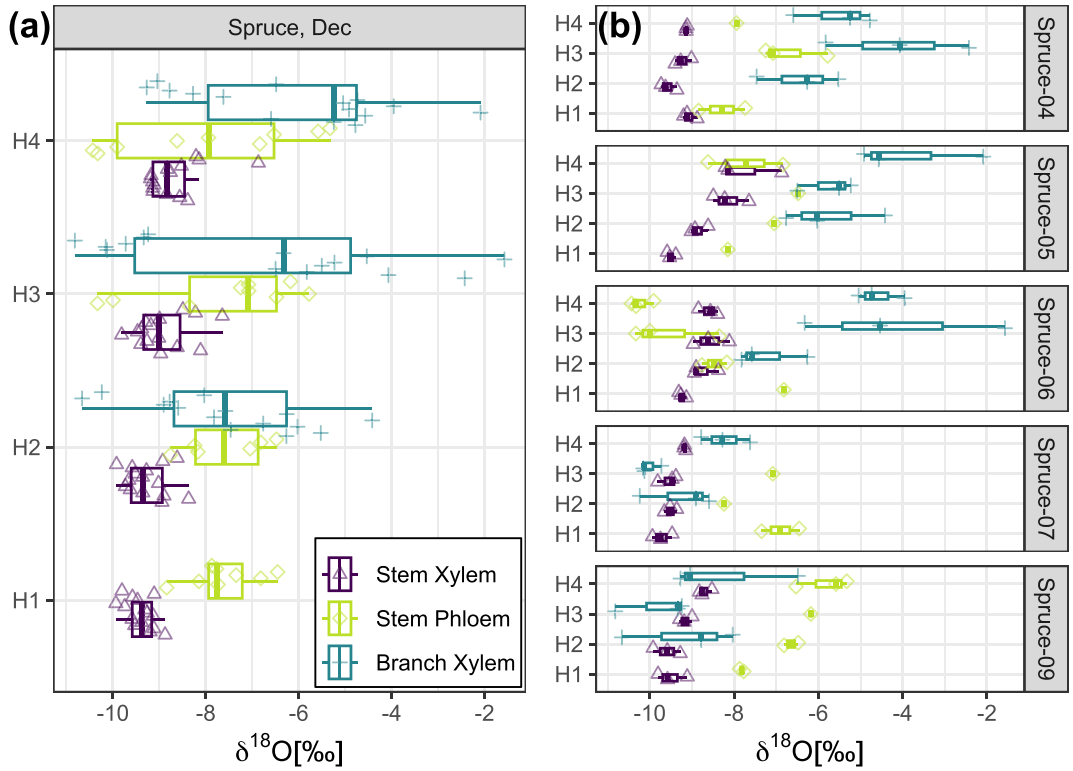

FIGURE 4 $\delta^{18}O$ isotope signatures of stem xylem, stem phloem and branch xylem waters across all spruce trees **(a)** and for individual spruce trees **(b)**. For some heights, multiple stem phloem samples from the same tree were combined into composite samples for extraction and analysis, due to the low extraction volume. Stem phloem water samples were systematically more enriched compared with the stem xylem water samples. The variability was largest in the branch xylem water samples.

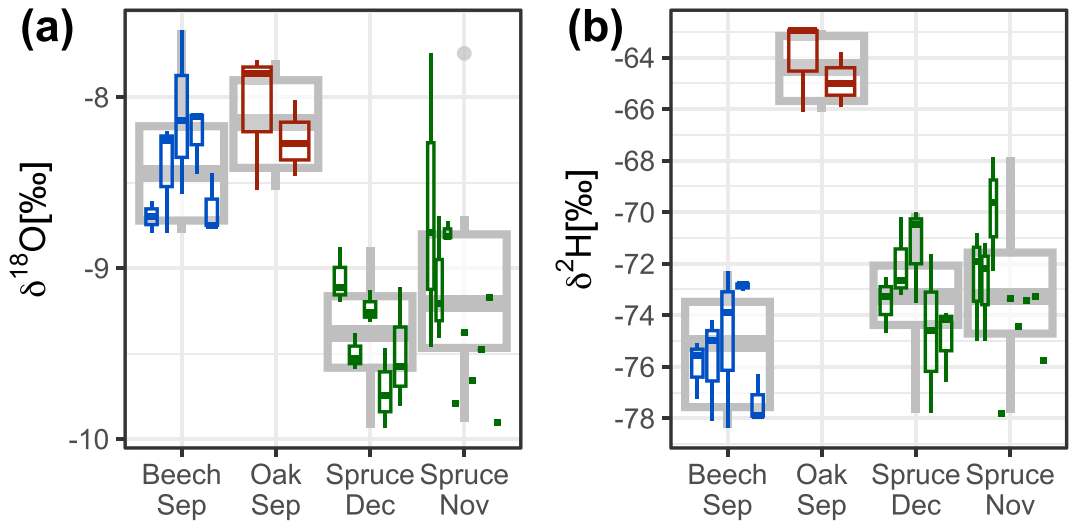

FIGURE 5 Variability of stem xylem water $\delta^{18}O$ **(a)** and $\delta^{2}H$ **(b)** isotope signatures of individual trees for beech (blue boxplots), oak (red) and spruce (green) and all samples of the respective stand (dark grey boxplots). Samples were taken at heights above ground of 1.3 m for beech, 0.2 m for oak and 0.5 m for spruce (corresponding to H1). Variabilities between individual trees at H1 are generally small, indicating that a few samples would yield a representative average for the whole stand.

3.4 | Differences in stem xylem water isotope signatures between neighbouring trees

In each of the three species, we compared the stand-scale isotopic variability in stem xylem water at height H1 with the variability found in individual trees at height H1 (Figure 5 & Table 1). Stand-scale standard deviations at H1 for $\delta^{18}O$ (and, in parentheses, $\delta^{2}H$) were 0.3‰ (2.2‰) for beech, 0.3‰ (1.4‰) for oak and 0.3‰ (2.2‰) for spruce (Table 1). These stand-scale variabilities were only marginally larger than those observed within individual trees. The maximum $\delta^{18}O$ (and

δ^2H) deviation between the stand and the individual trees' medians at height H1 was 0.3‰ (2.8‰) for beech, 0.3‰ (1.4‰) for oak and 0.6‰ (3.6‰) for spruce. Kruskal-Wallis rank sum tests indicated that the medians of individual tree stem xylem signatures were not significantly different from the stand median for nearby trees of the same species (Table S5). Therefore, we conclude that the median stem xylem signature from an individual tree at height H1 can represent trees of the same species, at least over the small distances (<50 m) of our study site. Standard deviations of stem xylem signatures were comparable between tree- and stand-scale for beech and oak across all heights H1 to H4 (Table 1). For spruce, the tree- and stand-scale variations were comparable only for height H1, whereas for heights H2, H3 and H4, the stand-scale variations were larger than the tree-scale variations (Table 1). This was due to strongly varying individual height profiles in the spruce trees (Figure 4 and S9).

3.5 | Soil water sources inferred from plant water samples

As expected, bulk soil water isotope signatures varied across different soil depths. On both sampling dates, we found vertical gradients in bulk soil waters (Figures 6, 3a and S6a), showing a tendency towards more depleted values with depth for $\delta^{18}O$ and to a smaller extent for δ^2H. $\delta^{18}O$ ranged from −11 to −8‰ in September and from −11.5 to −7‰ in December, when we also took samples in the top soil layer (i.e., between 0 and 10 cm depth). The variability relative to the overall gradient was larger in δ^2H than in $\delta^{18}O$. Bulk soil waters sampled around spruce were most isotopically enriched in the top soil layer from 0 to 10 cm (Figure 6). This soil layer also exhibited greater variability among different cardinal directions than the soil layers at 30 or 60 cm depth did, but variability in the bulk soil water increased again below 90 cm depth (Table 2). The variability in bulk soil water $\delta^{18}O$ and δ^2H among cardinal directions around beech and oak trees was largest in the upper soil and tended to decrease with increasing depth (Table 2). Variabilities in bulk soil water $\delta^{18}O$ and δ^2H among different cardinal directions around individual trees were larger than the tree-scale variability observed in xylem waters among different cardinal directions. Across all sampled depths, tree-scale bulk soil water $\delta^{18}O$ standard deviations averaged 0.2–0.9‰ around spruce, 0.1–0.6‰ around beech and 0.3–0.6‰ around oak; tree-scale δ^2H standard deviations averaged 1.0–4.7‰ around spruce, 0.7–4.5‰ around beech and 1.2–4.4‰ around oak (Table 2). Readers are reminded that bulk soils surrounding beech and oak were sampled in September, and those around spruce were sampled in December; thus any species effects will be inherently confounded with seasonal effects.

Stem xylem $\delta^{18}O$ signatures of all three species were within the range of bulk soil waters. For δ^2H signatures, however, this was only the case for oak and spruce; for beech, δ^2H signatures in stem xylem waters were lighter than any of the soil water samples (Figure 6). Hence, comparing isotope signatures between stem xylem and bulk soil waters yielded different conclusions for $\delta^{18}O$ and δ^2H. In oak and spruce, δ^2H signatures of stem xylem water corresponded best to soil waters from layers that were deeper than the layers that gave the best match in $\delta^{18}O$ (Figure 6). In dual isotope space (Figure 6c), stem xylem signatures plotted below the global meteoric water line, whereas the bulk soil signatures plotted closer to it. When δ^2H signatures were compared with the soil water line (SW), median values of

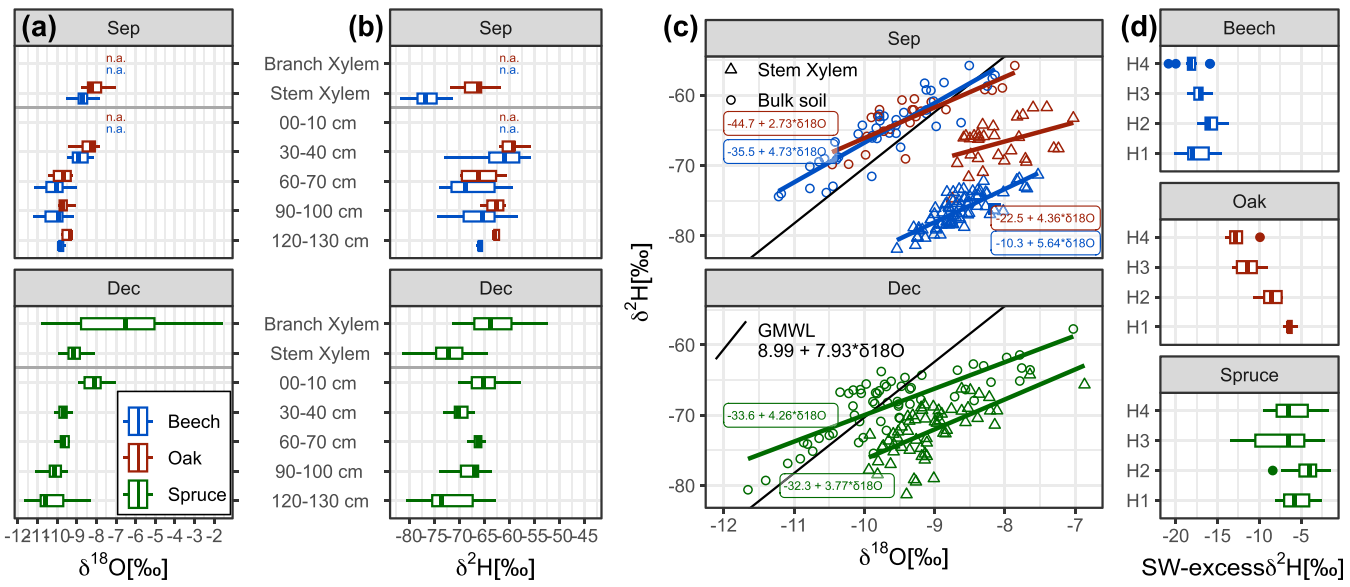

FIGURE 6 Boxplots of stand-scale variability of $\delta^{18}O$ **(a)** and δ^2H **(b)** isotope signatures for bulk soil water sorted by depth and compared with stem xylem water lumped for all heights H1 to H4. For spruce, branch xylem water is additionally shown in **(a)** and **(b)** lumped from H2 to H4. δ^2H offset between stem xylem and bulk soil water signatures is shown in dual isotope space **(c)** and as boxplots of SW-excess in δ^2H relative to the bulk soil water regression line **(d)**. Most δ^2H isotope signatures measured in the stem xylem of beech lie outside the range observed in bulk soil waters **(b)**. Substantial, species-specific offsets are evident between stem xylem and bulk soil water **(d)**.

excess δ^2H (SW-excess δ^2H) for the stem xylem waters were −18‰ for beech, −8.3‰ for oak and −5.5‰ for spruce (Figure 6d). In oak, SW-excess δ^2H became more negative with increasing sampling height, whereas, in beech and spruce, median SW-excess δ^2H varied less across the sampling heights (Figure 6d). Branch xylem signatures in spruce showed a considerably larger spread than stem xylem signatures, and most signatures were outside the range of the bulk soil water pools, i.e., the potential water sources. Note that this lack of overlap might be linked to the snapshot nature of our sampling strategy and the residence time of water in the tree.

Maximum spatial gradients in bulk soil water signatures were quantified between the 0–10, 30–40 and 60–70 cm layers and were 1.5‰ and 5.5‰ per 30 cm depth for $\delta^{18}O$ and δ^2H, respectively. Throughfall precipitation signatures varied throughout the year from −24‰ to −3‰ for $\delta^{18}O$ and from −180‰ to −18‰ for δ^2H. Maximum temporal gradients in throughfall waters were estimated with fitted sinusoidal functions (with seasonal amplitudes of 3.6‰ and 27.6‰) at 1.7‰ and 13.3‰ per month for $\delta^{18}O$ and δ^2H, respectively. Therefore, if we use (e.g.) three stem $\delta^{18}O$ xylem values to estimate the stand mean, the resulting standard error of the mean (0.17‰) will propagate with the difference in the potential source waters in space (1.5‰ per 30 cm depth) or in time (1.7‰ per month), yielding an uncertainty in the fractional contributions. For example, if two hypothetical source waters are separated by approximately 30 cm or 1 month, they could be expected to differ by roughly 1.5–1.7‰, and thus if the xylem water $\delta^{18}O$ is known to within 0.17‰, the standard error of the fractional contributions would be approximately 10%. This represents an optimistic uncertainty estimate for source-water determination under favourable maximum gradients. Errors in the source-water measurements (i.e. $SE(\delta_a)$ *and* $SE(\delta_b)$), cryogenic extraction biases and mixing of throughfall waters in the soil (leading to a reduction of the temporal gradient) would all further increase the error of the estimated fractional contribution.

4 | DISCUSSION

4.1 | Tree scale water isotope signatures: how large are sampling uncertainties within individual trees?

While the *variations* of isotope signatures in different cardinal directions around the stem at a given height were similar across sampling heights, the *average* isotope signatures changed with height along the stem. This might suggest that at the time of sampling, the stem xylem contained waters from different proportions of different source water pools at different heights along the stem. Sampling stem xylem instead of branch xylem greatly reduced the variability of our sample replicates. It appeared that stem sampling in the lower part of the stem provides isotopic water samples that are arguably the least altered from recent root water uptake by transport or storage effects (see discussion in sections 4.1.3 and 4.5).

4.1.1 | Effects of sampling time

Observed sub-daily variations were small when comparing stem xylem samples taken around 9 AM and 3 PM from supposedly transpiring beech and oak trees during the September sampling campaign. Therefore, different sampling times during the day did not affect the obtained isotope signatures. This is in line with previous findings in Zhao et al. (2016), Magh et al. (2020) and De Deurwaerder et al. (2020) that found no systematic sub-daily variations in stem xylem of poplar (*Populus euphratica)*, silver fir (*Abies alba*) and beech (*F. sylvatica*). Results from other studies that did show systematic sub-daily variations were derived from compartments that are not only much smaller than our main stems but also closer to the parts of the tree where transpiration occurs, such as suberized branches (Martín-Gómez et al., 2017), a range of suberized to unsuberized branches (Dawson & Ehleringer, 1993) or leaves (Cernusak et al., 2005). Size (i.e. stem or branch diameter) and distance can both reduce the magnitude of fractionation: Dawson and Ehleringer (1993) showed that sub-daily variations in branch xylem samples decreased with distance from the tips of the branches. For geometrical reasons, for a given area of potentially evaporating surface (and thus a given rate of evaporation), larger-diameter branches expose more wood (and also a larger volume of water) to evaporation than smaller-diameter branches do, resulting in smaller fractionation effects (Dawson & Ehleringer, 1993). The same scaling arguments apply to surfaces that potentially exchange water with phloem (or heartwood), for which we again expect larger-diameter branches to show smaller fractionation effects due to phloem-xylem (or heartwood-xylem) exchanges. Thus, we would expect larger sub-daily variations in xylem water isotope signatures in compartments with smaller storage volumes, and those that are closer to the parts of the tree where transpiration occurs.

4.1.2 | Effects of sampling orientation

Already at the lowest sampling height H1, N/E/S (North/East/South) variability in stem xylem was smaller than the N/E/S variability in bulk soil waters at 2 m distance from the stem. Trees' root systems obtain water from a soil volume that extends both vertically and laterally. Integration of source waters over this volume not only compensates for spatial variability in soil moisture (Guswa, 2012), but also averages over variations in soil water isotope signatures. Alternatively, tree internal mixing may be strong enough to reduce the lateral N/E/S variability found in bulk soil waters, yielding more homogeneous stem xylem waters at H1. This agrees with previous findings of reduced variability in stem xylem waters relative to root xylem waters (Vega-Grau et al., 2021). Species differ in their xylem structure, with different interconduit pit connectivity between adjacent vessels causing varying amounts of radial and circumferential xylem sectorality (Sperry, 1995; Zanne et al., 2006). For example, Norway spruce xylem only consists of tracheids, whereas oak xylem and beech xylem consist of both tracheids and vessels. Further, in beech trees, vessels are distributed across the whole xylem (*diffuse-porous*) whereas in oak

trees, vessels are located in earlywood and tracheids in latewood (*ring-porous*). Moreover, species can differ in xylem layer thickness, which is delimited by heartwood that does not actively facilitate water transport. Given the observed homogenization of the bulk soil water (either through integration by the root system or through circumferential mixing) in all three of our observed species, lateral variability due to the sampling orientation of the stem xylem core is expected to result in negligible variations when ambient isotope signatures are used for source water estimation. This result is in line with the rather small isotopic differences with sampling orientation observed in previous studies with ambient isotopes (Goldsmith et al., 2019; Treydte et al., 2021) and applied tracers (Nehemy et al., 2021). Studies that have shown dependency on sampling orientation (Treydte et al., 2021; Volkmann et al., 2016) relied on tracer-based approaches that likely caused much stronger spatiotemporal (and, specifically, lateral) isotope gradients in soil waters than are typically found under natural conditions in a temperate climate.

4.1.3 | Effects of sampling height

Within the tree stems, we found variations with sampling height. For most individual trees these variations were monotonically increasing or decreasing, forming gradual trends (with the exception of δ^2H in spruce; Figure S7). The direction of these trends for $\delta^{18}O$ differed between the species and sampling campaigns (i.e., enriched isotope signature with height for spruce in December and depleted isotope signature with height for beech in September; Table 1). We could not detect isotopic differences between H4 and H1 when separately testing the N, E and S directions, but by pooling these directions together we could detect significant differences between H4 and H1 (for $\delta^{18}O$ in beech and spruce and for δ^2H for all species). Differences between stem xylem waters at heights H1 and H4 could be caused by (i) a transport lag combined with a change in the source water (Seeger & Weiler, 2021; Mennekes et al., 2021; Nehemy et al., 2022) or, if the source water remained similar, (ii) exchanges of the xylem water with waters stored in the tree or flowing through the phloem (Barbeta et al., 2020; Nehemy et al., 2022) or (iii) increasing evaporative enrichment with increasing height, due to increasing proximity to the parts of the tree where transpiration occurs. For instance, Cernusak et al. (2005) and Dawson and Ehleringer (1993) showed that the enrichment signal of xylem waters decreased from the tip of the branch towards the base of the branch, disappearing a few nodes away from the tip of the branch (Dawson & Ehleringer, 1993). Hence, these previous results suggest that the effect of evaporative enrichment should be small in xylem samples from the main stem. Furthermore, the opposite height trend at the two sampling dates and the lack of clear evaporation signals in the dual isotope space (Figure 6c and Supplementary Figure S1c) make hypothesis (iii) – evaporative enrichment – an unlikely explanation for the differences between stem xylem waters from the lowest and highest sampling positions (H1 and H4). In previous studies, no systematic height effect was observed in willow (Nehemy et al., 2021), poplar (Zhao et al., 2016), olive trees (Amin et al., 2021) or *Corymbia* or *Eucalyptus* trees (Vega-Grau et al., 2021). Neither Zhao et al. (2016) nor Amin et al. (2021) found differences in isotope signatures between stem xylem and branches. All these previous findings are further evidence against evaporative fractionation systematically affecting stem xylem isotope signatures, supporting the remaining hypotheses of (i) a lag effect due to transport along the stem and (ii) exchanges between flowing xylem waters and stem storage or phloem.

The lack of systematic height effects in the previous studies could potentially be explained by the availability of a temporally stable water source (e.g. poplar trees potentially source groundwater; Zhao et al., 2016) or smaller tree sizes resulting in shorter water residence times (e.g. potted trees; Amin et al., 2021; Nehemy et al., 2021). Source waters with a relatively stable isotopic composition could not only hide the effect of lags due to transport through the stem (i) but could further lead to an isotopic steady state and thereby mask potential exchanges with waters stored in the tree (ii). Conversely, we hypothesize that temporal variations in precipitation and soil water isotopes at our sites were a prerequisite for observing the height effect along the stem. However, connecting the magnitude or direction of the observed height trends to previous stem xylem waters or seasonal evolution of precipitation is difficult with our limited data set. Given the absence of diel (i.e., 9 AM vs 3 PM) variations, the observed vertical variation is likely due to slower processes than the sub-daily variation postulated by De Deurwaerder et al. (2020). Model results of travel times in beech by Seeger and Weiler (2021) based on measurements of stem xylem isotopes at the stem base and a height of 8 m, also indicate that variations of $\delta^{18}O$ and δ^2H signatures within the stem xylem occur at longer timescales (i.e., larger than daily time scales). Their model indicated that the median xylem water age since root uptake at 8 m stem height was between 2 and 8 days, while at the stem base, xylem water age since root uptake was between 0.4 and 4 days.

Although our study design cannot determine the causes of the observed height differences in stem xylem isotopes, we assume that the potential causes – whether a temporal lag in water uptake from variable sources or exchange effects with water stored in the tree or flowing through phloem – would most likely be smaller at lower heights along the stem. Thus, sampling at lower heights could mitigate the increasing uncertainties along the upper parts of the stem for studies of root water uptake. As an alternative, dynamic soil water balance models explicitly simulating the evolution of soil water and root water uptake over time, and that incorporate transport times and tree hydraulics (incl. exchanges with potential storage volumes), could be used to study root water uptake patterns as well as to test potential causes of the observed height differences (Seeger & Weiler, 2021; Knighton et al., 2020; De Deurwaerder et al., 2020).

Potential causes of the height effect should also be examined in further studies using high-frequency measurements of stem xylem signatures at multiple heights, combined with occasionally sampled, cryogenically extracted stem cores. Most samples gathered during our study represent a single snapshot in time for each species. Thus, patterns arising through dynamic interactions between source water

pools might be missing or hard to discern. Repeated sampling was only done for spruce (a week after) and for oak and beech by sub-daily repetitions. Future campaigns with repeated sampling or continuous measurements of soil and plant water isotopic composition with automated in-situ systems (e.g., Gessler et al., 2022) could provide better evidence of transient effects in root water uptake.

4.1.4 | Effects of sampling compartment

The tree compartment from which plant water was extracted affected the variability of plant isotope signatures. For spruce species, branch xylem signatures at the tree scale showed two- to four-fold greater variability than stem xylem signatures (up to 1.2‰ and 4.8‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively, in branch xylem, compared with up to 0.3‰ and up to 2.2‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively, in stem xylem; Table S4). Goldsmith et al. (2019) measured standard deviations of 0.8‰ and 3.6‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively, in branch xylem in the crowns of individual spruce trees ($n = 3$). These tree-scale branch xylem standard deviations were comparable to our tree-scale observations in the branch xylem of spruce trees. Additionally, Goldsmith et al. (2019) measured beech tree-scale branch xylem variability of 0.3‰ and 1.8‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively, i.e. about half as large as the variabilities they observed in spruce trees at the same forest stand. Nehemy et al. (2021) found standard deviations of similar magnitudes (of 0.4‰ and 1.65‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively) within a potted willow tree across multiple main stems and branches and multiple heights.

Waters from stem phloem were enriched compared with stem xylem waters (Figure 4), agreeing with Cernusak et al.'s (2005) observations of $\delta^{18}O$ signatures in phloem waters, while contrasting with most (Nehemy et al., 2022) or all (Treydte et al., 2021) observations of other studies. In addition, isotopic variability was larger in the stem phloem than in the stem xylem, both in Cernusak et al. (2005) and in our study. An enrichment suggests that phloem waters can be affected by evaporative enrichment; however, it is unclear whether the mixing of water between phloem and xylem via the Münch counter flow has any measurable effect on stem xylem water signatures, as water volumes and flux rates in the xylem are more than an order of magnitude higher in the xylem than in the phloem (Hölttä et al., 2006).

4.2 | Stand-scale isotopic variability

4.2.1 | Branch xylem waters

Across individual spruce trees ($n = 5$), we measured stand-scale standard deviations in branch xylem for each sampling height H2 to H4 to be ~1.7 to 3.0‰ and ~3.7 to 7.7‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively.

Brinkmann et al. (2019) determined branch xylem standard deviations at a forest site approximately 12 km from our *Waldlabor* site on multiple sampling days over three vegetation seasons. They measured stand-scale standard deviations comparable to ours, ranging from 0.5 to 1.6‰ and 1.7 to 8.1‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively, across four individual spruce trees. In a mixed forest, Goldsmith et al. (2019) found standard deviations in branch xylem of spruce trees ($n = 23$) of 1.0‰ and 5.7‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively, over an area of 100 m by 100 m.

4.2.2 | Stem xylem waters

In stem xylem waters, the stand-scale standard deviations for $\delta^{18}O$ at H1 in all three tree species were drastically smaller than they were in branch xylem waters of the spruce trees. The stand-scale standard deviations of $\delta^{18}O$ in stem xylem waters observed in this study are comparable to the standard deviations observed in eucalyptus plantations by Cernusak et al. (2005) ($n = 5$ to 6), ranging from 0.2 to 1.0‰ for $\delta^{18}O$. Zhao et al. (2016) also found that the stand-scale standard deviations of isotope signatures in *stem* xylem samples at 1 m height were smaller than in *branch* xylem samples across three poplar trees for $\delta^{18}O$ and $\delta^{2}H$. Vega-Grau et al. (2021) also found smaller stand-scale variability in stem xylem samples from breast height than in branches across six *Corymbia* trees, whereas stem and branch variabilities were similar across six *Eucalyptus* trees in a mixed forest stand. Overall, stem xylem sampling at low stem heights could provide an alternative, more robust signal for root water uptake studies than branch xylem sampling, both at the tree scale and at the stand scale.

4.3 | Potential influence of transpiration rate or other seasonal factors

During late autumn sampling, transpiration rates may be lower than during the summer and residence times of water within the tree stem may be correspondingly longer. However, sap-flow measurements in trees located 700 m away indicated little reduction in spruce sap-flow rates during early December 2021 and 2022, whereas beech sap-flow rates dropped in September to approximately half their peak rates in June. It is likely that transpiration rates in 2020 at our study site behaved similarly.

If transpiration rates decrease, the resulting increase in residence times could lead to an enhancement of height-specific variations in isotopic plant water signals within a single tree. Moreover, variabilities in residence times *across* a tree stand could lead to larger variabilities of isotope signatures at the stand scale. For example, Brinkmann et al. (2019) measured lower stand-scale variability during the summer months, when residence times are expected to be at their minimum. Longer residence times might complicate the determination of plant water sources because of transport lags. Moreover, longer residence times could have increased the height effect in isotope signatures we found along the stem. Hence, the tree- and stand-scale variabilities reported here – observed at the selected study date in autumn – probably represent an upper limit for the variabilities that would be expected during the rest of the vegetation season.

Besides reduced transpiration rates, other seasonally varying physiological processes could further affect observed spatiotemporal variability of xylem water isotope signatures. As an example, winter hardening can not only (a) affect transpiration in evergreen coniferous trees (Christersson, 1972), but also (b) lead to stem dehydration and affect water distribution in the stem and (c) increase water flow between heartwood and both phloem and sapwood (driven by osmotic pressure due to sugar/sugar alcohol accumulation). As a result, winter hardening could potentially favour the fractionation of stem xylem water and therefore alter the direction of isotopic differences and the magnitude of isotopic spatial variability during wintertime.

4.4 | Propagation of stem-xylem uncertainty to source-water determination

We estimated the uncertainties associated with using a single stem xylem measurement to represent an entire tree or tree stand. The uncertainty associated with using a single sample to represent the tree's source water is assumed to be on the order of the observed tree-scale standard deviation at H1 of <0.3‰ for $\delta^{18}O$ and <1.8‰ for $\delta^{2}H$. At the stand scale, H1 stem xylem signatures showed only marginally larger variability than at the tree scale (with standard deviations of ~0.3‰ in beech, oak and spruce for $\delta^{18}O$ and 1.4‰ in oak and 2.2‰ in beech and spruce for $\delta^{2}H$). These values are between 2 and 3 times the analytical accuracy of our lab equipment. Even though our estimates of stand-scale variability were similar to those in other studies, factors influencing this variability might depend on the soil heterogeneity and stand structure (e.g., tree age distribution). For our site and conditions, a study design with single stem cores sampled at breast height regardless of orientation or time of day appears to provide a good estimate of the stand-scale isotope signatures.

Whether the error of that estimate is sufficiently small depends on the intended application. For source water attribution, one compares plant waters, usually xylem waters, with soil waters. A systematic offset in one of them constitutes an additional source of error in addition to the variability. For oak and spruce, potential source water depths tended to shift to deeper soil layers when assessed based on stem xylem water $\delta^{2}H$ instead of $\delta^{18}O$, whereas for beech, stem xylem water $\delta^{2}H$ values were beyond the range of the sampled soil water. This pattern could potentially be explained by a $\delta^{2}H$ offset that affected the xylem waters but not the bulk soil waters (see discussion in section 4.5).

Under the observed spatiotemporal isotope gradients and variabilities in this study, the uncertainty of the stand-scale estimate of xylem water signature would translate to a standard error of ~10% in estimating the fractional contributions from two hypothetical water sources corresponding to a depth shift of 30 cm or a temporal shift of 1 month. Such error propagation considerations illustrate that at sites where potential source waters show only small or no differences in space or time, i.e., sites with small seasonal gradients in precipitation isotopic composition and relatively homogeneous source water isotope signatures, source water attribution from isotopes will be more uncertain (Allen & Kirchner, 2022). Large errors in the result could either be mitigated by sampling more replicates and thereby reducing the standard error of the stand-scale estimates of soil and plant water isotope signatures, or by considering additional information such as soil moisture measurements or mechanistic water balance models (Seeger & Weiler, 2021; Meusburger et al., 2022).

4.5 | Potential sources for $\delta^{2}H$ offsets between bulk soil and stem xylem waters

Similarly to previous studies, we found that the plant water isotope signatures did not lie along the global meteoric water line (GMWL) in dual isotope space, potentially reflecting an offset in $\delta^{2}H$ values between plant and soil water samples (Figure 6 c,d). Interestingly, the $\delta^{2}H$ offset showed distinct values between species: Median SW-excess in $\delta^{2}H$ was quantified between −5.5 and −18‰ for the three species. These values were within the range of observations from previous studies (Barbeta et al., 2020, 2019; Chen et al., 2020; de la Casa et al., 2022; Diao et al., 2022; Tetzlaff et al., 2021; Zhao et al., 2016).

The differences we found in SW-excess $\delta^{2}H$ between species contrasted with observations from previous studies, which found either no differences (Barbeta et al., 2019; de la Casa et al., 2022) or effects opposite to what we observed in gymnosperms and angiosperms, respectively (Tetzlaff et al., 2021). de la Casa et al. (2022) did not find differences in SW-excess $\delta^{2}H$ between gymnosperms and angiosperms, or when assessing wood properties across a global data set. Barbeta et al. (2019) did not find species-specific differences in SW-excess $\delta^{2}H$ when comparing twig samples between *F. sylvatica* and *Q. robur* over a vegetation season. However, Barbeta et al. (2019) could show significant differences in SW-excess between twig samples and root samples across beech and oak trees. SW-excess $\delta^{2}H$ might not be a static quantity but evolve dynamically throughout a season, conditioned by shifts in source waters for the specific study site (Tetzlaff et al., 2021). This might make it more difficult to investigate mechanisms leading to SW-excess $\delta^{2}H$ when analysing globally or seasonally aggregated data sets such as in the two cited studies. Tetzlaff et al. (2021) observed significant SW-excess $\delta^{2}H$ across five temperate sites with more negative median SW-excess $\delta^{2}H$ in gymnosperms than in angiosperms at three of their five sites. This contrasts with our finding of more negative SW-excess $\delta^{2}H$ in beech and oak (angiosperms) than in spruce (gymnosperm). Potential explanations for this contrast might be linked to differences in the size of the sampled trees or bushes, the sampling method (stem cores vs branches) and the timing of the sampling, rather than the vegetation type (gymnosperm vs angiosperm). Snapshot samplings with high spatial resolution or controlled experiments could provide data sets with fewer confounding factors.

Differences that have been observed in SW-excess $\delta^{2}H$ could potentially be linked to species- and height-specific wood anatomical properties of the stem or the sampled core. In a recent, controlled steady-state experiment with potted beech trees, Barbeta et al.

(2020) found δ^2H offsets between soil and plant water samples that covered a range from −20 to +20‰. Barbeta et al. (2020) attributed these offsets to plant internal processes, such as mixing xylem-vessel water with stored water in other stem tissues and considered fractionation processes during root water uptake to be unlikely.

Potential factors for our observed variations in SW-excess δ^2H could be (i) the ratio of sapwood to heartwood or (ii) the fraction of radial and axial parenchyma (RAP). Both of these factors influence the amount of stored water within the stem. RAP can increase radial transfer between sapwood (xylem) layers, heartwood and inner bark. These factors might also interact with the sampling method and water extraction method. The fraction of heartwood in the stem cores could increase with sampling height, due to the constant coring depth of 5 cm. This could influence the isotope signatures (Fabiani et al., 2022), as cryogenic extraction is likely to extract water from the xylem vessels together with other waters present in the sample. Gymnosperms (such as Norway spruce) contain considerably less RAP than angiosperms (such as oak and beech) (Godfrey et al., 2020; Morris et al., 2016; Plavcová & Jansen, 2015; Zhang et al., 2022). The fraction of RAP cells in spruce (Picea) is around 5%, whereas in oak and beech trees it is typically above 20% (Plavcová & Jansen, 2015; Rezaie et al., 2023). RAP fraction could vary with sampling height: Barbaroux et al. (2003) showed that total nonstructural carbohydrates in wood (a proxy for RAP) gradually increased with height throughout the stem and in the branches of beech and oak trees. This is consistent with direct observations of RAP fraction by Rezaie et al. (2023) that measured larger RAP fractions in coarse roots than in the stem. Thus, RAP and/or heartwood fractions could potentially explain the variations of SW-excess δ^2H between species and sampling heights in our study. Note that besides RAP, cell walls can also bind water molecules, separating them from the free water in the transpiration stream (Berry & Roderick, 2005) and thus potentially inducing another memory effect.

Methodological artefacts from cavity ring-down spectrometry (CRDS) (e.g. if the MCM incompletely removed organic compounds) or from cryogenic extraction cannot entirely be ruled out as alternative explanations for offsets in $\delta^{18}O$ or δ^2H. Comparison of CRDS measurements and isotopic ratio mass spectrometer (IRMS) measurements on a subset of samples reveals no systematic differences in stem xylem signatures (Figure S1). Hence, it is unlikely that a CRDS measurement artefact introduced the observed δ^2H offsets. However, cryogenic extraction artefacts arising from exchangeable H atoms in woody material (Chen et al., 2020; Diao et al., 2022) could provide an alternative explanation for a measurable offset in δ^2H (and none in $\delta^{18}O$). Exchangeable H atoms in woody material might differ between tree species, and might also vary with sampling height, thus potentially explaining the pattern in SW-excess δ^2H we observed in oak. In a previous study with a poplar species accessing an isotopically stable water source (i.e., groundwater), Zhao et al. (2016) showed a clear negative δ^2H offset of around −10‰ from groundwater towards more depleted signatures in the cryogenically extracted stem, root and branch xylem waters (with maximum offsets up to −20‰). Syringe-sampled stem xylem samples from the same trees did not show any δ^2H offset. The same samples did not show any significant differences in $\delta^{18}O$. These observations illustrate the utility of alternative sampling techniques accessing xylem vessel water in a more direct way and suggest consideration of plant internal processes, e.g. exchanges between heartwood and sapwood (Barbeta et al., 2022; Fabiani et al., 2022). Overall, the use of $\delta^{18}O$ signatures for source water attribution might be more reliable than using δ^2H signatures.

5 | CONCLUSION

The analysis of isotope signatures in xylem and soil waters can aid in estimating the sources of tree water uptake. However, the number of samples and replicates is typically limited, and uncertainties arising from the sampling design are often not comprehensively reported. In targeted field campaigns, we collected multiple xylem and soil water samples along the different compartments of beech, oak and spruce trees to assess the potential sources of uncertainties in using isotope signatures for water source attribution.

Our results suggest that sub-daily variations and variations in the cardinal direction of stem xylem waters within a single tree were neither systematic nor very large. Thus, sampling time during the day and orientation along the stem are factors that can potentially be ignored when designing sampling campaigns at sites with similar latitudes, climates and species. The sampled compartment (i.e., stem xylem, branch xylem) and sampling height affected both plant water isotope signatures and their variability. We found distinct differences in the isotope signatures with height along the stem. The trend of the height differences showed opposite directions between our two sampling campaigns. These height trends in the isotope signatures suggest that a mixture of waters – taken up at different times or from different soil layers – was present simultaneously in the tree stem at different heights during sampling. The observed height trends were potentially consistent with a shift in source water isotopic composition within the time lag of vertical transport through the tree, or with exchanges of flowing water with water stored in the tree (e.g. xylem parenchyma, heartwood tissue, phloem, …), but not with evaporative enrichment. Stem xylem was much less variable than branch xylem or stem phloem among individual trees. However, coring stems is potentially more invasive than cutting branches, so study designs will require considering how the choice of sampling method will affect the number of replicates that are needed to achieve a desired level of precision. Even at breast height or below, the isotopic variability of the stem xylem waters was smaller than that of the bulk soil waters surrounding the tree. Thus, xylem waters from the lower stem provide isotope signatures that are likely to be little altered from recent root water uptake, but also mixed well enough to represent the integrated signature of recent root water uptake. Tree-scale standard deviations at low stem heights were small, and uncertainties were comparable to previous studies. Trees of the same species growing close to each other had similar stem xylem water isotope signatures. Thus, stand-scale variabilities were rather small, suggesting that small numbers of stem

xylem samples can provide a good approximation to the stand-scale xylem water isotopic composition.

We found discrepancies between δ^2H and $\delta^{18}O$ isotope signatures when comparing cryogenically extracted samples from bulk soil and xylem. For cryogenically extracted samples, $\delta^{18}O$ might be more reliable than δ^2H for source water attribution, and height- and species-specific variation of SW-excess δ^2H of stem xylem needs to be considered. Future work is needed to characterize the origin of δ^2H variations for cryogenically extracted xylem water. This study demonstrates that for trees in mixed temperate forests, sampling stem cores close to the ground can give a more precise and less altered signal of recent root water uptake than sampling branch xylem or stem cores higher up, and can constitute a viable alternative to branch xylem sampling for source water attribution studies.

AUTHOR CONTRIBUTIONS

F.B. and M.F. designed the study. F.B., M.F., and K.M. performed the sampling. F.B. processed the samples in the laboratory and did the formal analysis. F.B. prepared the original draft manuscript with contributions from all co-authors.

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DATA AVAILABILITY STATEMENT

The stable water isotope data and the gravimetric water content of the samples are available in supplementary material as a .csv file and reported in Table S1. Gravimetric water content was determined by weighing the samples before and after cryogenic extraction.

ORCID

Fabian Bernhard https://orcid.org/0000-0003-0338-0961
Marius G. Floriancic https://orcid.org/0000-0001-5108-4580

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SUPPORTING INFORMATION

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

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APPENDIX A: Additional study site information

Figure S4 shows an additional overview of the sampling heights, tree heights and tree diameters for each individual tree, as well as their approximate crown extents.

APPENDIX B: Validation of Picarro cavity ring-down spectrometer versus mass spectrometer (IRMS)

Based on a subset of bulk soil, phloem and xylem samples, Figure S1 confirms that $\delta^{18}O$ and $\delta^{2}H$ values measured on a Picarro cavity ring-down spectrometer with a combustion module generally agree well with those measured on an isotope ratio mass spectrometer (IRMS).

APPENDIX C: Correction of evaporation through pierced septa

Evaporation through pierced septa between multiple analyses was observed (Figure S2a and S2b) and was accounted for (Figure S2c,d,e) with a correction based on the Craig-Gordon model taking into account the sample amounts and the storage duration. The model used for computing initial isotope signatures $\delta_{init.}$ based on measured evaporated signatures $\delta_{evap.}$ was (Gonfiantini et al., 2018):

$$\frac{\delta_{evap.}+1}{\delta_{init.}+1}=f^{B},\ \text{where}\ B=\frac{\gamma}{\alpha_{eq}\alpha_{dif}^{X}(\gamma-h)}-1\ \text{and}\ f=\frac{m_0-t\times rate_{evap}}{m_0}$$

Fractionation factors $\alpha_{eq}(T)$ and α_{dif} were taken from Gonfiantini et al. (2018) based on Majoube (1971) and Merlivat (1978). Further, activity $\gamma=1$ and relative humidity $h=0$, were assumed. The amount of extracted water was used as initial mass m_0. The evaporation rate (g/week) was estimated to be 0.7 mg/week, based on a separate experiment (data not shown). The turbulence index X was assumed to be 0.5. The sensitivity of the model with respect to $rate_{evap}$ and X is small and is shown in Figure S3.