https://doi.org/10.5194/essd-2024-409
Preprint. Discussion started: 17 October 2024

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Soil and stem xylem water isotope data from two pan-European sampling campaigns

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https://doi.org/10.5194/essd-2024-409
Preprint. Discussion started: 17 October 2024

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Abstract. Stable isotope ratios of hydrogen (δ2H) and oxygen (δ18O) are crucial for studying ecohydrological dynamics in forests. However, most studies are confined to single sites, resulting in a lack of large-scale isotope data for understanding tree water uptake. Here, we provide a first systematic isotope dataset of soil and stem xylem water collected during two pan-European sampling campaigns at 40 beech (Fagus sylvatica), spruce (Picea abies), or mixed beech-spruce forest sites in spring and summer 2023 (Lehmann et al., 2024). The dataset is complemented by additional site-, soil-, and tree-specific metadata. The samples and metadata were collected by different researchers across Europe following a standardized protocol. Soil samples were taken at up to 5 depths (ranging from 0 to 90 cm) and stem xylem samples from three beech and/or spruce trees per site. All samples were sent to a single laboratory, where all analytical work was conducted. Water was extracted using cryogenic vacuum distillation and analyzed with an isotope laser spectrometer. Additionally, a subset of the samples was analyzed with an isotope ratio mass spectrometer. Data quality checks revealed a high mean total extraction efficiency, mean absolute water amount (> 1 mL), as well as high analytical accuracy and precision. The water isotopic signature of soil and stem xylem water varied as a function of the geographic origin and changed from spring to summer across all sites. While δ^2H and $\delta^{18}O$ values were strongly correlated, the soil water data plotted closer to the Global Meteoric Water Line (GMWL) than the stem xylem water. Specifically, the δ^2H values of the stem xylem were more enriched than those of the soil water, leading to a systematic deviation from the GMWL. Isotopic enrichment of the stem xylem water was larger for spruce than for beech trees at mixed forest sites. This dataset is particularly useful for large-scale studies on plant water use, ecohydrological model testing, and isotope mapping across Europe.

Keywords: Critical Zone Science, Europe, Forest, Hydrology, Hydrogen Isotopes, Oxygen Isotopes, Root Water Uptake, Soil Water Recharge, Water Stable Isotopes, Water Sources.

1 Introduction

Understanding how tree water uptake from soils varies with species, site characteristics, time, and across climate zones is essential to assess forest resilience to climate change; particularly the response of forests to the increasing frequency and intensity of droughts (Lindner et al., 2010; Spinoni et al., 2014; Büntgen et al., 2021). Despite some uncertainties, the stable isotope ratios of hydrogen (δ^2H) and oxygen ($\delta^{18}O$) in water extracted from soil and plants allow for the determination of the sources of water that are used by plants and to quantify the relative contribution of different water sources to plant water use (Rothfuss and Javaux, 2017; Beyer and Penna, 2021). Determination of water uptake patterns based on isotope data assumes that roots do not discriminate against the heavier hydrogen and oxygen stable isotopes during water uptake (Poca et al., 2019). Additionally, it is assumed that: (i) the sampling design captures the spatiotemporal variability of the isotopic composition of soil water sources, (ii) the water extracted from the plant xylem is a mixture of the different water sources taken up from the soil profile without isotopic alteration (e.g., due to stem evaporation or leaf transpiration, see Ellsworth and Sternberg (2015), and (iii) soil and xylem samples are collected, transported, stored, and extracted in a manner that avoids isotope fractionation (Ceperley et al., 2024). Although these assumptions are not always met, the method described here—

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whether used independently or in combination with others—can effectively test our understanding of the mechanisms driving plant responses to both short- and long-term droughts. It is also now affordable enough for practical applications beyond the field of isotope ecohydrology (Penna et al., 2018). Isotope-based analyses in forest ecosystems have, for example, been used to determine the changes in root water uptake depths of trees in response to drought (Brinkmann et al., 2018; Gessler et al., 2022), whether trees use summer or winter precipitation (Allen et al., 2019; Floriancic et al., 2024a), soil water, groundwater, or streamwater (Bowling et al., 2017; Engel et al., 2022), or to assess competitive or complementary water use strategies (Penna et al., 2020; Kinzinger et al., 2024). However, systematic datasets at large scales, i.e., spanning continents or multiple countries, are lacking. This hampers our understanding of how water uptake strategies for the same tree species vary across space and time (Beyer and Penna, 2021; Orlowski et al., 2023; Dubbert and Werner, 2019; Bachofen et al., 2024).

There are established networks for the observation of isotopes in freshwater systems, such as precipitation by the International Atomic Energy Agency (IAEA) Global Network of Isotopes in Precipitation (GNIP), which currently contains data for 300 active sites in 93 countries (Terzer-Wassmuth et al., 2023). The Global Network of Isotopes in Rivers (GNIR) contains data from 750 sites in 35 countries (Halder et al., 2015). Both networks have proven to provide valuable input data for modeling of the local to regional climate or surface-atmosphere water interactions with process-based (e.g., CLM, Wong et al. (2017), ISOLSM Cai et al. (2015), ECHAM5-JSBACH Haese et al. (2013)) or statistical models (e.g., Isoscapes (Bowen, 2010; Terzer et al., 2013; Allen et al., 2018; Koeniger et al., 2022), and time series analyses (Nelson et al., 2021; Erdélyi et al., 2023; Reckerth et al., 2017). They have furthermore helped to assess water flow pathways and the fraction of young water in streamflow (Von Freyberg et al., 2018; Floriancic et al., 2024b). The Moisture Isotopes in Biosphere and Atmosphere (MIBA) network, initiated by the IAEA in 2003-2004, is, to our knowledge, the only international network to survey the isotopic composition of water across different ecosystem compartments (i.e., soil, plant stems and leaves, soil, and atmospheric vapor). However, despite the global distribution of sites at the time of the establishment and a local application in Australia (Twining et al., 2006), the network is currently inactive.

Building on the idea of the MIBA and the proven usefulness of national large-scale sampling campaigns to determine regional differences in tree water uptake (Allen et al., 2019), the COST Action "WATer isotopeS in the critical zONe: from groundwater recharge to plant transpiration WATSON" (CA19120) organized two sampling campaigns across Europe in 2023. The effort took advantage of the European network of researchers to establish a unique systematic water isotope dataset and corresponding metadata. More specifically, the goal of the sampling campaigns was to obtain soil and stem xylem water isotope data of two tree species, namely beech (*Fagus sylvatica* L.) and spruce (*Picea abies* (L.) H. Karst) across a large climate gradient for the spring (25th May to 16th June) and summer (17th August to 18th September) of 2023. The two time points were selected to compare tree water uptake patterns under different soil moisture conditions (e.g., lower soil moisture in summer). The two species were selected because of their wide geographical distribution across Europe (Figure 1) and their important ecological and economical relevance, as well the expected differences in water uptake depth (Allen et al. 2019; Brinkmann et al. 2018; Goldsmith et al. 2019) with beech having a deeper rooting system than spruce.

During the European sampling campaigns, a total of 381 soil and 311 stem xylem samples were taken from 40 sites across 18 countries, following a standardized protocol. The water of these samples was cryogenically extracted and isotopically analyzed in a single laboratory. The simultaneous collection of soil and stem xylem

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samples across all European sites, combined with a centralized processing of the samples, ensures the uniqueness of this dataset. Using one laboratory prevents inconsistencies that might arise from varying sample handling and analysis methods, which can influence isotopic offsets (Orlowski et al., 2016; Orlowski et al., 2018). The isotope dataset is accompanied by site-, soil-, and tree-specific metadata at each location. Together, the metadata and isotope data provide a strong foundation for research on tree water use, model testing, and isotope mapping. This manuscript outlines the sample collection process, cryogenic water extraction, and isotope analysis, and details the dataset organization and metadata. Finally, we give an overview of the data and discuss potential applications. The full dataset is freely available (Lehmann et al., 2024).

2 Material and Methods

2.1 Organization of the WATSON pan-European sampling campaigns

During the initial phase (spring 2023), the members of the WATSON community (~200 members at that time) were contacted to assess their interest in participating in a coordinated sampling campaign. Based on the large interest, a core team was formed. The core team asked researchers from a similar region to form one team to keep the laboratory and analytical work manageable, while still obtaining samples from a broad geographic region. The core team wrote detailed instructions to ensure systematic sampling. The instructions provided detailed standardized protocols for collecting soil and stem xylem samples, including specifications for sampling depths, core dimensions and numbers, and the maximum number of samples. The protocols also covered short-term sample storage and shipment to the Swiss Federal Institute for Forest, Snow, and Landscape Research in Birmensdorf, Switzerland (WSL Birmensdorf), where all cryogenic water extractions and isotopic analyses were performed. In addition, participants were given instructions on how to take pictures for canopy cover analysis and the list of required metadata (e.g., geographical parameters, soil properties, tree diameter and height). The instructions were emailed to all interested contributors prior to the first sampling campaign in spring 2023 (Section S1). For the second campaign in summer 2023, the sampling protocol was slightly updated for clarity (i.e., weather conditions at sampling day, bark removal during stem xylem sampling, labelling of exetainers, taking photos) and emailed to all interested contributors again (Section S2). In addition, we held an online meeting between the two sampling campaigns to provide feedback to the participants, clarify any field issues, and answer questions.

2.2 Description of the sampling sites

Samples were taken from 40 different mono-specific and mixed forest sites with beech trees (*Fagus sylvatica*; 14 sites), spruce trees (*Picea abies;* 13 sites), or both tree species (13 sites) in 18 European countries (Figure 1; Table 1): 36 sites were sampled in the spring and 39 sites in the summer. For 35 of the 40 sites, samples were collected during both campaigns. In three of the sampling sites, separate beech (LIZ1, GLS1, WEI1) and spruce (LIZ2, GLS2, WEI2) stands were found close to each other (i.e. the sampling sites share the same geographic coordinates). Although there was a good cover of sites across central Europe for both species, most north-eastern sites were sampled for spruce only, while the spread of sampled beech trees extended more to south-western Europe. The sampling sites correspond to the natural and naturalised ranges of the tree species across Europe (Figure 1) and cover a range of temperate (Köppen-Geiger Cfa, Cfb, Csb) and cold (Köppen-Geiger Dfb, Dfc) climates. The

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sampling sites also differed in elevation (14 to 1870 m a.s.l.; Table 1). The sampling sites were evenly distributed across different slopes (i.e., flat, gentle, and steep). Most sites were located on Cambisols or Leptosols; with just one Histosol (i.e., peat at the site ROT in Finland). The maximum existing soil depth varied between 0.3 m and > 1 m and for half of the sites, the maximum soil depth was > 0.6 m. Canopy cover was determined for 30 of the 40 sampling sites from non-hemispherical photographs taken with a phone camera, as described in Section S3. Most of the pictures were taken during the spring campaign, however, for some sites, pictures were taken during the summer campaign or both campaigns. For the sites for which canopy cover could be determined, it was generally higher for the beech trees than the spruce trees (Table 1).

Table 1: Summary statistics for sampling campaigns across 40 European beech and spruce study sites, including 13 sites with both species. *Köppen-Geiger classification based on Beck et al. (2023).

		Beech	Spruce
Number of sites		27	26
Number of sites sampled during both campaigns		24	23
Elevation [m a.s.l.]	Min	63	14
	Mean	756	648
	Max	1541	1870
Climate* (Köppen-Geiger classification) [number of sites]	Cfa	1	0
	Cfb	10	6
	Csb	1	0
	Dfb	14	14
	Dfc	1	6
Tree height [m]	Min	7	4
	Mean	22	23
	Max	44	39
Diameter at breast height [cm]	Min	11	8
	Mean	39	36
	Max	87	65
Canopy cover (%)	Min	58	54
	Mean	88	80
	Max	100	94

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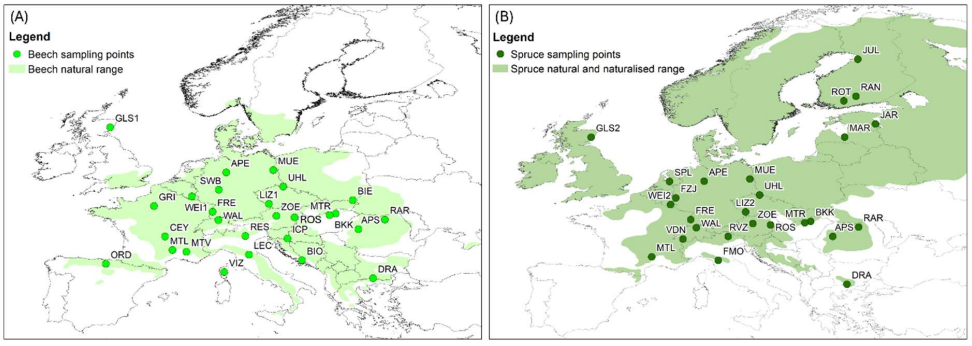

Figure 1. Maps showing the sampling sites (circles) for beech (A) and spruce (B) trees and their natural and naturalised ranges across Europe (shaded areas; data from Caudullo et al. (2017)).

2.3 Sampling, transport, and storage of stem xylem and soil samples

At each sampling site, three beech (*Fagus sylvatica*) and/or three spruce (*Picea abies*) trees were selected based on their representativeness for the stand. The selected spruce and beech trees ranged in size but were similar in mean height (22-23 m) and diameter at breast height (36-39 cm, Table 1). Stem xylem samples were taken from each selected tree at breast height using a 0.5 cm increment borer. Each sample (one per selected tree) consisted of two to three ~5 cm long stem sapwood samples. Most samples consisted of fully intact wood cores; but 9.8% of all stem xylem samples were non-intact stem xylem samples. The outer and inner bark of the wood cores were removed from the cores, yet, bark residue was observed in 40% of all stem xylem samples after cryogenic water extraction. The same three trees were sampled during both campaigns at each site, except at the beech site GRI, where different trees were sampled in spring and summer, and at the beech site MTV, where six samples were taken. This resulted in a total of 311 stem xylem samples.

In addition to the stem xylem samples, soil samples were taken at each site for each sampling campaign with a manual soil auger. The samples were typically taken from one soil core at three to five depths spanning 10 cm intervals (0-10, 10-20, 20-30, 50-60, and 80-90 cm below the surface), but occasionally also for other depths. The number of soil samples and the depth of the deepest soil sample depended on the maximum existing soil depth at the sampling site. The soil samples were taken from a location close to the selected trees. The litter was removed before taking the 0-10 cm soil sample. For some sites and sampling campaigns, soil samples from an additional two to four soil cores were taken. For a few sites with both species (i.e., DRA, FRE, UHL, ZOE), soil cores were separately taken for beech, spruce, and both species. This resulted in a total of 381 soil samples.

Stem xylem and soil samples were transferred into 12 mL gas-tight glass vials (“Exetainers”, Labco, Lampeter, UK). For the soil samples, exetainers were filled with 50-80% of their volume with soil. Some soil and stem xylem samples (13% of all 692 samples) were stored in other types of gas-tight plastic or glass vials. Most samples were taken midday on dry and sunny days. Samples were handled as fast as possible to avoid evaporative fractionation. Back in the laboratory, all samples were stored in a refrigerator to avoid moisture loss to evaporation and subsequent isotope fractionation until transportation. All samples were then shipped without cooling and arrived

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within four weeks of the final day of each sampling campaign at the laboratory at WSL Birmensdorf in Switzerland, where they were kept at -20°C until cryogenic water extraction.

2.4 Cryogenic vacuum water extraction

Water was extracted from all 692 samples at WSL Birmensdorf using a cryogenic vacuum distillation method as described in Diao et al. (2022). In brief, the exetainers with the samples were taken from the freezer and fitted with polypropylene fiber filters (Nozzle protection filter, Socorex Isba SA, Ecublens, Switzerland) to prevent particles from being drawn into the extraction line. Samples originally stored in other types of vials were transferred to exetainers that fit the cryogenic vacuum distillation system. Samples were then heated to 80°C in a water bath, while the extraction line was kept under a vacuum of < 5 Pa (BS2212, Brook Crompton Ltd, Doncaster, UK). The extracted water was trapped in U-shaped glass tubes, constantly kept in liquid nitrogen. After a minimum of 2 hours, the water extraction was stopped and atmospheric pressure was established in the extraction line by passing dry nitrogen gas through it. Then, the U-tubes were removed, the ends of the tubes were closed with rubber plugs and the water samples were thawed at room temperature. Depending on the extracted water amount, the water was pipetted to 350 µL or 2 mL glass vials (Infochroma AG, Goldau, Switzerland) and kept frozen at -20°C until isotope analysis. A few samples that appeared turbid after extraction were filtered with 0.45 µm nylon syringe filters (Infochroma AG).

We determined the sample weight before water extraction ("fw"), after water extraction ("dw1"), and after drying at 105°C for 24 hours (dw2) to estimate the absolute water amount ("awa"), the total extraction efficiency ("tef"), and the gravimetric water content (gwc) for each sample (for equations, see Table 3). The sample weights (i.e., "fw", "dw1", "dw2") were corrected for the weight of the exetainer ("exe_weight", Table 3). The latter was based on the mean weight of approximately thirty exetainers for 10 different types ("exe_type", Table 3; i.e., different combinations of glass vials, cap with a rubber seal, and label), which averaged around 13.0 g and varied by a maximum of 0.3 g. Across all soil and stem xylem samples (Figure 2A), "awa" averaged around 1.4 mL, and was well above the critical thresholds for extracted water volume in the vast majority of samples (Diao et al., 2022). The average value for "tef" was 100.6%, and was for most samples (N = 543) within the optimal range of 98-102% (Ceperley et al., 2024). The "gwc" varied between soil samples and stem xylem samples of beech and spruce, averaging around 40.9%, 61.3%, and 83.9%, respectively (Figure 2C). Note that variations in "awa", "tef", and "gwc", and "tef" values > 100%, may partly be due to uncertainties arising from the estimation of the exetainer weight ("exe_weight"; Table 3), reflecting an average value rather than the actual weight of each exetainer.

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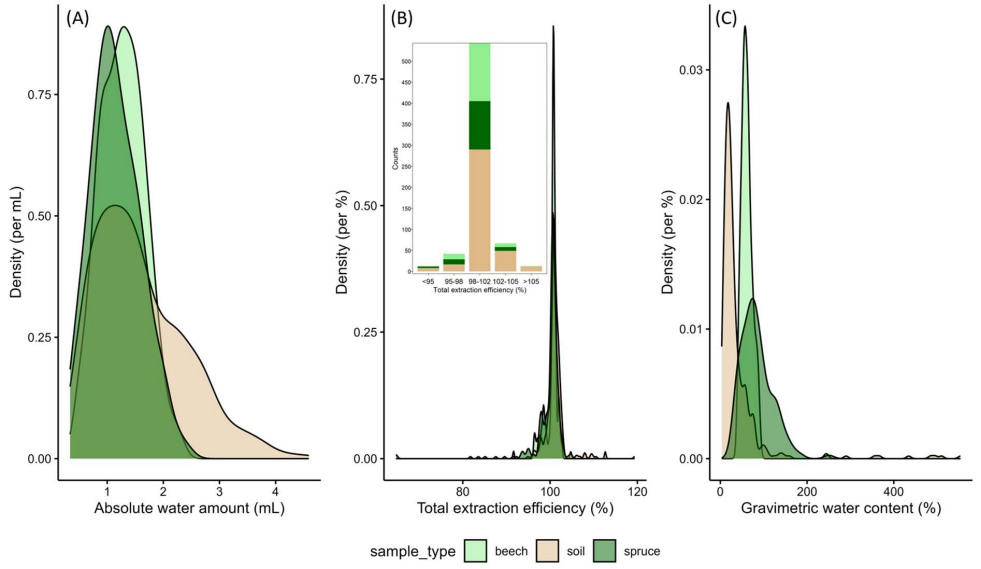

Figure 2: Density plots for (A) the extracted absolute water amounts, (B) the total extraction efficiency (tef), and (C) the gravimetric water content (gwc) for stem xylem (beech and spruce) and soil samples for all samples analysed (i.e., all sites and sampling campaigns). The insert in figure (C) shows the sample count for different types of samples across five different tef classifications.

2.5 Isotope analysis with laser spectrometer and IRMS

The stable isotope ratios of hydrogen (δ^2H) and oxygen ($\delta^{18}O$) of the cryogenically extracted water were measured at WSL Birmensdorf using a cavity ring-down spectrometer (L2140i, Picarro Inc., Santa Clara, USA) connected to a micro-combustion module (MCM) to eliminate sample artefacts caused by co-extracted organic compounds (Martín-Gómez et al., 2015). Each sample was injected eight times and the average of the final five injections was taken to minimize memory effects (Penna et al., 2012). Samples were calibrated with four reference isotope standards spanning from -10.5‰ to -120.2‰ for δ^2H and from -3.0‰ to -16.1‰ for $\delta^{18}O$ (LGR; Envitec NV, Lessines, Belgium) and normalized to the international Vienna Standard Mean Ocean Water (VSMOW-2) scale. The maximum deviation (i.e., accuracy) of an interspersed in-house laboratory standard (analysed every ~25 samples, $\delta^{18}O$: -9.6‰, δ^2H: -84.9‰) from the expected value was ≤ 0.2‰ for $\delta^{18}O$ and ≤ 0.5‰ for δ^2H. The standard deviation (SD) of the repeated measurements of the laboratory standards (i.e., precision) was ≤ 0.1‰ for $\delta^{18}O$ and ≤ 0.6‰ for δ^2H.

To check for spectral interferences with plant-produced volatile organic compounds during the isotope analysis with laser spectrometer, a subset of 83 samples were also analyzed using a thermal combustion/elemental analyzer (TC/EA) coupled to a DeltaPlus XP isotope ratio mass spectrometer (IRMS, Finnigan MAT, Bremen, Germany), with a typical precision of 1.0‰ for δ^2H and 0.2‰ for $\delta^{18}O$. This subset was representative for both sampling campaigns, sample types (stem xylem vs. soil), tree species, geographic locations, and range of isotopic values. The IRMS data were highly correlated with the data of the laser spectrometer (Figures 3A, 3B). Most of the data

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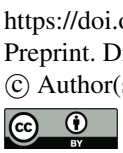

were within the range of ± 1 SD and showed a positive offset for both elements (Figure 3C). The δ^2H and $\delta^{18}O$ offset between the two types of analysis had mean values around 0.7‰ and 0.3‰ across all samples (Figure 3C), respectively. These mean offsets represent the average of the differences between the two methods, accounting for both positive and negative values. The SD of these offsets were 1.4‰ for δ^2H and 0.5‰ for $\delta^{18}O$, indicating the variability around the mean offsets, not zero. Paired t-tests across the samples of the subset show that the δ^2H and $\delta^{18}O$ differences between the two analytical methods were significantly ($P < 0.05$) larger for spruce (mean = 0.7‰ and 1.1‰) than for beech (mean = 0.4‰ and 0.7‰) and soils of all depth (only significant for δ^2H; mean = 0.6‰).

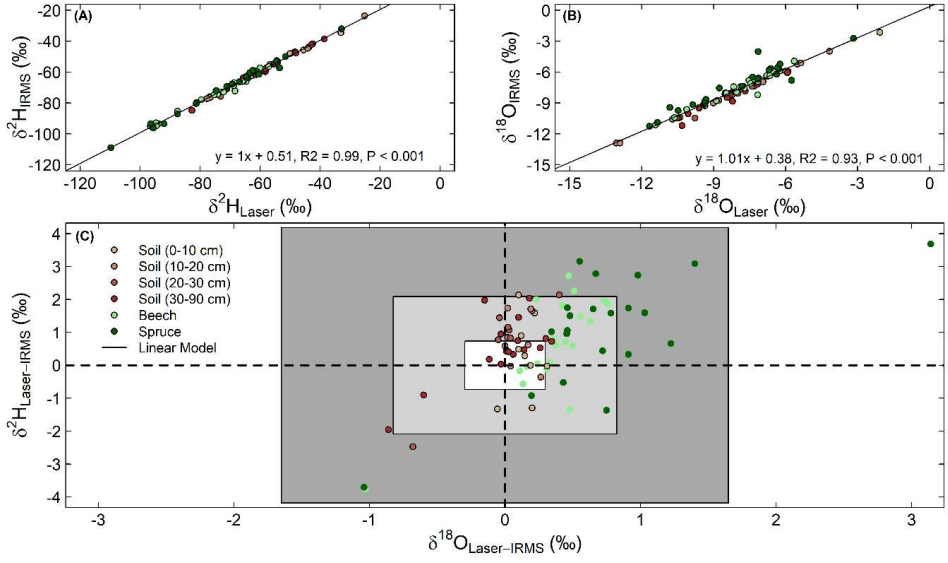

Figure 3: Linear relationships between hydrogen (A; δ^2H) and oxygen (B; $\delta^{18}O$) isotopic composition for the water samples analyzed using a laser spectrometer (Laser) and an isotope ratio mass spectrometer (IRMS). Panel (C) displays a biplot of the differences in the $\delta^{18}O$ and δ^2H values for the two instruments. The small white box in the middle of C represents the mean isotopic difference, while the light grey and dark grey boxes denote ± one and two standard deviations for the isotopic difference, respectively.

2.6 Description of the dataset

The dataset consists of three comma-separated files and one zip file with photos of the canopy at the sampling sites. The first datafile ("WATSON_Metadata.csv") contains all the metadata about the sampling sites including site-, soil- and tree-specific information (Table 2), the second file ("WATSON_Isotopedata.csv") contains the information about sample weights, cryogenic water extraction and the actual hydrogen and oxygen isotope data (Table 3), and the third file ("WATSON_Canopydata.csv") contains the information on the canopy cover (Table 4). The photos on which the canopy cover data are based are stored in the "WATSON_Canopy_Pictures.zip" file. All files can be linked by the "site_id", which is a three-letter identifier of the sampling sites.

Table 2: Description of the columns in the "WATSON_Metadata.csv" file containing all the meta-information about the sampling sites [and units].

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Column name	Description
site_id	A three-letter identifier of the sampling site. Note that for the three sites (LIZ, GLS, WEI), an additional number was added indicating the species: "1" refers to beech and "2" to spruce.
site_name	Full site and country name
country_id	A two-letter country code, as defined in ISO 3166-1
latitude	Latitude in decimal degree rounded to three decimals, WGS84 coordinate system
longitude	Longitude in decimal degree rounded to three decimals, WGS84 coordinate system
elevation	Elevation of the sample site [m above sea level]
slope_type	Descriptor of the slope: "flat", "gentle" or "steep"
spruce_site	Descriptor highlighting whether spruce trees were sampled at the site ("yes") or not ("no").
beech_site	Descriptor highlighting whether beech trees were sampled at the site ("yes") or not ("no").
stand_type	Descriptor highlighting whether the stand is a mixed species stand ("mixed") or a monoculture stand ("mono"). Note that "mixed" refers to stands with various species, not limited only spruce and beech.
understory	Descriptor highlighting the presence of understory vegetation ("yes") or not ("no").
soil_type	Soil type according to the FAO classification
soil_texture	Soil texture based on either measurement of the sand, silt and clay content or hand tests in the field (see Section S1, S2).
soil_depth_max	Maximum soil depth [m], for soils deeper than 1 m, > 1 is used.
sampling_doy_spring	Day of the year of sample collection for the spring sampling campaign
sampling_doy_summer	Day of the year of sample collection for the summer sampling campaign
sampling_daytime_spring	Time of the day of sample collection (local time) for the spring sampling campaign. When a start and end time were given, the middle point is recorded.
sampling_daytime_summer	Time of the day of sample collection (local time) for the summer sampling campaign. When a start and end time were given, the middle point is recorded.
height_spruce1	(Estimated) Height of spruce tree 1 [m]
height_spruce2	(Estimated) Height of spruce tree 2 [m]

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height_spruce3	(Estimated) Height of spruce tree 3 [m]
height_beech1	(Estimated) Height of beech tree 1 [m]
height_beech2	(Estimated) Height of beech tree 2 [m]
height_beech3	(Estimated) Height of beech tree 3 [m]
dbh_spruce1	Diameter at breast height (DBH) of spruce tree 1 [cm]
dbh_spruce2	Diameter at breast height (DBH) of spruce tree 2 [cm]
dbh_spruce3	Diameter at breast height (DBH) of spruce tree 3 [cm]
dbh_beech1	Diameter at breast height (DBH) of beech tree 1 [cm]
dbh_beech2	Diameter at breast height (DBH) of beech tree 2 [cm]
dbh_beech3	Diameter at breast height (DBH) of beech tree 3 [cm]
koppen	Three letter Köppen-Geiger climate code extracted from Beck et al. (2023).
canopy_cover_picture	Descriptor highlighting whether pictures of the canopy cover (see Table 4) are available in the WATSON_canopy_photos.zip file (“yes”) or not (“no”).
canopy_cover	Mean canopy cover (C) for the sampling site, reflecting the average value for all photos for a sampling site (varying *n* per sampling site). Calculation of C as described in Section S3.
gap_fraction	Average gap fraction. One minus the average canopy cover, 1-C
network	Comment field, indicating to which monitoring network the site belongs
website_link	URL of a website describing the sampling site
paper_1	DOI of paper 1 describing the sampling site
paper_2	DOI of paper 2 describing the sampling site
paper_3	DOI of paper 3 describing the sampling site

Table 3: Description of the columns in the “WATSON_Isotopedata.csv” file containing all the isotope data and additional information about the extraction [and units].

Column name	Description
site_id	A three-letter identifier of the sampling site. Note that for the three sites (LIZ, GLS, WEI), an additional number was added indicating the species: “1” refers to beech and “2” to spruce.
country_id	A two-letter country code, as defined in ISO 3166-1

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sampling_date	Date that the sample was collected in yymmdd format
sampling_campaign	Descriptor indicating whether the sample was collected during the “spring” or “summer” sampling campaign.
sample_type	Descriptor indicating whether the sample was a “beech”, “spruce” or “soil” sample
replicate	Number to indicate the tree from which the sample was taken (varying between 1 to 3, and occasionally between 4 to 6) or the replicate of the soil sample (typically only 1, but occasionall varying between 1 and 4).
spruce	Descriptor indicating if the sample was a vegetation sample from a spruce tree or if the soil was taken from a site that has spruce trees (“yes”), otherwise left blank
beech	Descriptor indicating if the sample was a vegetation sample from a beech tree or if the soil was taken from a site that has beech trees (“yes”), otherwise left blank
both	Descriptor indicating if the soil sample was taken from a site that has both beech and spruce trees (“yes”), otherwise left blank
species	Descriptor of the vegetation: “beech” and “spruce” for beech and spruce sites, respectively, or “both” if the soil samples were taken at a site where there are beech and spruce trees
soil_depth	Depth of the soil sample. Numbers ranging between 10 and 90, indicating the maximum depth of an interval, e.g. 10 for 0-10 cm, 20 for 10-20 cm, and 75 for 65-75 cm. For the vegetation samples, the field is left blank.
sample_id	A sample identifier used for all laboratory analyses
bark	“yes” when the sample included (remaining) pieces of bark, otherwise "no”
original_vial	The vial type in which the sample was received: exetainer that fit the cryogenic extraction line (“exetainer”) or other types of gas-tight glass and plastic vials (“others”)
extractionist	ID for the person responsible for cryogenic water extraction (A to D). Note that person D was only responsible for a very small subset.
cvd_slot_id	Slot ID of the cryogenic water extraction line at which a sample was placed during the extraction
exe_type	Numbers (1 to 10) indicate the type of exetainers (i.e., various combinations of glass vials, caps with rubber seals, and labels)
exe_weight	The mean weight of an empty exetainer of the exe_type, including glass vials, caps with rubber seals, and labels [mg]
fw	The fresh (field) weight of the sample [mg]

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dw1	The dry weight of the sample after cryogenic extraction [mg]
dw2	The dry weight of the sample after cryogenic extraction and oven drying at 105°C for 24 h [mg]
awa	Absolute water amount extracted from the sample during cryogenic extraction [mL], calculated as: awa=(fw-dw1)/1000
gwc	The gravimetric water content of the sample [%], calculated as: gwc = (fw-dw1)/dw1)*100)
tef	Total extraction efficiency [%], calculated as: tef = ((fw-dw1)/(fw-dw2))*100)
d18O	The $\delta^{18}O$ value (relative to VSMOW-2) as determined by the laser spectrometer [‰]
d2H	The $\delta^{2}H$ value (relative to VSMOW-2) as determined by the laser spectrometer [‰]
d18O_irms	The $\delta^{18}O$ value (relative to VSMOW-2) as determined by the isotope ratio mass spectrometer [‰]
d2H_irms	The $\delta^{2}H$ value (relative to VSMOW-2) as determined by the isotope ratio mass spectrometer [‰]

302

303 **Table 4:** Description of the columns in the "WATSON_Canopydata.csv" file describing the canopy cover for the
304 sampling sites for which canopy pictures were available.

Column name	Description
site_id	A three-letter identifier of the sampling site. Note that for the three sites (LIZ, GLS, WEI), an additional number was added indicating the species: "1" refers to beech and "2" to spruce.
country_id	A two-letter country code, as defined in ISO 3166-1
species	Descriptor iinndicating the species for which the pictures were taken, either "beech" or "spruce" or "canopy" if the picture represents a picture of a mixed site or the overall canopy of the sampling site.
photo	Name of the file of the photo as given in the WATSON_canopy_photos.zip file. The general structure of each file name is: country_site_date_speciesm_xxx.JPG, where "country" indicates the country_id, "site" indicates the site_id, "date" the date that the picture was taken in yymmdd format, "species" the tree species (beech or spruce), "m" the tree number, and "xxx" refers to additional information, such as the distance from the tree in meters (1, 3, 5) or the direction in which the picture was taken (N, E,

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	S, W). Where "canopy" is used for the "species", the picture shows the overall canopy of the forest site.
gap_fraction	One minus the canopy cover, 1-C
canopy_cover	The canopy cover (C), calculated as described in Section S3 [-]

3 Results and discussion

3.1 Isotopic variation for the spring and summer sampling campaigns

The isotopic composition of the soil and the stem xylem water samples varied spatially (Figure 4). The samples were more depleted in heavy isotopes at sites located further north and inland. Multiple linear regression analyses showed that latitude, longitude, and elevation were all important variables explaining the observed spatial variation in the isotopic composition of soil and stem xylem water (Table 5). Among the three geographic variables, longitude and latitude explained most of the variance for seven of the eight cases shown in Table 5. Since the total variance explained by latitude, longitude, and elevation was relatively low in most cases (R^2 = 0.17 to 0.6), other factors likely contributed to the variation in the isotopic composition of the samples. In combination with the gravimetric water content of the soil (e.g., "gwc"; Table 3), gridded climate data, and precipitation isotope data (Nelson et al., 2021), the data could be useful for new soil and stem xylem water isoscape models or function as additional data in hydrological studies.

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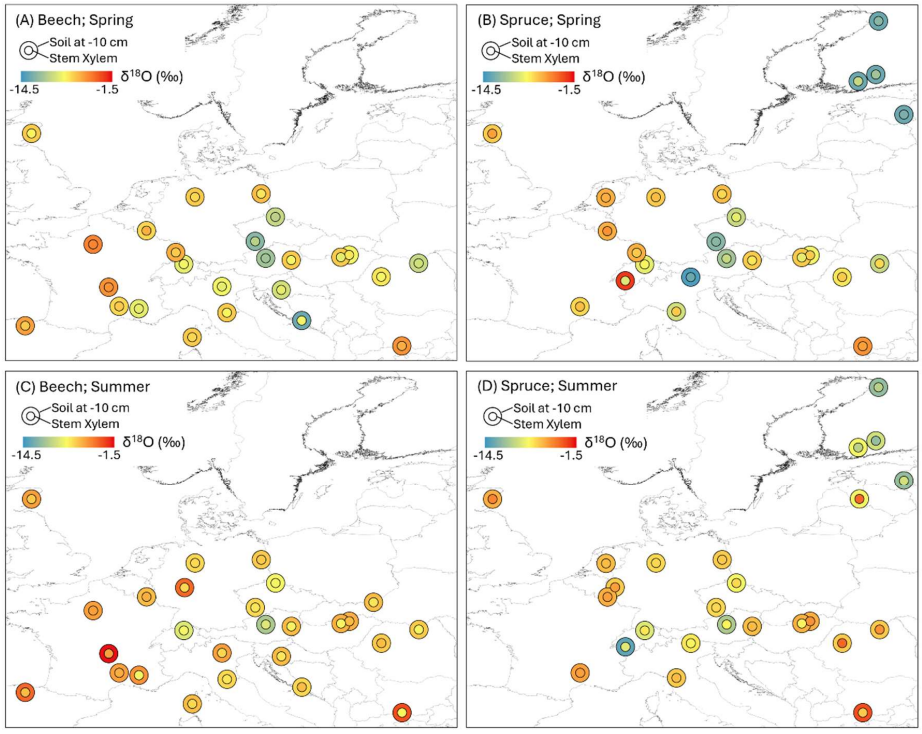

Figure 4: Map showing the $\delta^{18}O$ values for stem xylem water (inner circle) and soil water at 0-10 cm (outer circle) for the spring (A,B) and summer (C,D) sampling campaigns. Results for beech trees are reported on the left and spruce trees on the right. For some sites, the isotopic composition of the stem xylem samples was similar to that of the shallow soil (0-10 cm depth) (both circles have the same color); for others, the differences were large (i.e., the color of the inner and outer circle differs) indicating water uptake from a different (e.g. deeper) water source.

Table 5: Percentage of variance in $\delta^{18}O$ values explained by latitude, longitude, and elevation, as determined by multiple linear regression analyses. Values in bold indicate the highest relative contribution of a geographical parameter to the total variance for each sample type for each campaign (Spring/Summer). R^2 reflects the total variance explained by latitude, longitude, and elevation. All linear models were statistically significant ($P < 0.001$).

Campaign	Sample	R^2	Longitude (%)	Latitude (%)	Elevation (%)
Spring	Stem xylem (spruce)	0.48	25	50	25
	Stem xylem (beech)	0.34	29	33	38
	Soil (0-10 cm)	0.35	50	38	12
	Soil (30-90 cm)	0.60	35	46	19
Summer	Stem xylem (spruce)	0.32	13	66	21
	Stem xylem (beech)	0.17	56	13	31

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Preprint. Discussion started: 17 October 2024

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	Soil (0-10 cm)	0.29	19	64	17
	Soil (30-90 cm)	0.38	72	23	5

The isotopic composition of the soil and stem xylem water samples also varied between the two sampling campaigns (Figures 4 and 5). For instance, $\delta^{18}O$ values were higher (i.e., less negative) in summer compared to those of the spring for the different soil depths and the two tree species (unpaired t-test, $P < 0.05$), except for soils in the depth range of 30-90 cm for which there was no significant difference between spring and summer (unpaired t-test, $P > 0.05$; Figure 5). For the $\delta^{18}O$ values of stem xylem water, the median seasonal difference (summer-spring), averaged per site, was 0.8‰ across all spruce sites (ranging from -1.4 to 4.8‰) and 0.6 ‰ across all beech sites (ranging from -1.9 to 2.9‰). In comparison, the average median seasonal $\delta^{18}O$ difference was larger and/or showed higher a variability for soil water, e.g., 1.3‰ at 0-10 cm depth (ranging from -10.8 to 6.1‰) and 0.6 ‰ at 30-90 cm depth (ranging from -3.3 to 9.6‰). In spring, the $\delta^{18}O$ values of deep soils (30-90 cm) were only lower (i.e., more negative) compared to those of the shallower soils (0-10 cm), while in summer, $\delta^{18}O$ values of deep soils were lower compared to all other soil depths above 30 cm (unpaired t-test, $P < 0.05$). Similar seasonal differences for stem xylem and soil water were observed for the $\delta^{2}H$ values (Figure 5). The data may, therefore, be used to investigate the infiltration of precipitation and snowmelt into the soil, but also evaporative enrichment of the shallow soil water, or to test models that simulate these processes.

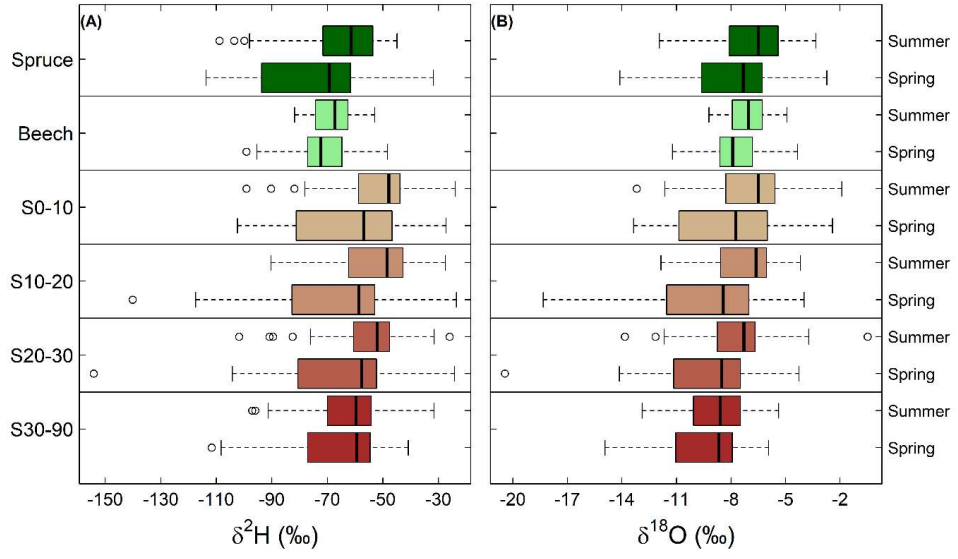

Figure 5: Boxplots for (A) hydrogen and (B) the oxygen isotopic composition ($\delta^{2}H$, $\delta^{18}O$) of stem xylem water of both tree species (beech and spruce) and soil water at different depths for the spring and summer campaigns. Soil depths are shown for 0-10 cm (S0-10), 10-20 cm (S10-20), 20-30 cm(S20-30) and 30-90 cm (S30-90). The vertical line within the box indicates the median (50th percentile). The box represents the interquartile range (IQR),

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spanning from the 25th percentile to the 75th percentile. The whiskers extend to the furthest data points within 1.5 times the IQR from the quartiles. Symbols outside the whiskers represent outliers.

Further, we found that the isotopic composition of the stem xylem water plotted in the range of soil water at the site level ("overlap"), though not consistently across all sites (Figure 6). The mean $\delta^{18}O$ values overlapped for more beech sites (68% in spring, 84% in summer) than for spruce sites (41 in spring, 48% in summer). The number of sites for which the $\delta^{18}O$ values of the soil and stem xylem water overlapped was also larger for the summer than for the spring sampling campaign. In contrast, the overlap in mean $\delta^{2}H$ values was higher for spruce sites (58% in spring, 68% in summer) than beech sites (28% in spring, 23% summer). A lack of overlap may indicate that the trees used water from other sources, such as recent precipitation events, water stored in organic surface layers, deeper, unsampled soil layers or groundwater. Another explanation might be related to cryogenic water extraction artefacts (see section on "Cryogenic water extraction biases").

The soil and stem xylem data could be used to test models that simulate plant-soil-water dynamics (Klein et al., 2014; Brinkmann et al., 2018; Knighton et al., 2020) and to test how this depends on site-, soil-, and tree-specific information (Table 3). When the data are combined with isotope data of precipitation, such as those from the GNIP network (e.g.,Terzer-Wassmuth et al., 2023), or models, such as Piso.AI (Nelson et al., 2021), the data can also be used to study the seasonal origins of tree water uptake, as well as the spatial and temporal patterns associated with it (Allen et al., 2019; Floriancic et al., 2024a). For sites without overlap, the application of mixing models, such as IsoSource (Phillips and Gregg, 2003) or MixSIAR (Stock et al., 2018), might be limited. However, alternative mixing models with incomplete end-members could be tested (Kirchner, 2023).

For sites with both species, the isotopic data for the stem xylem water of the two species appear to be different (Figure 6). The median difference between species across all sites for the mean $\delta^{2}H$ and $\delta^{18}O$ values (spruce-beech), averaged per site, was 4.1‰ and 0.7‰ in spring and 10.1‰ and 1.1‰ in summer, respectively. Thus, the stem xylem water in spruce tended to be isotopically enriched compared to ones in beech, which is consistent with the generally shallower root system of spruce compared to beech. The data can therefore be used to study species-specific differences in root water uptake depth across Europe.

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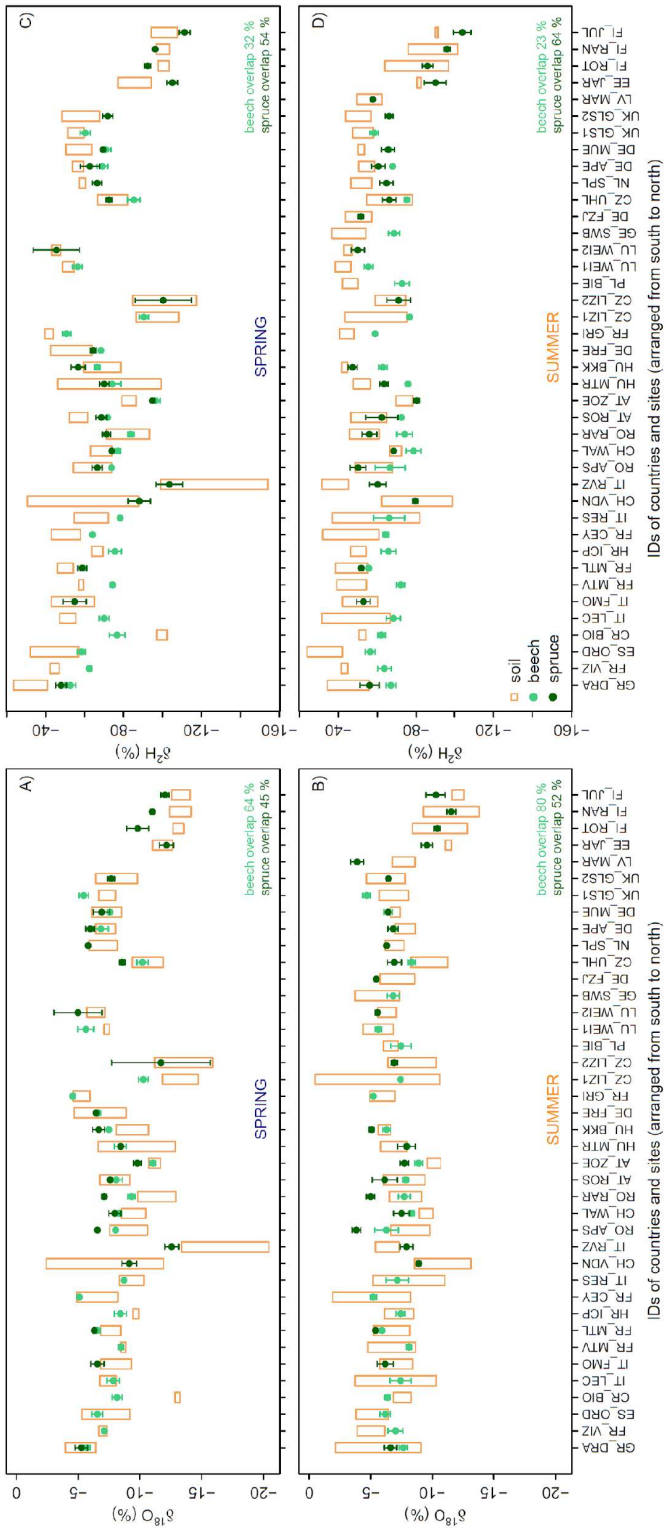

Figure 6: Overlap between the isotopic composition of soil and stem xylem water for spring (A, C) and summer (B, D) campaign. Oxygen ($\delta^{18}O$) and hydrogen ($\delta^{2}H$) isotope data are shown in the left and right panels, respectively. Orange bars indicate the minimum to maximum range of soil water isotope values. Mean values and standard errors are shown for the isotopic composition of stem xylem water.

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3.2 Cryogenic water extraction biases

The dual isotope plots show that the isotope ratios of the soil were closer to the GMWL than those of stem xylem water for both species (Figure 7). However, particularly in summer, the isotope ratios of the shallower soils at some locations also deviated from the GMWL. This may indicate that the water in the shallow soil was affected by evaporation and that the trees used this enriched water. While evaporation might be responsible for some of the offset between the soil and stem xylem samples, there was no evaporative enrichment for most soil samples. Nevertheless, it should be considered that soil organic matter can bias the isotopic composition of the extracted water (Ceperley et al., 2024; Orlowski et al., 2016), as well as the presence of volatile organic compounds that may interfere isotopic analysis with laser spectrometers (Martín-Gómez et al., 2015). The latter, however, should be reduced by the use of the micro-combution modul in our study. Furthermore, given the relatively small isotopic differences between the laser and IRMS measurements (Figure 3), the overall large δ^2H deviation from the GMWL for the stem xylem samples is more likely caused by methodological issues related to the cryogenic vacuum distillation method (Chen et al., 2020; Diao et al., 2022; Barbeta et al., 2022). According to these studies, biases might be related to stem water content, differences in the isotopic composition of the xylem water and water in plant cells, exchange of H-atoms between organic material and water or water vapour, and isotope fractionation related to evaporation and sublimation during the extraction procedure.

To address these issues, we performed further quality checks for the cryogenic extraction (Figure 8). Although there was a significant difference in the total extraction efficiency for the samples handled by the three main lab technicians (one-way ANOVA, $P < 0.001$; Figure 8A), the efficiency did not depend on the cryogenic vacuum distillation slot (Figure 8B) and showed no systematic effect on the δ^2H and $\delta^{18}O$ values (Figure 8C). The presence of bark residue in the samples did not significantly affect the isotope signals (unpaired t-test, $P > 0.05$), although the slopes of the dual isotope plots tended to be different ($P = 0.06$, Figure 8D). Comparing the δ^2H and $\delta^{18}O$ values between samples stored in exetainers and other vials (Table 3, "original_vial") revealed no visual or statistical differences either, suggesting that sampling, transport, and transfer of samples from other vials to exetainers before cryogenic water extraction in the laboratory did not notably affect the isotope results. The data of this study can be used to further explore the cryogenic water extraction biases with the additionally provided site-, soil- and tree-specific information (Zhao et al., 2024; Sobota et al., 2024). Alternatively, they can be used to support other studies on methodological issues related to cryogenic water extraction.

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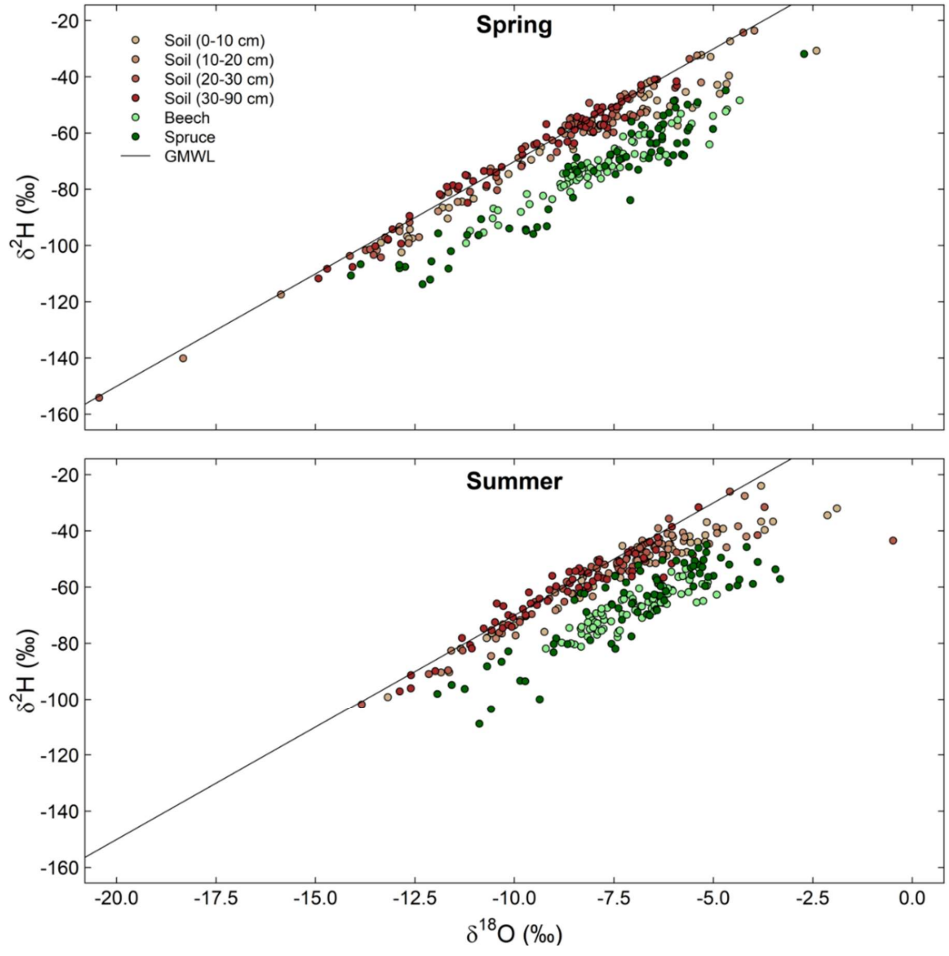

405

406 **Figure 7:** Dual isotope plots of oxygen and hydrogen isotope ratios (δ^2H, $\delta^{18}O$) for all soil and stem xylem water
407 samples for the spring (top panel) and the summer (bottom panel) campaigns. Isotope values for soil samples are
408 color coded according to soil depth. GMWL = Global Meteoric Water Line: $\delta^2H = 8\ \delta^{18}O + 10$.

409

410

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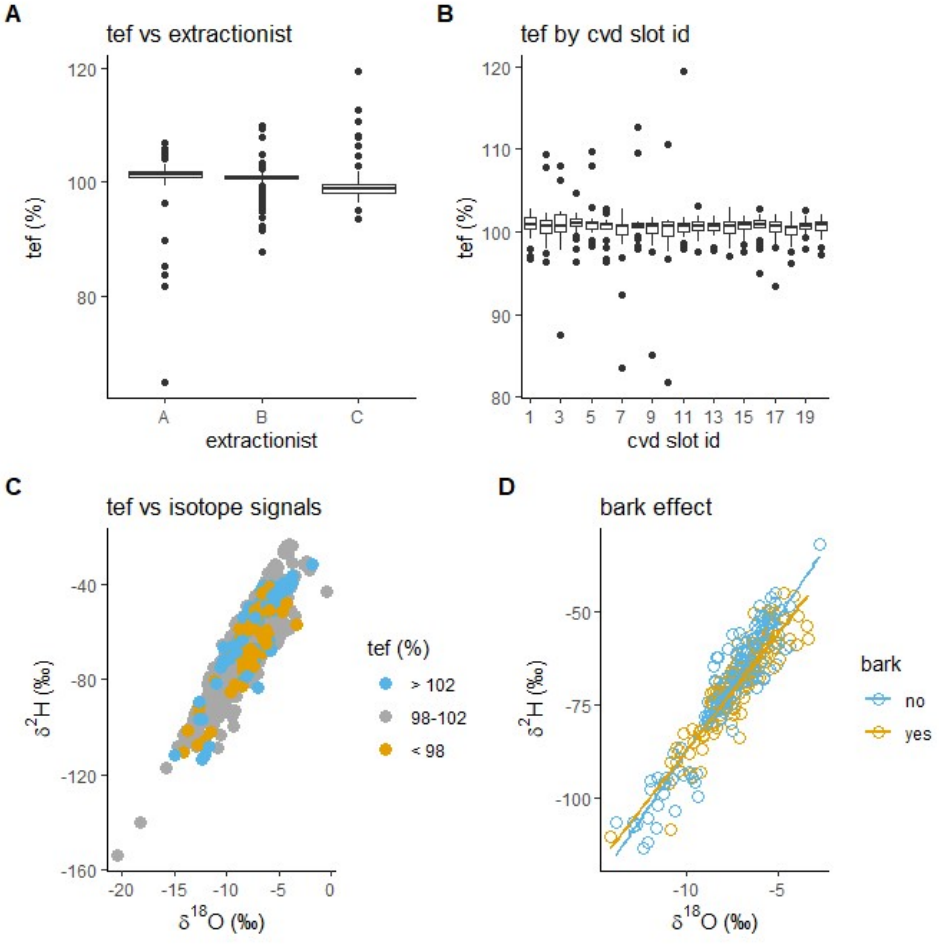

411

412 **Figure 8**: Total extraction efficiency (tef, %) quality checks: (A) tef values categorized by extractionist (Person
413 A, B, or C) and (B) by cryogenic vacuum distillation slot IDs. Correlation between oxygen ($\delta^{18}O$) and hydrogen
414 ($\delta^{2}H$) isotope values for (C) all samples colored by different tef categories and for (D) stem xylem samples with
415 ("yes") and without presence of bark ("no"), including fitted trend lines.

416 **4 Concluding remarks**

417 We present a large pan-European dataset of soil and stem xylem water isotopes of two common tree species
418 collected during spring and summer 2023. Since our observations are standardized according recently published
419 sampling and extraction procedures (Ceperley et al., 2024; Scandellari et al., 2024), this data can serve as a baseline
420 for future ecohydrological studies. This dataset is freely available and represents a valuable resource for different
421 research topics. These may include identifying the factors that affect tree water uptake depth and the seasonal
422 sources of water used by trees, calibrating and constraining isotope-aided ecohydrological models, incorporating

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423 the data into isoscape models, or studying how biases caused by cryogenic water extraction vary by species, soil
424 type, or climate.

425 **Statistics**

426 For all statistical analyses we used R version 4.3.1 (R Core Team, 2023). For our multiple linear regression
427 analyses, we applied a cube root transformation to the data to address non-normality. We then utilized the R
428 package "relaimpo" (Grömping, 2006) to assess the relative importance of the geographical parameters in our
429 model. If data is presented for soil at a depth of 30-90 cm, it represents all available data points for soil depths
430 greater than 30 cm, without any additional modifications of the data.

431 **Funding**

432 This study was financially supported by the COST Action: “Water isotopes in the critical zone: from groundwater
433 recharge to plant transpiration - WATSON” CA19120 (www.cost.eu). The extraction of the water and isotope
434 analyses were financially supported by the Swiss National Science Foundation (“TreeWater”, No. 205492;
435 “InsightForest”, No. 213367) and by WSL (“Innovative project Oxygen17”). Alicia Correa was supported by the
436 German Academic Exchange Service (DAAD) from funds of Federal Ministry for Economic Cooperation (BMZ),
437 SDGnexus Network (No. 57526248). Aurel Persoiu and Andrei Popa were supported by UEFISCDI Romania (No.
438 PN-III-P2-2.1-PED-2019-4102 & No. PN-III-P4-ID-PCE-2020-2723). Maren Dubbert acknowledges the funding
439 by the Deutsche Forschungsgemeinschaft (No. 501530203) and by the Leibniz collaborative excellent grant (No.
440 K444/2022), supporting Alberto Iraheta, Charlotte Koeber, and Clara Rohde. Katja Rinne was funded by the
441 Academy of Finland (No. 343059). Research part at University of Oulu was supported by Research Council of
442 Finland (No. 347348 and No. 356043), and Marie Skłodowska-Curie Postdoctoral Fellowship (No. 101111527).

443 **Acknowledgements**

444 The pan-European sampling campaign and the data collection initiative was developed during a workshop of the
445 COST Action: “WATSON” CA19120 (http://www.cost.eu/; https://watson-cost.eu/) held in March 2023 in
446 Dubrovnik, Croatia. We thank Timon Dufner, Sophia Ezhold, Noemi Kammerlander, Alligin Gazhoul, Jan Ziegler,
447 Jonathan Frei, Roger Köchli, David Schweizer, Manuela Oettli for the laboratory assistance, as well as Enara
448 Aldai, Wisam Almohamed, Hatice Türk, Patricia Vieira Pompeu, Fernanda Gianasi, Konstantinos Voudouris,
449 Ionel Popa, Martine Helfer, Anna Meier, Ladina Gaudy, Laura Kinzinger, Dominik Gerber, Simon Bürki, Dominik
450 Dubach, Paavo Ojanen, Ellinoora Ekman, Christiaan van der Tol, and Joni Koivula for their help with site
451 selection, and/or sample- or metadata collection.

452 **Data availability**

453 All data is freely available under the agreement “Creative Commons Zero - No Rights Reserved (CC0 1.0)” in the
454 data repository EnviDat: Lehmann, M. M., Geris, J., van Meerveld, I., Penna, D., Rothfuss, Y., Verdone, M., Ala-
455 Aho, P., Arvai, M., Babre, A., Balandier, P., Bernhard, F., Butorac, L., Carrière, S. D., Ceperley, N. C., Chen, Z.,
456 Correa, A., Diao, H., Dubbert, D., Dubbert, M., Ercoli, F., Floriancic, M. G., Gimeno, T. E., Gounelle, D.,

https://doi.org/10.5194/essd-2024-409
Preprint. Discussion started: 17 October 2024

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Competing interests

The authors declare that they have no conflict of interest.

Author contribution (CRediT)

The WATSON sampling campaign core organization and writing team consisted of Marco M. Lehmann (MML), Josie Geris (JG), Ilja van Meerveld (IvM), Daniele Penna (DP), Youri Rothfuss (YR) and Katrin Meusburger (KM). Conceptualization: MML, JG, IvM, DP, YR, KM; Data curation: MML, MV; Formal Analysis: MML, JG, IvM, DP, YR, MV, KM; Funding acquisition: MML, JG, IvM, DP, YR, KM; Investigation: MML, JG, IvM, DP, YR, KM; Methodology: MML, JG, IvM, DP, YR, KM; Project administration: MML, JG, IvM, DP, YR, KM; Resources: MML, KM; Validation: MML, JG, IvM, DP, YR, KM; Visualization: MML, JG, IvM, DP, YR, KM; Writing – original draft: MML, JG, IvM, DP, YR, KM; Writing – review & editing: all.

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