Plant and root-zone water isotopes are difficult to measure, explain, and predict: some practical recommendations for determining plant water sources

Jana von Freyberg*[1,2,3], Scott. T. Allen[4], Charlotte Grossiord[5,6], Todd E. Dawson[7]

[1] Department for Environmental Systems Sciences, ETH Zurich, Zurich, Switzerland

[2] Laboratory for Ecohydrology, School of Architecture, Civil and Environmental Engineering, EPFL, Lausanne, Switzerland

[3] Mountain Hydrology and Mass Movements, Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf, Switzerland

[4] Department of Geology and Geophysics, University of Utah, Salt Lake City, USA

[5] Plant Ecology Research Laboratory, School of Architecture, Civil and Environmental Engineering, EPFL, Lausanne, Switzerland

[6] Functional Plant Ecology, Community Ecology Unit, Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf, Switzerland

[7] Department of Integrative Biology, University of California, Berkeley, Berkeley, California, USA

* Corresponding author: jana.vonfreyberg@epfl.ch

Abstract

1) Stable isotope ratios of water ($\delta^{18}\mathrm{O}$, $\delta^{2}\mathrm{H}$) have long been used to study a core question in plant ecology and ecohydrology: “From where do plants take up water?” Indeed, decades of research has involved sampling potential plant water sources in the subsurface, classifying those sources as distinct endmembers (e.g., deep versus shallow soil waters), and then evaluating their contributions to a xylem water sample through mixing-model analysis to identify the depths of root water uptake.
2) However, more detailed interrogations of the subsurface and plant domains have revealed under-considered transport and isotopic-fractionation phenomena. These now apparent complexities raise new questions and challenge the many past assumptions inherent in endmember-mixing models that now seem overly simple.

3) Here, we introduce discussions of these recent insights and provide an overview of isotope effects that occur naturally in the root zone and in the plant, as well as artificially during sample handling. Better accounting for these complexities and their associated uncertainties can lead to more accurate and robust study designs, analytical frameworks, and, ultimately, inferences.
4) Finally, to more robustly characterize plant water sources using $\delta^{18}\mathrm{O}$ and $\delta^{2}\mathrm{H}$, we provide some practical recommendations that aim at maximizing the isotopic contrast between endmembers and/or minimizing potential uncertainties.

Key-words: ecohydrology; endmember-mixing models; plant water uptake; review; root zone; uncertainties; stable water isotopes

1. Introduction and motivation

Plant water use can represent up to 90% of terrestrial evapotranspiration (Jasechko et al., 2013), and therefore is an important driver of the global water cycle (Sellers et al., 1997). Because plant water relations are closely linked to carbon and nutrient relations (Schulze, 1982), understanding plant-water supplies is also key to predict global carbon and nutrient cycles (Lange, Kappen, & Schultze, 2012), and to scale plant functions to the ecosystem- and land-surface levels (Feddes et al., 2001; Grossiord et al., 2017; Javaux, Couvreur, Vanderborght, & Vereecken, 2013).

Root morphology (e.g. root diameter, root branching, root suberization, root hairs, rooting depth) and the ability of roots to adjust their structure and physiology to environmental factors are two major drivers of plant water uptake (Jackson, Sperry, & Dawson, 2000). These multiple rooting properties have been poorly assessed thus far compared to aboveground functions and structures of plants, in part because of the difficulty to access and measure belowground compartments (Isaac & Anglaaere, 2013). Destructive methods, such as excavating the whole root system of plants, inform how roots occupy soils; however, knowledge about where roots are located does not necessarily imply where water uptake occurs from (Ehleringer & Dawson, 1992). Whereas excavation studies can be useful to understand plant-physiological variations across climates, species and soils, they do not provide insights into how zones of active root water uptake vary in time and space, and how they correspond with soil-water conditions and plant functions.

For nearly 50 years, the analysis of stable isotope ratios of water ($\delta^{18}O$, $\delta^{2}H$) has provided a powerful tool to study plant water uptake processes (e.g., Dawson & Ehleringer, 1998; Dawson & Pate, 1996; Ehleringer & Dawson, 1992; Flanagan, Ehleringer, & Marshall, 1992; Penna et al., 2018; Phillips & Gregg, 2003; Unkovich, 2001; White, Cook, Lawrence, & Broecker, 1985; U. Zimmermann, Ehhalt, & Muennich, 1967 and many more). Isotope data of xylem and root-zone water are often used in linear endmember-mixing models, under the assumption that the isotopes represent conservative tracers with no fractionation occurring during water uptake by the roots (U. Zimmermann et al., 1967) so that the isotope ratios in xylem water reflects the mixture of water sources that supply functional roots.

The simplest case of a linear endmember-mixing model that uses one isotope ratio (e.g., $\delta^{18}O$) to differentiate between the relative contributions (*f*) of two sources *1* and *2* (e.g., deep and shallow soil water) to a mixture (e.g., plant xylem water) takes the form (Phillips & Gregg, 2001)

$$\delta_{mix} = f_1 \cdot \delta_1 + f_2 \cdot \delta_2 , \qquad (1)$$

where

$$1 = f_1 + f_2 \qquad (2)$$

Through combining equations (1) and (2), the relative fractions of source *1* in the mixture can be quantified:

$$f_1 = \frac{\delta_{mix} - \delta_2}{\delta_1 - \delta_2} \qquad (3)$$

An analytical solution for f_1 and f_2 can only be obtained if the number of sources is *n+1*, with *n* being the number of isotopic tracers; even when measurements of both $\delta^{18}O$ and $\delta^{2}H$ are available, they often cannot be treated as independent tracers because they strongly co-vary (Craig, 1961). In cases where a mixture contains more than two water sources but only one isotope tracer ($\delta^{18}O$ or $\delta^{2}H$) can be used, the bounds for each individual fraction $f_1, f_2, \ldots, f_{n+i}$ can be estimated statistically (e.g., IsoSource, Phillips & Gregg, 2003; SIAR, Parnell, Inger, Bearhop, & Jackson, 2010). Often, these multi-source mixing models have been implemented into a Bayesian framework to include prior information about soil properties, root distribution, climate etc. (Moore & Semmens, 2008; Rothfuss & Javaux, 2017).

Most importantly, these mixing-model approaches allow for quantifying the uncertainties in the relative source contributions *f* on the condition that the uncertainties of the individual endmembers are

known (these endmember uncertainties due to analytical errors and spatiotemporal heterogeneity will be discussed in more detail below). Phillips and Gregg (2001) present the analytical solution for calculating the standard error (SE) in f_1 for a two-source mixture (Eq. 1):

$$SE_{f_1}^2 = \frac{1}{(\delta_1-\delta_2)^2} \cdot \left[SE_{\delta_{mix}}^2 + f_1^2 \cdot SE_{\delta_1}^2 + (1-f_1)^2 \cdot SE_{\delta_2}^2\right] \quad (4)$$

Additional methods for propagating the errors in endmember signatures into the uncertainty in *f* have been extensively reviewed by others (e.g., Evaristo, McDonnell, & Clemens, 2017; Rothfuss & Javaux, 2017; Wang, Lu, & Fu, 2019).

Using linear mixing models requires that the endmembers capture the sources of plant water and that those defined endmembers are fully isotopically distinct; thus, intensive sampling can be required. Usually this involves sampling the vertical isotopic profile of the root zone so that variations across depths are understood. Then, natural isotopic break points are selected so that the endmembers describing different subsurface water pools can be identified (e.g., <0.3 m versus >0.3 m deep soil water in Figure 1). Alternatively, instead of defining plant source water endmembers by depth, they can be defined with respect to seasonal precipitation, which can provide different insights into how root distributions interplay with infiltration patterns (e.g., Allen, Kirchner, Braun, Siegwolf, & Goldsmith, 2019; Ehleringer, Phillips, Schuster, & Sandquist, 1991). In any case, the endmembers must be carefully defined because this process often involves assuming a binary division of the subsurface when root-zone waters actually vary gradually and continuously. Indeed, intensive sampling of root-zone water often reveals isotopic heterogeneities within defined endmembers, which influences the errors, and thus the strength of inference obtained from endmember-mixing models (Goldsmith et al., 2019; Oerter, Siebert, Bowling, & Bowen, 2019).

Advances in experimental and analytical methods have revealed many challenges in sampling all potential plant water sources and defining non-overlapping endmembers. In recent years, the isotopic composition of plant root zones has been increasingly investigated across diverse settings (see reviews by Penna et al., 2018 and Sprenger, Leistert, Gimbel, & Weiler, 2016). Importantly, some studies have linked root-zone isotopic heterogeneities to small-scale heterogeneities in transport and mixing processes (Sprenger, Llorens, Cayuela, Gallart, & Latron, 2019), and suggested the occurrence of isotopic fractionation effects at mineral-water interfaces (Y. Lin & Horita, 2016) and root-water interfaces (Vargas, Schaffer, Li, & Sternberg,

2017). Whereas the effects of these and other phenomena should manifest in temporally and spatially (laterally and vertically) variable root-zone isotope ratios, most practical field sampling designs will not allow for the over-sampling required to fully characterize and account for those heterogeneities in mixing-model analyses (Goldsmith et al., 2019). Thus, careful design, application, and interpretation of root-water uptake studies is warranted.

In this review, we show the diversity of isotope ratios that can naturally occur in the root zone and in plants and how this diversity can influence the identification of potential plant source waters. We then discuss these isotopic variations and the measurement and prediction challenges they convey in the context of traditional mixing model analyses. Our ultimate objective is to offer practical guidance to facilitate inferring the water sources used by plants through using stable water isotopes.

2. Root-zone water: Characterizing and sampling across natural variations in isotope endmembers

Isotope endmembers of the root-zone water ($\delta_{root-zone}$) are typically characterized using one or several isotope profiles of the subsurface, which are determined by extracting water from soils or other porous media sampled at various depths. Conventionally, it has been assumed that these isotope profiles are near-monotonic, with heavier isotope ratios in shallower depths and lighter isotope ratios in greater depths (solid red line in Figure 1). This characteristic isotope-depth profile often occurs when shallow soil waters become enriched as isotopically lighter water is preferentially evaporated (U. Zimmermann et al., 1967; Barnes & Allison, 1984) or when isotopically heavier growing-season precipitation recharges the soils that supply evapotranspiration (however, not always the most-recent precipitation is evapotranspired; Allen, von Freyberg, Weiler, Goldsmith, & Kirchner, 2019). Beneath the maximum evaporation penetration depth, infiltrated precipitation water mixes with previously stored water over seasons and years so that the isotopic signature of soil water represents the long-term average of previous precipitation events that have recharged these soils; thus, deeper soil waters are usually isotopically lighter than shallow soil waters.

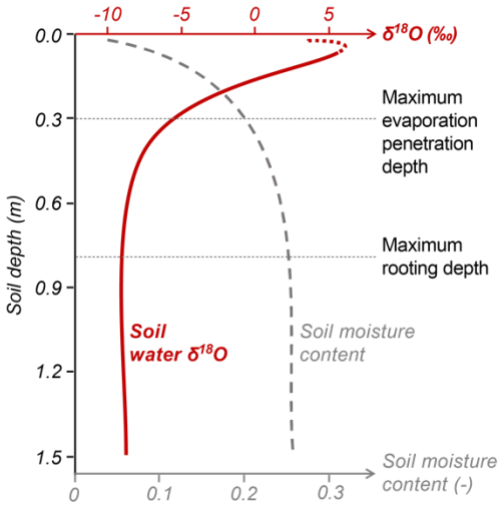


Figure 1: Hypothetical example of soil moisture content (grey dashed line) and soil-water $\delta^{18}O$ (red solid line) for a sandy-loam soil profile occupied by shallow-rooted plants. It is commonly assumed that the soil-water isotope profile is near-monotonic with disproportionate amounts of the heavier isotopologues in the shallow layers mainly due to evaporative fractionation. Isotopic inversions in the top few cm (red dashed line) result from fractionation associated with phase changes of water within the profile. Figure re-drawn after Barnes and Allison (1983).

2.1 Evaporation and transport result in lateral heterogeneities and non-monotonic isotope profiles

In most natural systems, $\delta_{root-zone}$ values do not monotonically decrease with depth if precipitation inputs are isotopically variable (and they usually are; Dansgaard, 1964, Munksgaard, Wurster, Bass, & Bird, 2012) or if evaporatively enriched pore waters are transported downward. Such variations are likely to be more extreme in regions with highly variable weather conditions; for instance, in a drying phase, the evaporation penetration front near the soil surface will move progressively downward into the deeper subsurface (Rothfuss et al., 2015). With precipitation, the (evaporatively enriched) isotope composition in the upper depth profile may become attenuated or displaced downward such that the pre-event and post-event isotope profiles differ (Sprenger et al., 2016). Figure 2a illustrates how dramatically $\delta_{root-zone}$ can vary across a desert soil profile in Arizona, USA; isotope ratios in root-zone water up to 25cm depth seasonally vary by more than 90‰ in $\delta^{2}\mathrm{H}$ and 20‰ in $\delta^{18}\mathrm{O}$. Even for wetter environments, such as on the humid Kohala pensinsula on Hawaii, the complex interplay between wetting and drying of the soil profile can result in up to 15‰ vertical variations in $\delta^{18}\mathrm{O}$ (Figure 2b). The occurrence of these isotope fluctuations in the subsurface imply that under-sampling certain depths could result in failing to identify the appropriate plant-water sources.

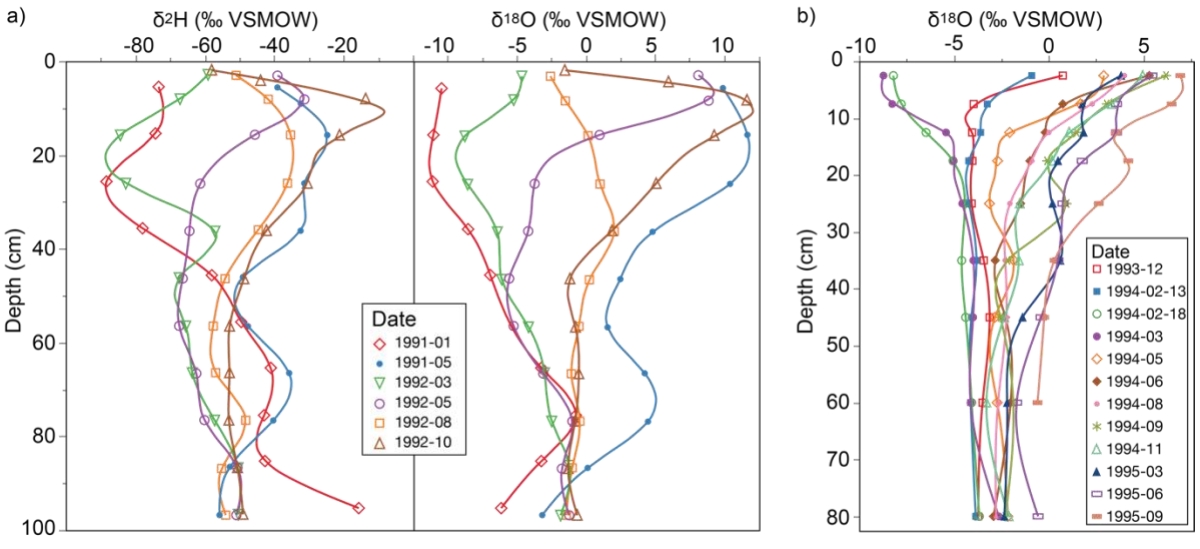
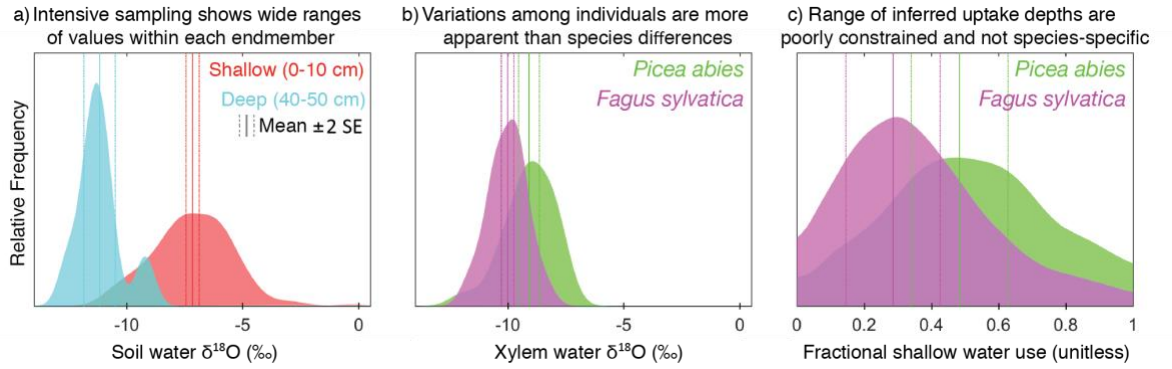


Figure 2: a) Seasonal variations of $\delta^{18}O$ and $\delta^{2}H$ in soil water in a nearly bare desert soil (figure re-drawn from Liu, Phillips, Hoines, Campbell, & Sharma, 1995). b) $\delta^{18}O$ values in soil water under pasture grass in a weathered volcanic ash soil (Andisol) on the island of Hawaii. The climatic conditions were humid with a high variability in rainfall. Isotope data from two samples per campaign were averaged (original data from Table 4 in Hsieh, Chadwick, Kelly, & Savin, 1998).

Beyond the vertical variability in $\delta_{root-zone}$ profiles, recent studies highlight substantial $\delta_{root-zone}$ heterogeneity in the lateral dimension. Lateral isotopic variations have been attributed to subsurface properties, canopy interception and shading effects (e.g., Goldsmith et al., 2019; McCole & Stern, 2007). For instance, Figure 3a shows extremely heterogeneous shallow $\delta_{root-zone}$ across a 1-ha forest plot; isotope ratios were weakly spatially autocorrelated, and thus their variations could not be easily predicted without this level of extensive sampling (Goldsmith et al., 2019).

Where lateral heterogeneities in $\delta_{root-zone}$ are prominent, mixing-model analyses should consider the full range of individual potential endmember values instead of simply averaging across all $\delta_{root-zone}$ values, which would dampen true variations and would yield a subsurface characterization that seems more well-mixed than probably ever actually exists (Figure 3a). When we calculate the relative source contributions *f* (Eq. 3) through including all potential endmember values we will obtain a range of solutions for each xylem water mixture (Figure 3c). Through combining each xylem water sample with each possible shallow-soil water endmember, one can see that the uncertainty in the two-component endmember mixing approach can be substantial, e.g., contributions of shallow soil water to *Picea abies* can vary from zero to 100% (Figure 3c). Thus, misinterpretations of endmember-mixing analyses are likely to happen if the heterogeneity of soil water (and xylem water) isotopic composition is not well characterized.



175

176 *Figure 3: δ¹⁸O of soil water from two depths, δ¹⁸O of xylem water from two species, and inferred use of shallow water from*
177 *mixing-model analysis, all from a single-day snapshot sampling across 1 ha of a forested hillslope in Switzerland (data are*
178 *from Goldsmith et al., 2019). All water samples (149 shallow soil samples, 8 deep soil samples, 22 Picea abies samples, and*
179 *35 Fagus sylvatica samples) were extracted by cryogenic vacuum distillation and then analyzed on a mass spectrometer;*
180 *detailed methods are described in the original Goldsmith et al. (2019) study. Here, we show these data to demonstrate that*
181 *intensive sampling can reveal wide ranges in isotope ratios within the endmember samples (panel a) and mixtures (b); these*
182 *ranges are often not revealed through typical sampling strategies, and means (± standard errors) can poorly capture the*
183 *distribution of actual potential values; the mixing-model standard errors were calculated using IsoError (Phillips & Gregg,*
184 *2001). Importantly (but not shown here), the shallow soil-water heterogeneity was not strongly structured, so any sample*
185 *could be associated with any tree; thus, the mixing-model analysis (c) shows the range of solutions for all trees for all*
186 *combinations of potential endmember values. While there are significant differences between the shallow and deep soil*
187 *water δ¹⁸O mean values, or the two species xylem δ¹⁸O water values, the distributions in panel (c) strongly overlap. While*
188 *the mixing-model analysis suggests that P. abies used shallower water than F. sylvatica, the ranges exceed 0-1 in both*
189 *(indeterminate values were cropped from the figure) and thus no clear characteristic depth of uptake could be inferred for*
190 *each species. Goldsmith et al.'s findings demonstrate that using mean values in the model could result in a*
191 *mischaracterization of the population (or even the sample). Furthermore, they suggest the need to approach such analyses*
192 *qualitatively, especially when samples sizes are insufficient for capturing the true isotopic variation.*

193

194 *2.2 In-situ sampling methods cannot mimic when and where plant roots access water*

195 The distribution of roots in the subsurface is often the first-order constraint over potential water
196 sources: uptake is unlikely to occur from zones that lack functional roots (but see papers on hydraulic
197 redistribution and mycorrhizal-mediated water transport; e.g., Augé, 2001; Dawson, 1993). It should be noted,
198 however, that roots differ in their functional or physical roles, e.g., anchorage and transport in suberized root
199 tissues versus resource acquisition in un-suberized tissues (Taiz & Zeiger, 2010). Furthermore, the role of a
200 root also depends on the interplay between its water potential and the surrounding soil's water potential, the
201 latter of which is a function of soil texture, water content and osmotic potential. These factors vary across
202 species, time and space, resulting in uptake dynamics with subtleties that are difficult to match with the
203 sampling methods used by researchers.

204 Some *in-situ* approaches sample water across a membrane using a pressure gradient, mimicking the
205 process used by plant roots (Steudle, 1994). For instance, suction lysimeters use buried porous ceramic cups
206 with a suction force applied to extract freely draining water and pore water. Depending on the ceramics'

porosity and the applied suction force, these lysimeters can extract water held at matric tensions reaching -10^3hPa (Sprenger, Herbstritt, & Weiler, 2015). Another benefit is that they allow repeated sampling of particular locations in the root zone, which can potentially be useful to monitor small-scale temporal isotopic variations of subsurface water. Importantly, where suction lysimeters differ from plant roots is the timing of water uptake and the sizes of sampled pores: much of the applied potential may be satisfied following rainfall events, and larger more conductive pores in the ceramic material may disproportionally transmit water (Hansen & Harris, 1975; Weihermüller et al., 2007). This contrasts with the natural behavior of plant roots when there is generally low transpirational demand after rainfall events (because atmospheric humidity is high) and when soil water bypasses the rooting zone as it drains rapidly downward through the largest pores (Brooks, Barnard, Coulombe, & McDonnell, 2010). Consequently, the isotopic composition of the water extracted by suction lysimeters may differ from the surrounding pore waters that are later used by plants; details of this process, however, have not been quantified experimentally.

Gas-permeable membranes have recently been used to extract subsurface water vapor *in-situ* for direct isotope analysis to characterize the isotopic composition of the unsaturated zone (Oerter, Perelet, Pardyjak, & Bowen, 2017; Rothfuss, Vereecken, & Brüggemann, 2013; Volkmann & Weiler, 2014; West, Patrickson, & Ehleringer, 2006). This approach allows for characterizing water vapor that has equilibrated with the liquid water in soils, reflecting water across a large variety of pores that surround the membrane probe. For translating vapor isotope measurements into liquid water isotope data (relative to Vienna Standard Mean Ocean Water, V-SMOW), calibration standards need to be generated by combining dry substrate from the study site with waters of known isotopic composition. With this, potentially-confounding effects of isotopic fractionation from clays or organic matter (Chen, Auerswald, & Schnyder, 2016; Oerter et al., 2014) can be implicitly included in the calibration. To correct for fractionation effects during equilibrium exchange between liquid water and vapor (Majoube, 1971), standards must be measured across a temperature range similar to that of each sampling depth (Rothfuss et al., 2013). Limitations in the practical application of the *in-situ* equilibration method can arise from condensation, mixing and diffusion of water vapor in the tubes leading from the membrane to the laser spectrometer.

Although these and other *in-situ* sampling methods have been developed to characterize isotope ratios in root-zone water (Orlowski, Pratt, & McDonnell, 2016; Sprenger et al., 2015), these methods may

sample differently across the subsurface spatiotemporal heterogeneities that we know to occur. It also needs to be recognized that plants may root across each soil horizon (Dekker & Ritsema, 1996; Stewart, Moran, & Wood, 1999), which may further complicate practical attempts to isotopically characterize plant water sources. In conclusion, we advocate for choosing the sampling technique(s) depending on the subsurface properties of the study site and we provide some practical recommendations in Sect. 5.

2.3 In-lab extraction methods homogenize water across functionally distinct pores

For applications where *in-situ* sampling is not ideal, root-zone water can also be extracted – as vapor or liquid – in the laboratory from samples collected in the field. Being relatively cheap and technically easy, augering into soils is common practice for collecting samples and characterizing subsurface properties up to a few meters depth. However, augering is destructive, and thus repeated augering in a small area could fundamentally alter the local infiltration pathways. Moreover, augering only provides integrated samples at any particular depth, and thus the mixture of waters with distinct isotope ratios in any chunk of soil cannot be resolved and the spatiotemporal resolution of such isotope data sets remains generally low (Landon, Delin, Komor, & Regan, 1999).

Various techniques are used for extracting water from porous media in the laboratory, most commonly cryogenic vacuum distillation and direct water vapor equilibration, that have been extensively tested and evaluated in multiple studies (e.g., Araguás-Araguás, Rozanski, Gonfiantini, & Louvat, 1995; Kübert et al., 2020; Orlowski et al., 2016; Sprenger et al., 2015; Thoma, Frentress, Tagliavini, & Scandellari, 2018; Volkmann, Kühnhammer, Herbstritt, Gessler, & Weiler, 2016). Analytical uncertainties of cryogenic vacuum distillation can result from incomplete extraction leading to partial distillation from the sample such that the extracted water is isotopically lighter than that in the original sample (Stoll, Hissler, & Legout, 2014; Thielemann, Gerjets, & Dyckmans, 2019). While cross-laboratory comparisons have attempted to quantify errors involved in cryogenic extraction, such tests usually use soil samples that have been oven-dried at very high temperatures (e.g., 105°C) and then spiked with water of known isotopic composition (e.g., Orlowski et al., 2018). This approach might, however, introduce variable isotopic fractionation processes associated with the re-hydration of oven-dried matrix materials, which is not representative of real-world soils (Gaj, Kaufhold, & McDonnell, 2017; Sprenger et al., 2015). This suggests that such tests of the cryogenic extraction method

might exaggerate the true isotopic uncertainties (Newberry, Nelson, & Kahmen, 2017). Furthermore, because of the extremely low water potentials used in cryogenic distillation, small residual water pools (e.g., hygroscopic and biologically-bound water) are collected that would rarely, if ever, be usable by plants. Thus, particularly for soil samples with low moisture and/or high clay contents, cryogenic extraction may not perfectly retrieve soil-water isotope ratios that match the water that is specifically available to plants. Although cryogenic extraction is widely used, systems and user protocols can vary across laboratories (Orlowski et al., 2018) and thus, uncertainties introduced by this method should always be quantified.

The direct equilibration method allows for water vapor of a moist soil sample to be measured directly with a laser spectrometer in the lab (Hendry, Schmeling, Wassenaar, Barbour, & Pratt, 2015; Mattei et al., 2019; Wassenaar, Hendry, Chostner, & Lis, 2008). Here, water vapor is extracted from a substrate sample in a tightly sealed bag or container, taking advantage of the equilibrium vapor-liquid isotopic offset (Majoube, 1971). Similar to the in-situ equilibration method, vapor measurements are calibrated relative to V-SMOW by measuring alternatingly water vapor from bags filled with isotope standards (Garvelmann, Kulls, & Weiler, 2012; Wassenaar et al., 2008). Uncertainties in the measured isotope ratios can increase when the water content in the bag becomes too small (e.g., <3g in a 1-L bag; Hendry et al., 2015); also, volatile organics in the water vapor, either already in the sample or due to microbial activity, can cause analytical interferences (Gralher, Herbstritt, Weiler, Wassenaar, & Stumpp, 2018; Hsieh, Savin, Kelly, & Chadwick, 1998).

Other, less frequently used water extraction methods, such as centrifugation, mechanical squeezing, azeotropic distillation and microwave distillation have also been evaluated for their capability to retrieve representative root-zone water samples for isotope analysis (e.g., Adams et al., 2020; Figuéroa-Johnson, Tindall, & Friedel, 2007; Kelln, Wassenaar, & Hendry, 2001; Munksgaard, Cheesman, Wurster, Cernusak, & Bird, 2014). Some limitations of the methods have been identified, e.g. that squeezing and centrifugation yield comparable results to direct equilibration only when used for coarse soils with >20% water content (Orlowski et al., 2016); however, more testing is needed across a large range of soil types and extraction conditions to facilitate a more widespread application of these alternative methods.

Whereas in-lab extraction methods typically cannot deliver the high-frequency measurements useful for matching xylem water to the constantly changing isotope values in the root zone – e.g., because frequent coring may fundamentally alter the infiltration properties of the subsurface – *in-situ* methods rarely provide

291 insights into the spatially variability in $\delta_{root-zone}$. Choosing one sampling or extraction strategy over others is
292 implicitly a compromise that requires considerations of its inherent limitations and the associated
293 uncertainties (but see Sect. 4 and 5).

294

295 3. Xylem water: Characterizing and sampling across natural variations in isotope
296 mixtures

297 Our review of the spatiotemporal heterogeneity within root-zone water endmembers points towards
298 the importance of their careful characterization; similarly, variability in xylem water isotopic composition
299 (δ_{xylem}) should be carefully considered when defining xylem water as a mixture of root-zone water sources.
300 An inability to attribute xylem water mixtures to potential root-zone water endmembers may not only be due
301 to under-sampling of subsurface water sources, but also due to challenges in determining δ_{xylem} values.
302 While it has been previously assumed that the isotope ratios of water extracted from plant xylem exactly
303 reflect that of the water taken up by roots, we discuss evidence suggesting that δ_{xylem} should be used as an
304 approximation – not an exact reflection – because fractionation effects and heterogeneity create
305 uncertainties.

306

307 *3.1 Uncertainties in xylem water isotope values associated with extraction, analysis and natural*
308 *variability within the plant*

309 Flow through vascular plants involves many of the same processes as flow through soils, such as
310 preferential flow through certain pores and mixing of new inputs with stored water. Thus, heterogeneities in
311 the xylem water properties arise and the best practices for sampling plants may be very similar to those for
312 soils: sample extensively and often. However, paralleling the measurement challenges in plants with those in
313 soils, plant-sampling methods are limited in their ability to extract the water flowing in xylem (without also
314 extracting stored waters) and are not weighted by the relative importance to the transpiration stream. Most
315 commonly, tree tissue is obtained from coring tree trunks or sampling thicker branches with bark (Dawson &
316 Ehleringer, 1993), from which xylem water is extracted via cryogenic vacuum distillation, microwave extraction
317 or high-pressure mechanical squeezing (e.g., Koeniger, Marshall, Link, & Mulch, 2011; Millar, Pratt, Schneider,

318 & McDonnell, 2018). Alternative methods include xylem-water vapor sampling with the direct equilibration
319 method (Millar et al., 2018), or with vapor-permeable membranes implanted into the tree's water-conducting
320 xylem (Marshall, Cuntz, Beyer, Dubbert, & Kuehnhammer, 2020; Volkmann et al., 2016). Analytical
321 uncertainties associated with each of these extraction methods and subsequent isotope analysis can be
322 substantial (but see reviews of Martín-Gómez et al., 2015; Millar et al., 2018; West, Goldsmith, Matimati, &
323 Dawson, 2011), and thus requires a careful selection of an approach based on the research's specific goal, as
324 well as on the sample types, costs and needed precision.

325 In addition to analytical errors, uncertainties in δ_{xylem} can arise due to the natural spatiotemporal
326 heterogeneity of water flow in plants. E.g., different parts of the tree crown can be supplied by different flow
327 pathways through the tree, potentially connected to roots tapped into different water pools (Schulte &
328 Brooks, 2003; M. H. Zimmermann, 1983). A single snapshot of 57 trees across 1ha (Goldsmith et al., 2019)
329 showed strong variation in δ_{xylem} among individual branches (intra-crown variability; Table 2), which was
330 attributed to sectorality. Xylem sectorality is also hypothesized to explain the 6.5-9.3‰ variation in $\delta^{18}O$
331 measured in single redwood trees that were 80-107m tall (T. E. Dawson, personal communication, May 2020).
332 Additionally, it is assumed that heartwood water does not substantially contribute to transpiration, but it is not
333 well known how hydraulically isolated heartwood water remains until it is needed, e.g., during drought (Scholz,
334 Philips, Bucci, Meinzer, & Goldstein, 2011). Systematic comparisons of sapwood versus heartwood are needed
335 to better inform best practices for stem sampling (but see White et al., 1985). While the generality of these
336 findings for all plant types and environments is yet unknown, they suggest that single xylem water samples can
337 only provide a partial view into any tree's water sources.

338 Post-uptake processes in the plants themselves can also affect the isotope composition of xylem
339 water. For herbaceous plants, transpiration occurs in most above-ground tissues, such that evaporative
340 fractionation at the leaf-atmosphere interface leads to isotopic enrichment compared to source water (Craig &
341 Gordon, 1965; Farquhar et al., 1993; Helliker & Ehleringer, 2000). In this case, herbaceous-plant tissue should
342 be sampled from root crowns as they are the least isotopically variable and seem most reliable (Barnard, Bello,
343 Gilgen, & Buchmann, 2006). For trees, evaporative fractionation should also be considered for sections close
344 to the leaf-atmosphere interface, or in green stems that contain stomata or lenticels where isotopically
345 enriched water may diffuse backwards and re-mix (Ehleringer, Roden, & Dawson, 2000; Lehmann et al., 2018).

As a result, δ_{xylem} values within small stems or leaves can differ substantially from each other and from those within the larger trunk.

There can also be meaningful variations in δ_{xylem} values among closely spaced individuals, that should not be ignored through averaging. While often site-level or species-level plant-source water inferences are sought, pursuing those should include accounting for among-tree (or even within-tree) variations in water sources (Figure 3). Combining sample data from across parts of the plant to capture this multi-scale hierarchical heterogeneity (e.g., twigs, branches or trunk cores of trees) should complement xylem water studies, to enable reporting uncertainties in individual δ_{xylem} values.

3.2 Water-transport lags within trees complicate endmember mixing analyses

Time lags from water traveling from roots to stem to twig exist, so we should understand the range of temporal variations in root-zone water over recent times and not just at the instant of sampling. Soil water and xylem water are often collected at the same time, an thus endmember-mixing analyses implicitly neglect the lags between time of uptake and time of water reaching the stem or twig that is sampled. Those travel times and transport velocities of water in the plants are not well quantified. Some studies have combined measurements of stable water isotopes and sap flux to estimate the time from uptake to transpiration, mostly showing that peak tracer concentrations lag inputs by one day to one week (e.g., Gaines, Meinzer, Duffy, Thomas, & Eissenstat, 2016; Schwendenmann, Dierick, Kohler, & Holscher, 2010), but also lag times as long as 2-3 weeks have been observed in 1-m diameter 50-m tall trees (Meinzer et al., 2006). As a consequence of the wide range of water residence times that can exist in trees, synchronizing the sampling of subsurface material with the sampling of plant material for subsequent isotope analysis remains challenging.

3.3 Fractionation during root water uptake should not be ignored

The assumption that the isotope composition of root-zone water exactly matches that of the plant's xylem water is based on early investigations that found no evidence for isotope fractionation during the uptake process (Washburn & Smith, 1934). Several subsequent studies also reported data that supported this claim (Dawson & Ehleringer, 1991; Walker & Richardson, 1991; U. Zimmermann et al., 1967). In the first paper

we could find on this topic, Washburn and Smith (1934) grew plants hydroponically and measured the change in density of the remaining water as a proxy for isotopic fractionation; no change in density was seen after approximately 99% of the hydroponic water was removed by transpiration. These results formed the initial basis for the assumption that plants do not discriminate against any isotopologues of water during uptake. However, many of the early investigations used only one isotope (either $^{2}\mathrm{H}$ or $^{18}\mathrm{O}$) because their analysis was not easy, reliable or routine as it has become today. This, in addition to smaller sample sizes, may be a reason why any isotope fractionation effects, if they were present, may have not been detected. Using both $\delta^{2}\mathrm{H}$ and $\delta^{18}\mathrm{O}$, with which the deuterium excess can be calculated, makes it far easier to identify whether isotopic fractionation has altered a water sample (Dawson & Simonin, 2011; Sprenger et al., 2016).

Furthermore, the studied plants in Washburn and Smith (1934) were never water limited; however, in unsaturated conditions where strong potentials are at play in the root-substrate interface, one may observe isotopic fractionation upon root water uptake. Evidence suggests that (hydrogen) isotopic fractionation during root water uptake can occur in some specialized groups of plants that can live in salt water or saline soils (Ellsworth & Williams, 2007; G. Lin & Sternberg, 1993). Recent potted plant experiments found that isotope values in xylem water were consistently lower than those in root-zone water (e.g., with absolute offsets in $\delta^{2}\mathrm{H}$ averaging 9.2‰ in Vargas et al. (2017), 10.6‰ in Barbeta et al. (2020), and ranging from 2.9 to 15.6‰ in Poca et al. (2019)). The processes leading to these isotopic offsets are not well understood, although the existing studies propose some possible explanations. While uptake in most plants is an advective mass-flow process (apoplastic flow), Poca et al. (2019) speculated that uptake can also involve transmembrane water transport through aquaporins, which may discriminate against heavy isotopes. In contrast, Barbeta et al. (2020) hypothesized that xylem water is isotopically lighter than root-zone water because xylem-water samples contain isotopically depleted water stored in stem tissue. It is also possible that isotopic fractionation occurs during liquid-vapor phase transitions in the root-zone pore spaces located in close proximity to the plant roots. As first outlined in Allison, Barnes, Hughes, and Leaney (1984) and later elaborated on by others including Vargas et al. (2017), temperature and flow dynamics in the subsurface can yield liquid-vapor exchanges that are not explained by equilibrium fractionation factors and have directional effects on subsurface isotope values.

These recent studies suggest that for some plant species and environmental conditions, root water uptake may cause xylem water to be isotopically depleted relative to its source water, whereas other studies indicate that source and xylem waters are seemingly mismatched because of isotope variations within the sampled materials. Experiments to better understand this depletion may need to focus on small-scale variations in water inside roots, xylem vessels or tracheids, and other storages. Otherwise, until the physical and chemical processes underlying these apparent fractionations are understood well enough to account for them mechanistically, errors should be assumed to avoid compromising the validity of endmember mixing-model analyses.

4. Uncertainties abound: Determining mixture and endmember isotopic signatures is technically challenging and associated uncertainties are often unknown

While the question often arises "Which method is the right one?" for identifying the root-zone water endmembers that plants may access, we should recognize that our tools are unlikely to be as flexible and exact as any plant root is. Furthermore, every step towards quantifying an isotope ratio introduces uncertainty. This poses real technical limitations for applying endmember measurements, rendering them only as an approximation and demanding the assumption that δ_{xylem} and $\delta_{root-zone}$ values are uncertain. These uncertainties – associated with natural variability, sampling limitations, and measurement or extraction analytical errors – vary widely, depending on the system conditions and how water is sampled, prepared, extracted, and analyzed.

Only few studies have specifically investigated the δ_{xylem} and $\delta_{root-zone}$ uncertainties that can naturally occur due to the heterogeneity inherent in natural systems, and some examples are shown in Table 1. These values can be large for both δ_{xylem} and $\delta_{root-zone}$, suggesting that many samples need to be collected and analyzed to quantify the natural uncertainties in the mixture and endmember isotopic signatures, which then allows for more robust endmember-mixing analyses (e.g., such as in Figure 3c).

425 *Table 1: Ranges of natural isotopic variability, expressed as 1 standard deviation (σ) or mean isotopic difference (Δ), that*
426 *can occur within trees and soils. These values only provide a limited selection of isotope uncertainties and more detailed*
427 *analyses have been carried out elsewhere (references in Sect. 2.1 and 3.3).*

			Observed natural variability (‰ VSMOW)		
	Source of variability	**Experimental details**	$\delta^{18}O$	$\delta^{2}H$	**Reference**
a	Within the tree crown	*P. abies* branch xylem water (σ of 5 samples, averaged across 3 trees)	1.6	4.4	Goldsmith et al. (2019)
b	Among-tree variability within plot	*P. abies* branch xylem water (σ of 4-8 trees per plot, averaged across 71 plots)	0.8	2.1	Allen, Kirchner, et al. (2019)
c	Laterally in deep soil	Soil water from 40-50cm depth across 1ha (σ, n=8)	1.0	7.1	Goldsmith et al. (2019)
d	Laterally in shallow soil	Soil water from 0-10cm depth across 1ha (σ, n=150)	1.7	10.6	Goldsmith et al. (2019)
e	Isotopic separation during root water uptake	Irrigated sealed pots with *Persea Americana*, $\Delta=\delta_{soil}-\delta_{xylem}$ (mean Δ, n=32)	1.1	9.2	Vargas et al. (2017)

428

429 Table 2 shows some typical uncertainty values due to sample extraction and analysis, which were
430 retrieved from studies specifically targeted to quantify these uncertainties. However, most of the studies that
431 partially tested and evaluated laboratory and field-based extraction methods for soils (Orlowski et al., 2016;
432 Orlowski et al., 2018; Sprenger et al., 2015; Sprenger et al., 2018; Thoma et al., 2018) and plants (Newberry et
433 al., 2017; Martín-Gómez et al., 2015; Millar et al., 2018) have targeted the discussion on uncertainties at
434 certain problems and thus are not directly transferable to other laboratory infrastructures or sample media. In
435 fact, such tests can be ambiguous; for example, studies that have attempted to quantify the difference
436 between δ_{xylem} and $\delta_{root-zone}$ face practical limitations such as when soils also undergo evaporation which
437 likely causes isotopic enrichment of soil water and confounds inferring analytical errors (Millar et al., 2018;
438 Newberry et al., 2017). The uncertainties in Table 2 are further compounded by the fact that the soil water
439 measurements are not necessarily measurements of the specific soil-water that a plant might extract from the
440 root zone; however, this additional uncertainty is not well quantified (Sect. 2). Therefore, the uncertainties in
441 Table 2 should rather be used to guide decisions about sampling- and analysis procedures.

442

443

444

445

Table 2: Analytical uncertainties of commonly-used extraction and measurement methods for stable water isotopes in soil and plant samples. Error was quantified as the mean absolute deviation from an isotope reference value (mostly that of spike water) and repeatability was quantified as one standard deviation of that mean. These values only provide a limited selection of isotope uncertainties and more detailed method comparisons have been carried out elsewhere (references in Sect. 2.2, 2.3 and 3.1).

	Extraction methods	Experimental details	Metric	$\delta^{18}O$ (‰ VSMOW)	$\delta^{2}H$ (‰ VSMOW)	Reference
1	Suction lysimeter (70–75kPa), IRMS	Soil water, spiked sandy loam (n=10)	Error Repeatability	0.68 0.71	1.9 1.5	Thoma et al. (2018)
2	Centrifugation (5000rpm, 15min), OA-ICOS	Soil water, spiked silty sand, 20% GWC (n=5)	Error Repeatability	0.19 0.06	1.08 0.36	Orlowski et al. (2016)
3	Microwave extraction (330W, 15min), OA-ICOS	Soil water, spiked silty sand, 20% GWC (n=5)	Error Repeatability	0.57 0.32	24.95 1.47	Orlowski et al. (2016)
4	Cryogenic vacuum distillation (98°C, 45min), OA-ICOS	Soil water, spiked silty sand, 20% GWC (n=5)	Error Repeatability	0.71 0.18	5.54 1.17	Orlowski et al. (2016)
5	Cryogenic vacuum distillation (100°C, 210min), IRMS	Xylem water, root crown, irrigated open pots with *Triticum aestivum* L., (n=5)	Error Repeatability	Not reported 0.35	 0.86	Millar et al. (2018)
-	Cryogenic vacuum distillation (90°C, 120min), IRMS	Xylem water, irrigated sealed pots with *Salix viminalis* (n=68)	Error Repeatability	0.84 1.13	Not signif. Not reported	Newberry et al. (2017)
6	Direct vapor equilibration method with bags (6d), OA-ICOS	Soil water, spiked coarse sand, medium sand, coarse silt, 8-50% GWC (n=9)	Error Repeatability	0.52 0.76	2.87 4.67	Mattei et al. (2019)
7	*In-situ* equilibration method with membranes (DDS, TI), IRIS	Soil water, slightly clayey silt (n=9)	Error Repeatability	0.12 0.15	1.10 1.32	Volkmann and Weiler (2014)
	Analysis methods					
8	IRMS (Thermo Fischer Delta Plus Advantage mass spectrometer (Thermo Fisher Scientific Inc., Massachusetts, USA) connected to a GFL 1086 equilibration device)	Water, 10 replicates (n=13)	Repeatability	0.02	0.46	Penna et al. (2012)
9	OA-ICOS (Los Gatos Research Inc., off-axis integrated cavity output spectroscope model DLT-100 version 908-0008 or newer)	Water, last 8 of 18 injections (n=72)	Repeatability	0.33	0.33	Penna et al. (2012)
10	IRIS (Picarro Inc., model L1102-*i* liquid analyzer or newer)	Water, last 8 of 18 injections (n=72)	Repeatability	0.1	0.13	Penna et al. (2012)

Figure 4 visually contrasts the values from Table 1 and Table 2 and demonstrates that the ranges of isotope variations attributed to natural heterogeneity mostly exceed analytical errors that have been the primary focus of past critiques on isotope-based plant-water uptake studies. Thus, potentially large uncertainties in δ_{xylem} and $\delta_{root-zone}$ values do not imply that stable water isotopes cannot be used to study plant water sources. Figure 4 rather suggests that errors and uncertainties must be recognized and incorporated into mixing-model analyses. Even if these errors and uncertainties cannot be quantified in a given study, assuming values with magnitudes similar to those in Table 1 and Table 2 would be a reasonable alternative.

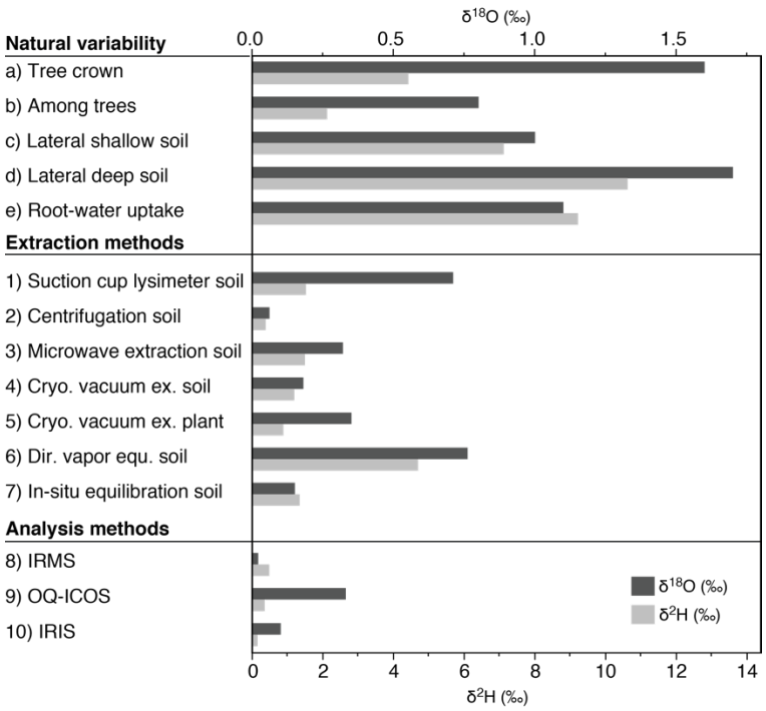


Figure 4: Natural variability of isotope values in soils and plants, and uncertainties of isotope values (repeatability) due to extraction and analysis methods. Information about the data and references are provided in Table 1 and Table 2.

5. Looking forward: designing useful experiments given endmember challenges

Isotope-based endmember mixing models are widely used to quantitatively determine which root-zone water sources are taken up by plants. Given that both the mixture and endmember terms can be challenging to characterize, we should assume mixing-model solutions to be inexact and design studies that maximize the isotopic contrast between endmembers and/or minimize potential uncertainties. As a first step, uncertainties should be estimated or assumed to theoretically determine what the smallest isotopic difference between two endmembers needs to be to enable a robust endmember-mixing analysis (see Sect. 4, and example in Rothfuss & Javaux, 2017).

To summarize and conclude, we provide some specific recommendations to maximize signal-to-noise ratios and thus to enable more confidently inferring plant source waters in physiological, ecological or eco-hydrologic studies. These approaches (and any sampling design) should always be adapted to the specific properties of the study site: the soil and/or substrate type and structure, the climate, and the hydrological setting (e.g., hypothesized range of water sources), and of course, any knowledge of rooting patterns.

1) Design experiments to maximize the isotopic contrast between endmembers

Exploit extreme isotopic anomalies in plant water sources

Stable water isotopes may be best suited for identifying potential plant water sources such as fog, mist or dew (Dawson, 1998; Hill, Dawson, Shelef, & Rachmilevitch, 2015), or rock moisture in the deep weathered bedrock underneath hillslope soils (Oshun, Dietrich, Dawson, & Fung, 2016; Schwinning, 2010), where values deviate strongly from soil water. Other isotopic contrasts between subsurface waters may occur due to mineral-water interactions that lead to strongly fractionated pore water (Y. Lin & Horita, 2016; Oerter et al., 2014), or when goundwater is more depleted than soil water because groundwater recharge was fed mostly by isotopically-light snowmelt (Dawson & Ehleringer, 1991). We can design sampling campaigns specifically to target these isotopic anomalies, or target circumstances where the isotopic differences between the endmembers are large, so that we can more robustly use mixing models.

Ask questions about root-zone water uptake during the driest conditions

During dry conditions, root-zone water isotope profiles will be more monotonic (mainly due to evaporative fractionation near the soil surface), compared to after-precipitation conditions when infiltrating water mixes with pre-event soil water (Sect. 2). Thus, distinguishing among shallow and deep root-zone water sources with an endmember-mixing model is inherently easier (i.e., less uncertain) during dry periods and in dry regions.

Study water uptake after precipitation events that follow dry periods

Rather than orienting research questions around depth of water uptake, similarly useful insights can be gained by asking whether plants are using recent precipitation (or snowmelt) event water. This approach requires that the isotope signal of the event water is very distinct from that already stored in the subsurface (i.e., pre-event water). Thus, sampling root-zone and xylem water before and after events enables us to see whether recent water is taken up by the plant roots (Oerter and Bowen (2017), Zhang, Jiang, Wang, Jiao, and Wang (2018)).

Perform artificial labelling experiments

Applying isotopically enriched or depleted water to the root zone can increase the isotopic differences between the plant source water endmembers in the mixing analyses. Labelled water can be sprinkled on experimental plots to better discern the contribution of “irrigation” versus “pre-irrigation” water

sources to plant water (Grossiord et al., 2014). Alternatively, labelled water can be used to mark specific locations of the root zone to investigate the distribution of active roots (Beyer et al., 2016) or hydraulic redistribution processes (Zapater et al., 2011).

2) Quantify and minimize uncertainties in endmembers and mixture

Quantify xylem water isotopic heterogeneity at the plant- or plot-level

While isotopic heterogeneity should be expected within soil samples and among soil samples at the same depths (Sect. 2), it can also be relevant among xylem samples within and among plants (Sect. 3). While identifying these heterogeneities may be of interest for some specific research questions, usually we are more interested in species-level or plot-level inferences, and thus want to include within-tree variability as an uncertainty term. Optimally, not only individuals are sampled, but also multiple twigs from individuals, so that all δ_{xylem} values can then be incorporated in any endmember-mixing analysis, e.g. by using iterations of mixing models for all permutations of individual sample values (Figure 3).

Use the dual-isotope approach

Both $\delta^{18}\mathrm{O}$ and $\delta^{2}\mathrm{H}$ can now be analyzed simultaneously at very low costs, so that isotopic fractionation effects can be tracked and quantified as deviations from precipitation using deuterium excess or line-conditioned excess (Landwehr & Coplen, 2006). If the relationship between $\delta^{18}\mathrm{O}$ and $\delta^{2}\mathrm{H}$ varies with depth across the root-zone profile (e.g., Figure 2a), both isotopologues can be used as individual endmembers in mixing models. Alternatively, the deuterium excess or line-conditioned excess values themselves can be used to characterize depth-dependent endmembers and the mixture.

Conduct potted-plant experiments

Potted-plant experiments allow for controlling and monitoring all input and output water fluxes, which is useful for tracking variations in δ_{xylem} and $\delta_{root-zone}$ at higher precision and resolution than would be possible in most natural systems. Because the complexity inherent in most natural systems is reduced in such experiments, we can study individual processes in greater detail (such as fractionation effects during plant-water uptake; Sect. 3.3). Although potted-plant experiments may

never fully represent natural systems, a well-designed set-up allows for informative comparison analyses between different environmental conditions and plant species (Kawaletz et al., 2014).

Monitor isotopic variations in root-zone and xylem water at high temporal resolution

New sampling techniques, such as the *in-situ* equilibration method with membrane probes, potentially allow for measuring δ_{xylem} and $\delta_{root-zone}$ at sub-hourly resolution over periods of weeks and months (Sect. 2.2). This might be particularly practical for detecting temporal isotopic anomalies in the root zone (due to fractionation or mixing of new water inputs), and thus may allow for more robust source water attribution. Isotope time series data collected with such methods may also be well suited for calibrating mechanistic models (Knighton et al., 2020).

6. Summary and conclusions

Stable isotopes of water can provide powerful insights into plant water sources, however, accurately determining from when and from where plants take up water requires us to account for the potential sources of uncertainties and limitations associated with the isotope approach. Isotope-based endmember mixing models should only be used to distinguish among highly distinct and well-characterized plant water sources. This means that the differences between endmembers need to be much larger than the uncertainties associated with sample extraction, analysis or modeling in order to yield robust and unambiguous results (Figure 4). Nonetheless, numerous studies have shown truly distinct endmembers, enabling robust inferences on plant water sources, and advancing our understanding of plant water uptake (see references in Sect. 1).

Ideally, all endmembers of a mixture should be known. However, sampling all endmembers is often not practicable due to high sample extraction costs, technical limitations, or unpredictable root distributions. In those cases when not all endmembers can be quantified, we know that uncertainties still exist and thus their consequences for the endmember-mixing model results should be acknowledged. Although we define distinct endmembers, and thus drastically simplify and discretize the complex subsurface water flow processes, endmember-mixing models still provide a route towards new understanding that is not always compromised by recent findings about isotopic variations in the root zone and in the plant. Suggestions for best using this this route are provided in Sect. 5.

559 In addition to the uncertainties associated with the endmembers of plant water sources, we need to
560 acknowledge the limitations of the concept behind isotope-based endmember mixing analyses. While
561 technology improves, it remains unlikely that mixing-model analysis will transition from a robust comparative
562 method to one that provides exact information on depths of root water uptake. In other words, isotope-based
563 endmember mixing models allow us to identify which water sources the plant "uses" but it cannot always help
564 us to identify the sources it "depends on". Nonetheless, new technologies have significantly increased
565 temporal and spatial sampling frequencies, which can mitigate and constrain the uncertainties discussed in
566 this paper. We are hopeful that continuous progress and method development will provide new insights on
567 plant- and ecosystem-level water relations.
568

569 ## Authors' contributions

570 JF, SA, CG and TD conceived the ideas and designed methodology, hence collected and reviewed the
571 references and data. JF and SA led the writing of the manuscript with substantial inputs from CG and TD. All
572 authors contributed critically to the drafts and gave final approval for publication.

573

574 ## Data accessibility

575 Data for this article consist of lists of natural variations and analytical uncertainties in stable water isotope
576 values that were pulled from 10 published studies and that are presented in Table 1 and Table 2.

577 ## Acknowledgements

578 We are thankful to Gregory R. Goldsmith, James W. Kirchner, Erik J. Oerter and Michael Bliss Singer for early
579 discussions on the challenges involves in using stable water isotopes in plant water studies. JF was supported
580 by the Swiss National Science Foundation SNF (PR00P2_185931) and CG was supported by the Swiss National
581 Science Foundation SNF (PZ00P3_174068).

582 ## References

583 Adams, R. E., Hyodo, A., SantaMaria, T., Wright, C. L., Boutton, T. W., & West, J. B. (2020). Bound and mobile soil water
584 isotope ratios are affected by soil texture and mineralogy, whereas extraction method influences their measurement.
585 *Hydrological Processes, 34*(4), 991-1003. doi:10.1002/hyp.13633

586 Allen, S. T., Kirchner, J. W., Braun, S., Siegwolf, R. T. W., & Goldsmith, G. R. (2019). Seasonal origins of soil water used by
587 trees. *Hydrology and Earth System Sciences, 23*(2), 1199-1210. doi:10.5194/hess-23-1199-2019

588 Allen, S. T., von Freyberg, J., Weiler, M., Goldsmith, G. R., & Kirchner, J. W. (2019). The Seasonal Origins of Streamwater in
589 Switzerland. *Geophysical Research Letters, 46*, 10425– 10434. doi:10.1029/2019gl084552

590 Allison, G. B., Barnes, C. J., Hughes, M. W., & Leaney, F. W. J. (1984). Effect of climate and vegetation on oxygen-18 and
591 deuterium profiles in soils. In *Isotope hydrology 1983. Proceedings of the "International symposium on isotope*
592 *hydrology in water resources development", Vienna (Austria), 12-16 Sep 1983* (Vol. IAEA-SM-270/20, pp. 105-122):
593 International Atomic Energy Agency, Vienna (Austria).

594 Araguás-Araguás, L., Rozanski, K., Gonfiantini, R., & Louvat, D. (1995). Isotope effects accompanying vacuum extraction of
595 soil water for stable isotope analyses. *JOURNAL OF HYDROLOGY, 168*(1), 159-171. doi:https://doi.org/10.1016/0022-
596 1694(94)02636-P

597 Augé, R. M. (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza, 11*(1), 3-42.
598 doi:10.1007/s005720100097

599 Barbeta, A., Gimeno, T. E., Clave, L., Frejaville, B., Jones, S. P., Delvigne, C., . . . Ogee, J. M. (2020). An explanation for the
600 isotopic offset between soil and stem water in a temperate tree species. *New Phytologist*. doi:10.1111/nph.16564

601 Barnard, R., Bello, F., Gilgen, A., & Buchmann, N. (2006). The δ18O of root crown water best reflects source water δ18O in
602 different types of herbaceous species. *Rapid communications in mass spectrometry : RCM, 20*, 3799-3802.
603 doi:10.1002/rcm.2778

604 Barnes, C. J., & Allison, G. B. (1983). The Distribution of Deuterium and O-18 in Dry Soils.1. Theory. *JOURNAL OF*
605 *HYDROLOGY, 60*(1-4), 141-156.

606 Barnes, C. J., & Allison, G. B. (1984). The Distribution of Deuterium and O-18 in Dry Soils. 3. Theory for Non-Isothermal
607 Water-Movement. *JOURNAL OF HYDROLOGY, 74*(1-2), 119-135.

608 Beyer, M., Koeniger, P., Gaj, M., Hamutoko, J. T., Wanke, H., & Himmelsbach, T. (2016). A deuterium-based labeling
609 technique for the investigation of rooting depths, water uptake dynamics and unsaturated zone water transport in
610 semiarid environments. *JOURNAL OF HYDROLOGY, 533*, 627-643. doi:https://doi.org/10.1016/j.jhydrol.2015.12.037

611 Brooks, J. R., Barnard, H. R., Coulombe, R., & McDonnell, J. J. (2010). Ecohydrologic separation of water between trees and
612 streams in a Mediterranean climate. *Nature Geoscience, 3*(2), 100-104. doi:10.1038/NGEO722

613 Chen, G., Auerswald, K., & Schnyder, H. (2016). 2H and 18O depletion of water close to organic surfaces. *Biogeosciences,*
614 *13*(10), 3175-3186. doi:10.5194/bg-13-3175-2016

615 Craig, H. (1961). Isotopic variations in meteoric waters. *Science, 133*(3465), 1702-1703.

616 Craig, H., & Gordon, L. (1965). Deuterium and oxygen-18 in the ocean and the marine atmosphere. In E. Tongiorgi (Ed.),
617 *Stable Isotopes in Oceanographic Studies and Paleotemperatures* (pp. 130). Spoleto, Italy.

618 Dansgaard, W. (1964). Stable isotopes in precipitation. *Tellus, 16*(4), 436-468. doi:10.1111/j.2153-3490.1964.tb00181.x

619 Dawson, T. E. (1993). Hydraulic lift and water use by plants: implications for water balance, performance and plant-plant
620 interactions. *Oecologia, 95*(4), 565–574. doi:10.1007/BF00317442

621 Dawson, T. E. (1998). Fog in the California redwood forest: ecosystem inputs and use by plants. *Oecologia, 117*(4), 476-485.
622 doi:10.1007/s004420050683

623 Dawson, T. E., & Ehleringer, J. R. (1991). Streamside Trees That Do Not Use Stream Water. *Nature, 350*(6316), 335-337.

624 Dawson, T. E., & Ehleringer, J. R. (1993). Isotopic enrichment of water in the "woody" tissues of plants: Implications for
625 plant water source, water uptake, and other studies which use the stable isotopic composition of cellulose. *Geochimica*
626 *Et Cosmochimica Acta, 57*(14), 3487-3492. doi:https://doi.org/10.1016/0016-7037(93)90554-A

627 Dawson, T. E., & Ehleringer, J. R. (1998). Plants, Isotopes, and Water use: A catchment-level perspective. In C. Kendall & J. J.
628 McDonnell (Eds.), *Isotope tracers in catchment hydrology* (pp. 165-202). Amsterdam; New York: Elsevier Science.

629 Dawson, T. E., & Pate, J. S. (1996). Seasonal water uptake and movement in root systems of Australian phraeatophytic
630 plants of dimorphic root morphology: A stable isotope investigation. *Oecologia, 107*(1), 13-20. doi:10.1007/Bf00582230

631 Dawson, T. E., & Simonin, K. E. (2011). The roles of stable isotopes in forest hydrology and biogeochemistry In D. F. Levia, D.
632 Carlyle-Moses, & T. Tanaka (Eds.), *Forest Hydrology and Biogeochemistry. Ecological Studies (Analysis and Synthesis)*
633 (Vol. 216, pp. 137-161). Dordrecht: Springer.

634 Dekker, L. W., & Ritsema, C. J. (1996). Preferential flow paths in a water repellent clay soil with grass cover. *Water*
635 *Resources Research, 32*(5), 1239-1249. doi:Doi 10.1029/96wr00267

636 Ehleringer, J. R., & Dawson, T. E. (1992). Water-Uptake by Plants - Perspectives from Stable Isotope Composition. *Plant Cell*
637 *and Environment, 15*(9), 1073-1082.

638 Ehleringer, J. R., Phillips, S. L., Schuster, W. S. F., & Sandquist, D. R. (1991). Differential Utilization of Summer Rains by
639 Desert Plants. *Oecologia, 88*(3), 430-434. doi:10.1007/Bf00317589

640 Ehleringer, J. R., Roden, J., & Dawson, T. E. (2000). Assessing Ecosystem-Level Water Relations Through Stable Isotope Ratio
641 Analyses. In O. E. Sala, R. B. Jackson, H. A. Mooney, & R. W. Howarth (Eds.), *Methods in Ecosystem Science* (pp. 181-
642 198). New York, NY: Springer New York.

643 Ellsworth, P. Z., & Williams, D. G. (2007). Hydrogen isotope fractionation during water uptake by woody xerophytes. *Plant*
644 *and Soil, 291*(1-2), 93-107. doi:10.1007/s11104-006-9177-1

645 Evaristo, J., McDonnell, J. J., & Clemens, J. (2017). Plant source water apportionment using stable isotopes: A comparison
646 of simple linear, two-compartment mixing model approaches. *Hydrological Processes, 31*(21), 3750-3758.

647 Farquhar, G. D., Lloyd, J., Taylor, J. A., Flanagan, L. B., Syvertsen, J. P., Hubick, K. T., . . . Ehleringer, J. R. (1993). Vegetation
648 Effects on the Isotope Composition of Oxygen in Atmospheric CO2. *Nature, 363*(6428), 439-443. doi:10.1038/363439a0

649 Feddes, R. A., Hoff, H., Bruen, M., Dawson, T., de Rosnay, P., Dirmeyer, P., . . . Pitman, A. J. (2001). Modeling Root Water
650 Uptake in Hydrological and Climate Models. *Bulletin of the American Meteorological Society, 82*(12), 2797-2810.
651 doi:10.1175/1520-0477(2001)082<2797:Mrwuih>2.3.Co;2

652 Figuéroa-Johnson, M. A., Tindall, J. A., & Friedel, M. (2007). A comparison of O-18 delta composition of water extracted
653 from suction lysimeters, centrifugation, and azeotropic distillation. *Water Air and Soil Pollution, 184*(1-4), 63-75.
654 doi:10.1007/s11270-007-9399-8

655 Flanagan, L. B., Ehleringer, J. R., & Marshall, J. D. (1992). Differential Uptake of Summer Precipitation among Cooccurring
656 Trees and Shrubs in a Pinyon-Juniper Woodland. *Plant Cell and Environment, 15*(7), 831-836.

657 Gaines, K. P., Meinzer, F. C., Duffy, C. J., Thomas, E. M., & Eissenstat, D. M. (2016). Rapid tree water transport and
658 residence times in a Pennsylvania catchment. *Ecohydrology, 9*(8), 1554-1565. doi:10.1002/eco.1747

659 Gaj, M., Kaufhold, S., & McDonnell, J. J. (2017). Potential limitation of cryogenic vacuum extractions and spiked
660 experiments. *Rapid Communications in Mass Spectrometry, 31*(9), 821-823. doi:10.1002/rcm.7850

661 Garvelmann, J., Kulls, C., & Weiler, M. (2012). A porewater-based stable isotope approach for the investigation of
662 subsurface hydrological processes. *Hydrology and Earth System Sciences, 16*(2), 631-640.

663 Goldsmith, G. R., Allen, S. T., Braun, S., Engbersen, N., González-Quijano, C. R., Kirchner, J. W., & Siegwolf, R. T. W. (2019).
664 Spatial variation in throughfall, soil, and plant water isotopes in a temperate forest. *Ecohydrology, 12*(2), e2059.
665 doi:10.1002/eco.2059

666 Gralher, B., Herbstritt, B., Weiler, M., Wassenaar, L. I., & Stumpp, C. (2018). Correcting for biogenic gas matrix effects on
667 laser-based pore water-vapor stable isotope measurements. *Vadose Zone Journal, 17*(1). doi:10.2136/vzj2017.08.0157

668 Grossiord, C., Gessler, A., Granier, A., Berger, S., Brechet, C., Hentschel, R., . . . Bonal, D. (2014). Impact of interspecific
669 interactions on the soil water uptake depth in a young temperate mixed species plantation. *JOURNAL OF HYDROLOGY,*
670 *519*, 3511-3519. doi:10.1016/j.jhydrol.2014.11.011

671 Hansen, E. A., & Harris, A. R. (1975). Validity of Soil-Water Samples Collected with Porous Ceramic Cups. *Soil Science Society*
672 *of America Journal, 39*(3), 528-536. doi:10.2136/sssaj1975.03615995003900030041x

673 Helliker, B. R., & Ehleringer, J. R. (2000). Establishing a grassland signature in veins: 18O in the leaf water of C3 and C4
674 grasses. *Proceedings of the National Academy of Sciences, 97*(14), 7894-7898. doi:10.1073/pnas.97.14.7894

675 Hendry, M. J., Schmeling, E., Wassenaar, L. I., Barbour, S. L., & Pratt, D. (2015). Determining the stable isotope composition
676 of pore water from saturated and unsaturated zone core: improvements to the direct vapour equilibration laser
677 spectrometry method. *Hydrology and Earth System Sciences, 19*(11), 4427-4440. doi:10.5194/hess-19-4427-2015

678 Hill, A. J., Dawson, T. E., Shelef, O., & Rachmilevitch, S. (2015). The role of dew in Negev Desert plants. *Oecologia, 178*(2),
679 317-327. doi:10.1007/s00442-015-3287-5

680 Hsieh, J. C. C., Chadwick, O. A., Kelly, E. F., & Savin, S. M. (1998). Oxygen isotopic composition of soil water: Quantifying
681 evaporation and transpiration. *Geoderma, 82*(1), 269-293. doi:https://doi.org/10.1016/S0016-7061(97)00105-5

682 Hsieh, J. C. C., Savin, S. M., Kelly, E. F., & Chadwick, O. A. (1998). Measurement of soil-water δ18O values by direct
683 equilibration with CO2. *Geoderma, 82*(1), 255-268. doi:https://doi.org/10.1016/S0016-7061(97)00104-3

684 Isaac, M. E., & Anglaaere, L. C. N. (2013). An in situ approach to detect tree root ecology: linking ground-penetrating radar
685 imaging to isotope-derived water acquisition zones. *Ecology and Evolution, 3*(5), 1330-1339. doi:10.1002/ece3.543

686 Jackson, R. B., Sperry, J. S., & Dawson, T. E. (2000). Root water uptake and transport: using physiological processes in global
687 predictions. *Trends in Plant Science, 5*(11), 482-488. doi:https://doi.org/10.1016/S1360-1385(00)01766-0

688 Jasechko, S., Sharp, Z. D., Gibson, J. J., Birks, S. J., Yi, Y., & Fawcett, P. J. (2013). Terrestrial water fluxes dominated by
689 transpiration. *Nature, 496*, 347. doi:10.1038/nature11983

690 https://www.nature.com/articles/nature11983#supplementary-information

691 Javaux, M., Couvreur, V., Vanderborght, J., & Vereecken, H. (2013). Root Water Uptake: From Three-Dimensional
692 Biophysical Processes to Macroscopic Modeling Approaches. *Vadose Zone Journal, 12*(4). doi:10.2136/vzj2013.02.0042

693 Kawaletz, H., Molder, I., Annighofer, P., Terwei, A., Zerbe, S., & Ammer, C. (2014). Pot experiments with woody species - a
694 review. *Forestry, 87*(4), 482-491. doi:10.1093/forestry/cpu017

695 Kelln, C. J., Wassenaar, L. I., & Hendry, M. J. (2001). Stable isotopes (delta O-18,delta H-2) of pore waters in clay-rich
696 aquitards: A comparison and evaluation of measurement techniques. *Ground Water Monitoring and Remediation,*
697 *21*(2), 108-116. doi:10.1111/j.1745-6592.2001.tb00306.x

698 Knighton, J., Kuppel, S., Smith, A., Soulsby, C., Sprenger, M., & Tetzlaff, D. (2020). Using isotopes to incorporate tree water
699 storage and mixing dynamics into a distributed ecohydrologic modelling framework. *Ecohydrology, 13*(3). doi:ARTN
700 e2201

701 10.1002/eco.2201

702 Koeniger, P., Marshall, J. D., Link, T., & Mulch, A. (2011). An inexpensive, fast, and reliable method for vacuum extraction of
703 soil and plant water for stable isotope analyses by mass spectrometry. *Rapid Communications in Mass Spectrometry,*
704 *25*(20), 3041-3048. doi:10.1002/rcm.5198

705 Kübert, A., Paulus, S., Dahlmann, A., Werner, C., Rothfuss, Y., Orlowski, N., & Dubbert, M. (2020). Water Stable Isotopes in
706 Ecohydrological Field Research: Comparison Between In Situ and Destructive Monitoring Methods to Determine Soil
707 Water Isotopic Signatures. *Frontiers in Plant Science, 11*. doi:10.3389/fpls.2020.00387

708 Landon, M. K., Delin, G. N., Komor, S. C., & Regan, C. P. (1999). Comparison of the stable-isotopic composition of soil water
709 collected from suction lysimeters, wick samplers, and cores in a sandy unsaturated zone. *JOURNAL OF HYDROLOGY,*
710 *224*(1), 45-54. doi:http://dx.doi.org/10.1016/S0022-1694(99)00120-1

711 Landwehr, J., & Coplen, T. B. (2006). Line-conditioned excess: a new method for characterizing stable hydrogen and oxygen
712 isotope ratios in hydrologic systems. In *Isotopes in Environmental Studies* (1 ed., pp. 132-135): IAEA.

713 Lange, O. L., Kappen, L., & Schultze, E.-D. (2012). *Water and Plant Life: Problems and Modern Approaches* (O. L. Lange, L.
714 Kappen, & E.-D. Schultze Eds. Vol. 19): Springer-Verlag Berlin Heidelberg.

715 Lehmann, M. M., Goldsmith, G. R., Schmid, L., Gessler, A., Saurer, M., & Siegwolf, R. T. W. (2018). The effect of 18O-labelled
716 water vapour on the oxygen isotope ratio of water and assimilates in plants at high humidity. *New Phytologist, 217*(1),
717 105-116. doi:10.1111/nph.14788

718 Lin, G., & Sternberg, d. S. L. L. (1993). 31 - Hydrogen Isotopic Fractionation by Plant Roots during Water Uptake in Coastal
719 Wetland Plants. In J. R. Ehleringer, A. E. Hall, & G. D. Farquhar (Eds.), *Stable Isotopes and Plant Carbon-water Relations*
720 (pp. 497-510). San Diego: Academic Press.

721 Lin, Y., & Horita, J. (2016). An experimental study on isotope fractionation in a mesoporous silica-water system with
722 implications for vadose-zone hydrology. *Geochimica Et Cosmochimica Acta, 184*, 257-271.
723 doi:10.1016/j.gca.2016.04.029

724 Liu, B. L., Phillips, F., Hoines, S., Campbell, A. R., & Sharma, P. (1995). Water-Movement in Desert Soil Traced by Hydrogen
725 and Oxygen Isotopes, Chloride, and Cl-36, Southern Arizona. *JOURNAL OF HYDROLOGY, 168*(1-4), 91-110.
726 doi:10.1016/0022-1694(94)02646-S

727 Majoube, M. (1971). Oxygen-18 and Deuterium Fractionation between Water and Steam. *Journal De Chimie Physique Et De*
728 *Physico-Chimie Biologique, 68*(10), 1423-+. doi:10.1051/jcp/1971681423

729 Marshall, J. D., Cuntz, M., Beyer, M., Dubbert, M., & Kuehnhammer, K. (2020). Borehole Equilibration: Testing a New
730 Method to Monitor the Isotopic Composition of Tree Xylem Water in situ. *Frontiers in Plant Science, 11*.
731 doi:10.3389/fpls.2020.00358

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

735 Mattei, A., Barbecot, F., Guillon, S., Goblet, P., Hélie, J.-F., & Meyzonnat, G. (2019). Improved accuracy and precision of
736 water stable isotope measurements using the direct vapour equilibration method. *Rapid Communications in Mass*
737 *Spectrometry, 33*(20), 1613-1622. doi:10.1002/rcm.8494

738 McCole, A. A., & Stern, L. A. (2007). Seasonal water use patterns of Juniperus ashei on the Edwards Plateau, Texas, based
739 on stable isotopes in water. *JOURNAL OF HYDROLOGY, 342*(3), 238-248.
740 doi:https://doi.org/10.1016/j.jhydrol.2007.05.024

741 Meinzer, F. C., Brooks, J. R., Domec, J.-C., Gartner, B. L., Warren, J. M., Woodruff, D. R., . . . Shaw, D. C. (2006). Dynamics of
742 water transport and storage in conifers studied with deuterium and heat tracing techniques. *Plant, Cell & Environment,*
743 *29*(1), 105-114. doi:10.1111/j.1365-3040.2005.01404.x

744 Millar, C., Pratt, D., Schneider, D. J., & McDonnell, J. J. (2018). A comparison of extraction systems for plant water stable
745 isotope analysis. *Rapid Communications in Mass Spectrometry, 32*(13), 1031-1044. doi:10.1002/rcm.8136

746 Moore, J. W., & Semmens, B. X. (2008). Incorporating uncertainty and prior information into stable isotope mixing models.
747 *Ecology Letters, 11*(5), 470-480. doi:10.1111/j.1461-0248.2008.01163.x

748 Munksgaard, N. C., Cheesman, A. W., Wurster, C. M., Cernusak, L. A., & Bird, M. I. (2014). Microwave extraction–isotope
749 ratio infrared spectroscopy (ME-IRIS): a novel technique for rapid extraction and in-line analysis of δ18O and δ2H
750 values of water in plants, soils and insects. *Rapid Communications in Mass Spectrometry, 28*(20), 2151-2161.
751 doi:10.1002/rcm.7005

752 Munksgaard, N. C., Wurster, C. M., Bass, A., & Bird, M. I. (2012). Extreme short-term stable isotope variability revealed by
753 continuous rainwater analysis. *Hydrological Processes, 26*(23), 3630-3634. doi:10.1002/hyp.9505

754 Newberry, S. L., Nelson, D. B., & Kahmen, A. (2017). Cryogenic vacuum artifacts do not affect plant water-uptake studies
755 using stable isotope analysis. *Ecohydrology, 10*(8), e1892. doi:10.1002/eco.1892

756 Oerter, E. J., & Bowen, G. (2017). In situ monitoring of H and O stable isotopes in soil water reveals ecohydrologic dynamics
757 in managed soil systems. *Ecohydrology, 10*(4). doi:10.1002/eco.1841

758 Oerter, E. J., Finstad, K., Schaefer, J., Goldsmith, G. R., Dawson, T., & Amundson, R. (2014). Oxygen isotope fractionation
759 effects in soil water via interaction with cations (Mg, Ca, K, Na) adsorbed to phyllosilicate clay minerals. *JOURNAL OF*
760 *HYDROLOGY, 515*, 1-9.

761 Oerter, E. J., Perelet, A., Pardyjak, E., & Bowen, G. (2017). Membrane inlet laser spectroscopy to measure H and O stable
762 isotope compositions of soil and sediment pore water with high sample throughput. *Rapid Communications in Mass*
763 *Spectrometry, 31*(1), 75-84.

764 Oerter, E. J., Siebert, G., Bowling, D. R., & Bowen, G. (2019). Soil water vapor isotopes identify missing water source for
765 streamside trees. *Ecohydrology*, e2083. doi:10.1002/eco.2083

766 Orlowski, N., Breuer, L., Angeli, N., Boeckx, P., Brumbt, C., Cook, C. S., . . . McDonnell, J. J. (2018). Inter-laboratory
767 comparison of cryogenic water extraction systems for stable isotope analysis of soil water. *Hydrology and Earth System*
768 *Sciences, 22*(7), 3619-3637. doi:https://doi.org/10.5194/hess-22-3619-2018

769 Orlowski, N., Pratt, D. L., & McDonnell, J. J. (2016). Intercomparison of soil water extraction methods for stable isotope
770 analysis. *Hydrological Processes, 30*(19), 3434-3449. doi:https://doi.org/10.1002/hyp.10870

771 Oshun, J., Dietrich, W. E., Dawson, T. E., & Fung, I. (2016). Dynamic, structured heterogeneity of water isotopes inside
772 hillslopes. *Water Resources Research, 52*(1), 164-189.

773 Parnell, A. C., Inger, R., Bearhop, S., & Jackson, A. L. (2010). Source Partitioning Using Stable Isotopes: Coping with Too
774 Much Variation. *PLoS ONE, 5*(3). doi:ARTN e9672

775 10.1371/journal.pone.0009672

776 Penna, D., Hopp, L., Scandellari, F., Allen, S. T., Benettin, P., Beyer, M., . . . Kirchner, J. W. (2018). Ideas and perspectives:
777 Tracing terrestrial ecosystem water fluxes using hydrogen and oxygen stable isotopes – challenges and opportunities
778 from an interdisciplinary perspective. *Biogeosciences, 15*(21), 6399-6415. doi:10.5194/bg-15-6399-2018

779 Penna, D., Stenni, B., Sanda, M., Wrede, S., Bogaard, T. A., Michelini, M., . . . Wassenaar, L. I. (2012). Technical Note:
780 Evaluation of between-sample memory effects in the analysis of delta H-2 and delta O-18 of water samples measured
781 by laser spectroscopes. *Hydrology and Earth System Sciences, 16*(10), 3925-3933. doi:10.5194/hess-16-3925-2012

782 Phillips, D. L., & Gregg, J. W. (2001). Uncertainty in source partitioning using stable isotopes. *Oecologia, 127*(2), 171-179.
783 doi:10.1007/s004420000578

784 Phillips, D. L., & Gregg, J. W. (2003). Source partitioning using stable isotopes: coping with too many sources. *Oecologia,*
785 *136*(2), 261-269. doi:10.1007/s00442-003-1218-3

786 Poca, M., Coomans, O., Urcelay, C., Zeballos, S. R., Bodé, S., & Boeckx, P. (2019). Isotope fractionation during root water
787 uptake by Acacia caven is enhanced by arbuscular mycorrhizas. *Plant and Soil, 441*(1), 485-497. doi:10.1007/s11104-
788 019-04139-1

789 Rothfuss, Y., & Javaux, M. (2017). Reviews and syntheses: Isotopic approaches to quantify root water uptake: A review and
790 comparison of methods. *Biogeosciences, 14*(8), 2199-2224. doi:10.5194/bg-14-2199-2017

791 Rothfuss, Y., Merz, S., Vanderborght, J., Hermes, N., Weuthen, A., Pohlmeier, A., . . . Brüggemann, N. (2015). Long-term and
792 high-frequency non-destructive monitoring of water stable isotope profiles in an evaporating soil column. *Hydrology*
793 *and Earth System Sciences, 19*(10), 4067-4080. doi:10.5194/hess-19-4067-2015

794 Rothfuss, Y., Vereecken, H., & Brüggemann, N. (2013). Monitoring water stable isotopic composition in soils using gas-
795 permeable tubing and infrared laser absorption spectroscopy. *Water Resources Research, 49*(6), 3747-3755.
796 doi:10.1002/wrcr.20311

797 Scholz, F. G., Philips, N. G., Bucci, S. J., Meinzer, F. C., & Goldstein, G. (2011). Hydraulic Capacitance: Biophysics and
798 Functional Significance of Internal Water Sources in Relation to Tree Size. In F. C. Meinzer, B. Lachenbruch, & T. E.
799 Dawson (Eds.), *Size- and Age-Related Changes in Tree Structure and Function* (Vol. 4, pp. 341-361). Dordrecht: Springer.

800 Schulte, P. J., & Brooks, J. R. (2003). Branch junctions and the flow of water through xylem in Douglas-fir and ponderosa
801 pine stems. *Journal of Experimental Botany, 54*(387), 1597-1605. doi:10.1093/jxb/erg169

802 Schulze, E. D. (1982). Plant life forms and their carbon, water and nutrient relations. In *Physiological plant ecology II* (pp.
803 pp. 615-676). Heidelberg: Springer.

804 Schwendenmann, L., Dierick, D., Kohler, M., & Holscher, D. (2010). Can deuterium tracing be used for reliably estimating
805 water use of tropical trees and bamboo? *Tree Physiology, 30*(7), 886-900. doi:10.1093/treephys/tpq045

806 Schwinning, S. (2010). The ecohydrology of roots in rocks. *Ecohydrology, 3*(2), 238-245. doi:10.1002/eco.134

807 Sellers, P. J., Dickinson, R. E., Randall, D. A., Betts, A. K., Hall, F. G., Berry, J. A., . . . Henderson-Sellers, A. (1997). Modeling
808 the exchanges of energy, water, and carbon between continents and the atmosphere. *Science, 275*(5299), 502-509.
809 doi:10.1126/science.275.5299.502

810 Sprenger, M., Herbstritt, B., & Weiler, M. (2015). Established methods and new opportunities for pore water stable isotope
811 analysis. *Hydrological Processes, 29*(25), 5174-5192.

812 Sprenger, M., Leistert, H., Gimbel, K., & Weiler, M. (2016). Illuminating hydrological processes at the soil-vegetation-
813 atmosphere interface with water stable isotopes. *Reviews of Geophysics, 54*(3), 674-704. doi:10.1002/2015RG000515

814 Sprenger, M., Llorens, P., Cayuela, C., Gallart, F., & Latron, J. (2019). Mechanisms of consistently disjunct soil water pools
815 over (pore) space and time. *Hydrology and Earth System Sciences, 23*(6), 2751-2762. doi:10.5194/hess-23-2751-2019

816 Sprenger, M., Tetzlaff, D., Buttle, J., Laudon, H., Leistert, H., Mitchell, C. P. J., . . . Soulsby, C. (2018). Measuring and
817 Modeling Stable Isotopes of Mobile and Bulk Soil Water. *Vadose Zone Journal, 17*(1). doi:10.2136/vzj2017.08.0149

818 Steudle, E. (1994). Water Transport across Roots. *Plant and Soil, 167*(1), 79-90. doi:10.1007/Bf01587602

819 Stewart, J. B., Moran, C. J., & Wood, J. T. (1999). Macropore sheath: quantification of plant root and soil macropore
820 association. *Plant and Soil, 211*(1), 59-67. doi:10.1023/A:1004405422847

821 Stoll, M., Hissler, C., & Legout, A. (2014). *Isotope fractionation in different types of soil water*. Paper presented at the AGU
822 Chapman Conference, 23-26 September 2014, Luxembourg City, Luxembourg.
823 https://www.researchgate.net/publication/312587890_Isotope_fractionation_in_different_types_of_soil_water

824 Taiz, L., & Zeiger, E. (2010). *Plant physiology* (5th ed.). Sunderland, MA: Sinauer Associates.

825 Thielemann, L., Gerjets, R., & Dyckmans, J. (2019). Effects of soil-bound water exchange on the recovery of spike water by
826 cryogenic water extraction. *Rapid Communications in Mass Spectrometry, 33*(5), 405-410. doi:10.1002/rcm.8348

827 Thoma, M., Frentress, J., Tagliavini, M., & Scandellari, F. (2018). Comparison of pore water samplers and cryogenic
828 distillation under laboratory and field conditions for soil water stable isotope analysis. *Isotopes in Environmental and*
829 *Health Studies, 54*(4), 403-417. doi:10.1080/10256016.2018.1437034

830 Unkovich, M. (2001). *Stable isotope techniques in the study of biological processes and functioning of ecosystems* (M.
831 Unkovich, J. S. Pate, A. McNeill, & J. Gibbs Eds.). Dordrecht: Kluwer Academic Publishers.

832 Vargas, A. I., Schaffer, B., Li, Y. H., & Sternberg, L. D. L. (2017). Testing plant use of mobile vs immobile soil water sources
833 using stable isotope experiments. *New Phytologist, 215*(2), 582-594. doi:10.1111/nph.14616

834 Volkmann, T. H. M., Kühnhammer, K., Herbstritt, B., Gessler, A., & Weiler, M. (2016). A method for in situ monitoring of the
835 isotope composition of tree xylem water using laser spectroscopy. *Plant, Cell & Environment, 39*(9), 2055-2063.
836 doi:10.1111/pce.12725

837 Volkmann, T. H. M., & Weiler, M. (2014). Continual in situ monitoring of pore water stable isotopes in the subsurface.
838 *Hydrology and Earth System Sciences, 18*(5), 1819-1833. doi:10.5194/hess-18-1819-2014

839 Walker, C. D., & Richardson, S. B. (1991). The Use of Stable Isotopes of Water in Characterizing the Source of Water in
840 Vegetation. *Chemical Geology, 94*(2), 145-158. doi:10.1016/0168-9622(91)90007-J

841 Wang, J., Lu, N., & Fu, B. J. (2019). Inter-comparison of stable isotope mixing models for determining plant water source
842 partitioning. *Science of the Total Environment, 666*, 685-693. doi:10.1016/j.scitotenv.2019.02.262

843 Washburn, E. W., & Smith, E. R. (1934). THE ISOTOPIC FRACTIONATION OF WATER BY PHYSIOLOGICAL PROCESSES. *Science,*
844 *79*(2043), 188-189. doi:10.1126/science.79.2043.188

845 Wassenaar, L. I., Hendry, M. J., Chostner, V. L., & Lis, G. P. (2008). High Resolution Pore Water delta H-2 and delta O-18
846 Measurements by H2O(liquid)-H2O(vapor) Equilibration Laser Spectroscopy. *Environmental Science & Technology,*
847 *42*(24), 9262-9267. doi:10.1021/es802065s

848 Weihermüller, L., Siemens, J., Deurer, M., Knoblauch, S., Rupp, H., Göttlein, A., & Pütz, T. (2007). In Situ Soil Water
849 Extraction: A Review. *Journal of Environmental Quality, 36*(6), 1735-1748. doi:10.2134/jeq2007.0218

850 West, A. G., Goldsmith, G. R., Matimati, I., & Dawson, T. E. (2011). Spectral analysis software improves confidence in plant
851 and soil water stable isotope analyses performed by isotope ratio infrared spectroscopy (IRIS). *Rapid Communications*
852 *in Mass Spectrometry, 25*(16), 2268-2274.

853 West, A. G., Patrickson, S. J., & Ehleringer, J. R. (2006). Water extraction times for plant and soil materials used in stable
854 isotope analysis. *Rapid Communications in Mass Spectrometry, 20*(8), 1317-1321. doi:10.1002/rcm.2456

855 White, J. W. C., Cook, E. R., Lawrence, J. R., & Broecker, W. S. (1985). The D/H Ratios of Sap in Trees - Implications for
856 Water Sources and Tree-Ring D/H Ratios. *Geochimica Et Cosmochimica Acta, 49*(1), 237-246.

857 Zapater, M., Hossann, C., Breda, N., Brechet, C., Bonal, D., & Granier, A. (2011). Evidence of hydraulic lift in a young beech
858 and oak mixed forest using O-18 soil water labelling. *Trees-Structure and Function, 25*(5), 885-894. doi:10.1007/s00468-
859 011-0563-9

860 Zhang, Y.-P., Jiang, Y., Wang, B., Jiao, L., & Wang, M. (2018). Seasonal water use by Larix principis-rupprechtii in an alpine
861 habitat. *Forest Ecology and Management, 409*, 47-55. doi:https://doi.org/10.1016/j.foreco.2017.11.009

862 Zimmermann, M. H. (1983). *Xylem structure and the ascent of sap*. Berlin u.a.: Springer.

863 Zimmermann, U., Ehhalt, D., & Muennich, K. O. (1967). Soil-water movement and evapotranspiration: Changes in the
864 isotopic composition of the water. In (pp. 567-585): International Atomic Energy Agency (IAEA).

865