Received: 17 November 2020 | Revised: 28 January 2021 | Accepted: 1 February 2021

DOI: 10.1002/hyp.14073

SCIENTIFIC BRIEFING

HP TODAY WILEY

On the use of leaf water to determine plant water source: A proof of concept

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Abstract

Source water apportionment studies using the dual isotopes of oxygen and hydrogen have revolutionized our understanding of ecohydrology. But despite these developments—mostly over the past decade—many technical problems still exist in terms of linking xylem water to its soil water and groundwater sources. This is mainly due to sampling issues and possible fractionation of xylem water. Here we explore whether or not leaf water alone can be used to quantify the blend of rainfall event inputs from which the leaf water originates. Leaf water has historically been avoided in plant water uptake studies due to the extreme fractionation processes at the leaf surface. In our proof of concept work we embrace those processes and use the well-known Craig and Gordon model to map leaf water back to its individual precipitation event water sources. We also employ a Bayesian uncertainty estimation approach to quantify source apportionment uncertainties. We show this using a controlled, vegetated lysimeter experiment where we were able to use leaf water to correctly identify the mean seasonal rainfall that was taken up by the plant, with an uncertainty typically within ±1‰ for $\delta^{18}O$. While not appropriate for all source water studies, this work shows that leaf water isotope composition may provide a new, relatively unintrusive method for addressing questions about the plant water source.

KEYWORDS
fractionation, isotopes, leaf, source water

1 | INTRODUCTION

Inferring plant water sources in time and space is a fundamental scientific challenge in ecohydrology (see recent community commentary in Berry et al. (2018) and Penna et al. (2018)).The stable isotopes of hydrogen and oxygen (^2H and ^{18}O) have become powerful tools for quantifying source water since the early work of Dawson and Ehleringer (1991). But even early on in this area of study, the limitations of the procedures used for determining the source of water used by plants were becoming apparent (Brunel, Walker, & Kennett-Smith, 1995). While many strides have been made in recent years on quantifying the subsurface soil water pools used by plants (Bowling, Schulze, & Hall, 2017; Brooks, Barnard, Coulombe, & McDonnell, 2010; Evaristo, Jasechko, & McDonnell, 2015), one continuing vexing issue is potential fractionation linked to the transpiration process itself (Barbeta et al., 2019; De Deurwaerder et al., 2020; Ellsworth & Sternberg, 2015; Martín-Gómez, Serrano, & Ferrio, 2017; von Freyberg, Allen, Grossiord, & Dawson, 2020). Incomplete knowledge about fractionation throughout the transpiration process is perhaps *the thing* that most affects our ability to trace plant water source. Ellsworth and Williams (2007) showed early on that *where* one samples in the transpiration stream may impact one's ability to map tree water sources (Barbeta et al., 2020; Vargas, Schaffer, Yuhong, & Sternberg, 2017). Of course, *how* one samples the soil and/or plant water source—that is, the extraction technique used—can also impact

the source calculation (Chen et al., 2020; Orlowski, Pratt, & McDonnell, 2016; Zhao et al., 2016).

Beyond the fractionation and extraction challenges, collection of xylem samples has always been central to isotope-based source water studies. It is labour-intensive and limited to suberized branch availability (Dawson & Ehleringer, 1993); and can disturb the plant, causing permanent injury as a result of sampling, particularly for high-frequency sampling or to integrate the spatial variability across branches. New xylem in-situ techniques have been developed that are less invasive for repeated sampling of a single position in the tree (Marshall, Cuntz, Beyer, Dubbert, & Kuehnhammer, 2020; Volkmann, Haberer, Gessler, & Weiler, 2016); but they are still mainly in development stage (Beyer, Kühnhammer, & Dubbert, 2020). Such techniques can also be impacted by fractionation associated with water transport within the tree (i.e. passage through membranes) and with co-extracted volatile substances that plague the laser-based in-situ instruments (Nehemy et al., 2019).

Here we explore a new proof of concept for a different way of coming at plant water uptake studies using stable isotopes—one focused on leaves. Our approach is much simplified compared to current source apportionment approaches. Rather than sampling soil water at different depths and xylem water from the plant, we use only leaf water and rainfall. This approach allows for larger temporal and spatial sampling resolution than current available methods, although with the caveat that it necessitates the use of a model which itself depends on additional measurements of humidity and temperature.

Leaves are an appealing sampling point for source water apportionment studies since they are easier to sample compared to xylem water and because they contain enough water for analysis (where xylem water can sometimes be problematic in terms of extraction volumes) and their collection is faster and much less invasive. This means that more samples can be collected in space and time. But leaf water undergoes severe isotope fractionation that completely transforms its isotopic signature. Early greenhouse work by Flanagan and Ehleringer (1991) showed how leaf water plots on an isotopic evaporation line in δ^2H–$\delta^{18}O$ space, intersecting the corresponding xylem water near the source water, but then increasing with a much shallower slope than the meteoric water line (Dongmann, Nürnberg, Förstel, & Wagener, 1974; Gonfiantini, Tongiorgi, & Gratziu, 1965). While the xylem water isotope composition reflects evaporation at the soil level over timescales of days to months, bulk leaf water enrichment can occur within minutes to hours and it is highly dependent on the atmospheric conditions and vapour concentration within the stomata (Cernusak et al., 2016; Farquhar & Cernusak, 2005; Roden & Ehleringer, 1999). Because of this, leaf water has been studied extensively to understand leaf cellulose isotope signature, and give insight into plant physiological processes. But as far as we know, no studies have yet been published that trace back to the original rainfall sources that make up the leaf water mixture.

So how can we retrieve the tree source water origin from this highly-fractionated leaf water? We hypothesize that coupling the isotopic composition of leaf water to a model of evaporative enrichment may allow retrieval of the average isotope composition of the original rainfall sources. We further hypothesize here that the mean rainfall origin estimated for xylem water and leaf water are the same, following removal of the fractionation effects. We do this with a new, vegetated lysimeter experiment where we control inputs with a labelled water injection and measure leaf and xylem water isotope composition. We control the issue of potential xylem water fractionation with a strong label. The objectives of this Scientific Briefing are thus to show our methodology for using leaf water to determine plant water source, show proof of concept that it works and to discuss the implications for isotope-based ecohydrological studies in forest, urban and agricultural areas.

2 | THEORY

The isotope composition of leaf water typically lies far away from the region that characterizes precipitation in dual-isotope space. Nevertheless, a connection between the belowground source water with leaf water can be reconstructed using models of evaporative enrichment such as the Craig and Gordon (1965) model (Cernusak et al., 2016; Gonfiantini, Wassenaar, Araguas-Araguas, & Aggarwal, 2018; Horita, Rozanski, & Cohen, 2008; Piayda, Dubbert, Siegwolf, Cuntz, & Werner, 2017). Simultaneous application of the Craig and Gordon model (hereafter referred to as the CG model) to both $\delta^{18}O$ and δ^2H provides the dual-isotope enrichment trajectory, often referred to as an 'evaporation line'. To infer the precipitation source that contributed to an enriched water sample, one can perform an inversion of the CG model. However, since the enrichment trajectory is typically linear (Gat, 1996; Gibson, Birks, & Edwards, 2008), full inversion of the CG model is not necessary because one simply needs the slope of the evaporation line to 'project' the sample composition back to its source on the local meteoric water line (LMWL). This is a significant simplification because estimating the evaporation slope is much easier than estimating the exact degree of enrichment along the evaporation trajectory (e.g., it does not matter whether isotopic steady-state is reached or not). Pointing directly to the precipitation source in this way is in fact a 'sidestepping' of xylem and soil water altogether; and the inferred source can be interpreted as the mean across the distribution of precipitation events that contributed to the sample. This approach assumes that any offset from the LMWL in plants is a result of evaporative fractionation.

Simple projection techniques have been used previously to compensate for the effect of evaporative enrichment in studies dealing with lake waters (Bowen et al., 2018; Cluett & Thomas, 2020), soil drainage (Benettin, Queloz, Bensimon, McDonnell, & Rinaldo, 2019), xylem water (Allen, Kirchner, Braun, Siegwolf, & Goldsmith, 2019; Dwivedi et al., 2020) and tap water (Good et al., 2014). For heavily fractionated samples like leaf water, however, small variations in the evaporation line slope can cause large variations in the projected source. This makes it imperative to evaluate the uncertainty introduced by the projection method. To account for these uncertainties, we build on the method of Bowen et al. (2018) to account for not just one evaporation line but for an entire distribution of evaporation line

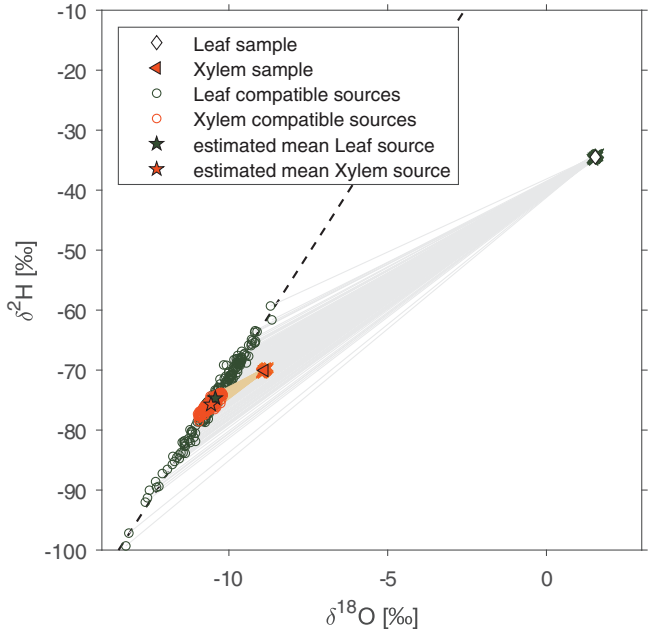

FIGURE 1 Illustration of the source water tracing procedure following the approach by Bowen et al. (2018). A water sample (red triangle for xylem sample and black diamond for leaf sample) is projected onto the local meteoric water line (i.e., where all sources belong) according to a distribution of enrichment trajectories (lines). Each of these trajectories identifies a source that is compatible with the sample (triangles). The mean of these potential sources is indicated by a star. Grey colours refer to leaf samples and red colours refer to xylem samples. The estimated mean potential sources are similar in this example because the two samples lie approximately on the same evaporation line. Leaf source has a larger uncertainty because the sample lies further away from the LMWL. This theoretical example was run with normally-distributed evaporation slopes with mean 3.3 and *SD* 0.3

slopes that are compatible with the measured sample. These are obtained by running a basic implementation of the CG model (Benettin et al., 2018) with multiple combinations of humidity, temperature, hydrodynamic transport parameter and atmospheric isotopic composition values (see Sections S1 and S2—Supplementary Information).

Figure 1 shows the procedure for a hypothetical xylem water sample (red dot) and leaf water sample (black dot). Both samples are assumed to have originated from a mean source around the LMWL and to have followed an evaporation line which is normal-distributed with mean slope 3.3 and *SD* 0.3. Since the leaf water sample lies farther away from the LMWL, the cloud of possible sources is larger. Yet, because the two samples lie roughly on the same evaporation line, the distributions of potential sources have similar means, i.e. the two samples likely originated from the same source.

3 | METHODS

To test our approach, we used data from xylem and leaf water samples from two small willow trees (*Salix viminalis*), collected during a lysimeter experiment in May–June 2018 near Lausanne, CH (Nehemy et al., 2021). The willow-planted lysimeter was left open to natural precipitation for over 2 years. At the beginning of the experiment the entire soil column was filled with winter precipitation (as confirmed by bulk soil water samples collected at different depths and locations). Then, the soil (which included about 500 mm of water) was irrigated with 25 mm of labelled water with distinctive isotopic signature ($\delta^{18}O$=+29.63‰, $\delta^{2}H$=+256.55‰). The goal of this experiment was to probe how a large—yet realistic—storm event would be taken up by the vegetation. Xylem samples were collected throughout the experiment (n = 58), while leaf samples were collected after the first 15 days from the start of the experiment (n = 25). A meteorological station located 5 m away from the lysimeter provided air temperature and relative humidity data.

We collected xylem and leaf samples from second or third order branches originating from the main stem on 23 occasions during a 1-month period. Sampling generally occurred every 2–3 days around midday, but during the period 21–24 June, samples were collected at sub-daily scale (four times a day): at predawn, during the morning, at midday, and in the evening. We sampled a different branch every time by clipping the branch at the node intersection with the higher order branch or main stem. After immediate removal of the branch, we covered the open section with silicone to avoid possible fractionation. Thus, an entire branch from node to leaves was removed. We collected xylem and leaf material from this same branch. We sampled from long and suberized branches with mature bark to avoid evaporative fractionation in the xylem (Dawson & Ehleringer, 1993). The xylem was collected close to the portion that was attached to the main stem and leaves from the tip of the branch. We immediately removed the phloem, quickly chopped the xylem and stored xylem and leaves in glass vials (12 ml Exetainer). This procedure was done quickly to avoid sample exposure to air. Vials with plant material were stored in a fridge at 4°C. We extracted xylem and bulk leaf water and determined their isotopic composition at the Hillslope Hydrology Laboratory, at the University of Saskatchewan. We extracted the water from all plant material using cryogenic vacuum distillation method, following Koeniger, Marshall, Link, and Mulch (2011). All plant samples were weighed before and after cryogenic extraction, as well as after an additional oven drying (48 hr at 105°C) to determine water extraction efficiency (Araguás-Araguás, Rozanski, Gonfiantini, & Louvat, 1995). All water samples were filtered (0.45 μm) and stored in 2 ml vials. Isotope analysis of extracted bulk leaf water and xylem were carried out using an Isoprime isotope ratio mass spectrometer (IRMS) (Elementar UK Ltd, Cheadle Hulme, UK). This was done to avoid any possible spectral contamination of co-extracted organic compounds from plant material in Isotope Ratio Infrared Spectrometer (IRIS) (Millar, Pratt, Schneider, & McDonnell, 2018; Nehemy et al., 2019). Xylem and leaf water hydrogen isotope composition were measured by on-line reduction of the water sample to hydrogen by reaction with elemental chromium (Morrison, Brockwell, Merren, Fourel, & Phillips, 2001). The oxygen isotopic composition of samples was determined using the CO_2-water equilibration method (Epstein & Mayeda, 1953). CO_2-water equilibrations were carried at 25°C using

a Gas Bench II interface, and sample preparation device (Thermo Fischer Scientific, Waltham, MA) connected to a Delta V IRMS instrument (Thermo Fischer Scientific, Waltham, MA). Measured raw delta value of hydrogen and oxygen were normalized to the VSMOW-SLAP scale by analyses of two calibrated reference waters. IRMS laboratory precision for this project was ±0.12‰ and ± 0.81‰ (n = 8; water standards) on $\delta^{18}O$ and $\delta^{2}H$, respectively.

The distribution of possible evaporation lines associated with each sample was obtained by running the CG model for 10,000 combinations of: temperature (in the range: measured −3°C to measured +3°C), relative humidity (range: measured −10% to measured +10%), hydrodynamics transport parameter (range 0.75–1 for soil evaporation and 0.85–1 for leaf evaporation), atmospheric isotope composition (parameter k in the range 0.75–1). The empirical slope distribution was then fitted through a lognormal distribution (see Section S1).

4 | RESULTS

Figure 2 shows that xylem samples plotted approximately parallel to the LMWL, suggesting a similar degree of fractionation (i.e. similar offset from the LMWL) and reflecting the contribution of the labelled irrigation to the plant water (variability along the LMWL). Leaf samples were more heavily and variously fractionated. Figure 3 shows how after we applied the isotope projection methodology (Section S1), samples whose isotope compositions were very distinct due to fractionation effectively collapsed into similar estimated sources. Uncertainty in the projection was quantified through the standard deviation of the projected source distribution. This was variable across the samples (reflecting different environmental conditions and different degrees of fractionation) but was on average 0.25‰ for $\delta^{18}O$ for xylem and 0.63‰ for $\delta^{18}O$ for leaves. The mean sources estimated through xylem and leaf samples were typically within 0.8‰ of each other for $\delta^{18}O$. Figure 4 shows that the estimated sources correctly fell within the region characterized by mixing between winter precipitation and the labelled irrigation.

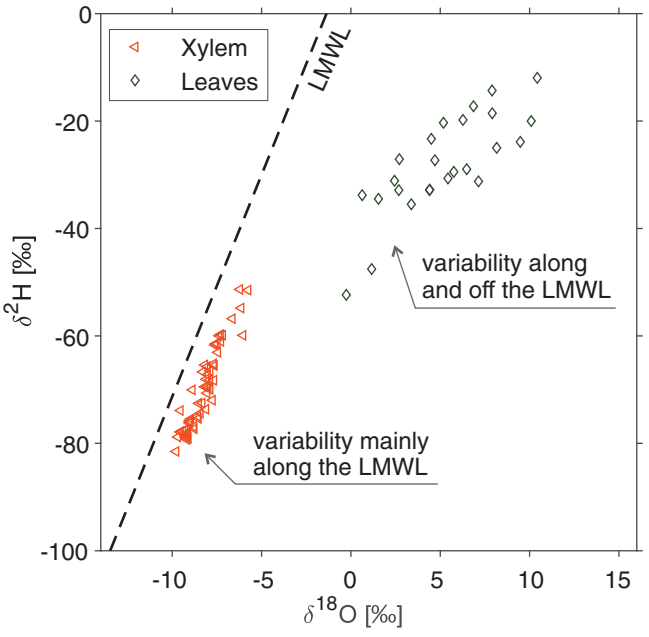

FIGURE 2 Xylem samples (red triangles) and leaf samples (black diamonds) collected during the lysimeter experiment. The variability in xylem isotope composition along the LMWL reflects the application of highly enriched water at the beginning of the experiment. Leaf samples reflect the source variability along the LMWL but they are also heavily and more variously fractionated

5 | DISCUSSION AND CONCLUSIONS

There is much recent, increased concern about fractionation associated with the transpiration stream (Barbeta et al., 2020, 2019; Ellsworth & Sternberg, 2015; Martín-Gómez et al., 2017; Vargas et al., 2017; von Freyberg et al., 2020). This Scientific Briefing has sought to outline a possible new methodology for using leaf water to determine plant water source. Our proof of concept has shown that the mean rainfall $\delta^{18}O$ sources estimated using xylem and leaf samples fell, on average, within 0.8‰ of each other and were always within 2‰. The estimated origin is fully consistent with the true origin at the site, which is given by winter rainfall mixed with heavy-water irrigation. While an accuracy of 1–2‰ on $\delta^{18}O$ may not be enough to understand short-term variations in some detailed water partitioning studies, it would appear to be accurate enough to identify the seasonal water origin. Thus, our initial hypothesis that leaf samples can be realistically linked to their mean rainfall sources is accepted. Of course, uncertainty evaluation is fundamental to the use of leaf water in this way and no estimate of source water should be given without the related uncertainty. The accuracy of the results is determined by the accuracy of key data like humidity and by how well the CG model describes the fractionated samples. For example, the dry experimental conditions around June 20 resulted in water stress conditions to the plant (see Nehemy et al., 2021) and during the same period the method tends to overestimate the true source, suggesting that the simple CG model may be insufficient to describe the evaporative enrichment in these circumstances. A full sensitivity analysis that explores the dependence of the results on model and data assumptions goes beyond this initial proof of concept, but would be the next critical follow-up to this preliminary work. In particular, key points that remain to be addressed are: how does the approach change when a two-pool model or Péclet effects are considered across species or over time? How does the lack of precise measurements at leaf level (e.g., leaf temperature) affect the results? How important is the assumption of leaf water being in isotopic steady state? Could this approach be used for calculating fractional contributions from different subsurface pools rather than the mean rainfall origin?

Our results suggest that leaves—so far avoided for any water apportionment studies—can be used to retrieve information on the original water used to sustain transpiration. The price to pay is the need for additional data to inform the fractionation model and a larger

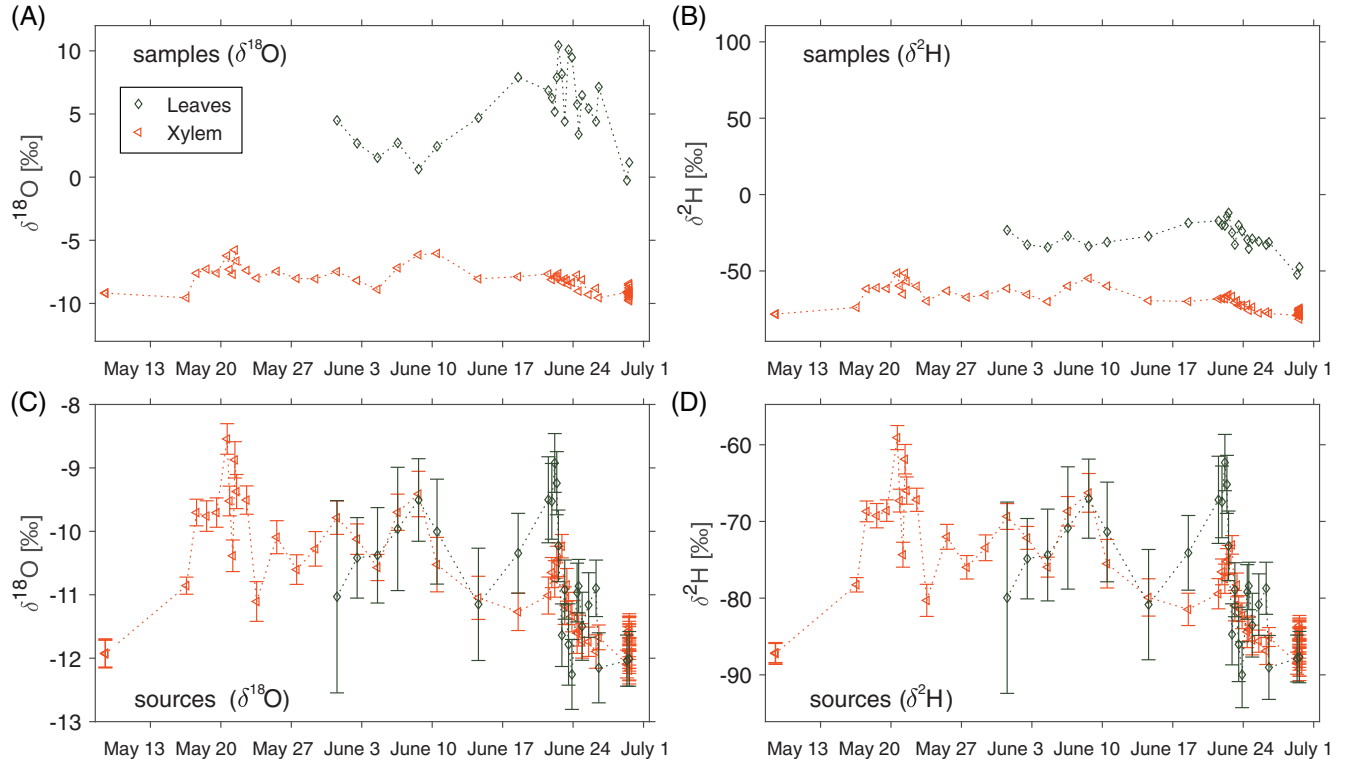

FIGURE 3 Top panels show the original δ^{18}O and δ^{2}H time series for xylem and leaf samples. Bottom panels show the estimated water sources (mean and *SD*) after projecting all samples on the LMWL. Vertical axes are scaled according to the LMWL to provide equal scaling for the two isotopes. The temporal dynamics in the estimated source reflect the breakthrough of the labelled irrigation

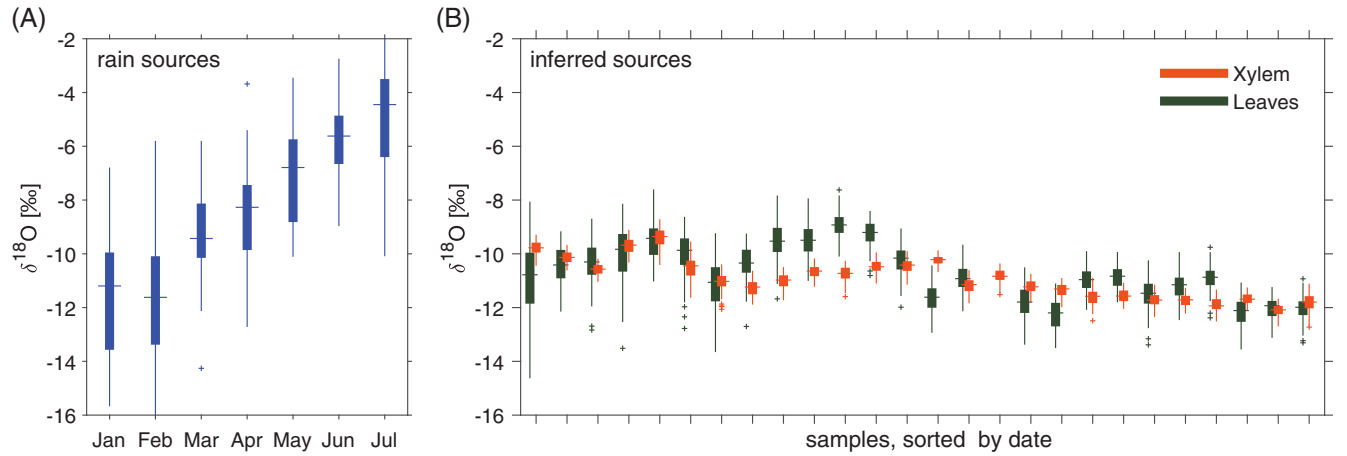

FIGURE 4 (a) Isotope composition of typical rainfall sources in the study area (data from Nyon station, IAEA/WMO GNIP dataset); (b) rainfall sources estimated from xylem and leaf samples collected in May–June 2018. The inferred origin is broadly consistent with the true sources (given by winter rainfall mixed with the labelled tracer irrigation) and the uncertainty is small compared to the yearly rainfall variability

uncertainty in the inferred source waters. In some cases, this could be a price that is worth paying. Despite the lower accuracy, there are situations where the collection of xylem samples can be problematic and the use of leaves and our approach would be advantageous. In agricultural and agroforestry systems, for example, stable isotopes have helped identify and quantify the relative contribution of different sources (rainfall, groundwater, irrigation) to plant uptake and to better understand the impact of different irrigation methods in crop development (Penna, Geris, Hopp, & Scandellari, 2020). For many crops like corn or vines, the collection of xylem samples typically compromises the plant's functionality. Our results suggest that higher-frequency and less-destructive sampling through leaves can be beneficial for our understanding of water source apportionment for such agricultural studies.

Given the practical advantages of collecting leaves, and the fact that they can map rainfall sources to a reasonable accuracy, we foresee potential for future water apportionment investigations. It is important to note that we do not suggest replacing xylem sampling in favour of leaf sampling. But we do think that leaves could be a low-effort, useful complement to xylem information. Heterogeneity in isotope ratios within an individual plant is known but rarely quantified (Goldsmith et al., 2019) because of the increased experimental effort and stress to the plant. Sampling leaves from multiple branches could be a way to improve representation of intra-crown isotopic variability. Finally, we envision at least two ways to move these preliminary results forward: (a) with experiments run under a range of controlled conditions to produce new data and test these proof of concept findings, and (b) by testing and developing more advanced theory for fractionation models that can improve the accuracy of the reconstructed water origin.

ACKNOWLEDGEMENTS

The authors thank Andrea Rinaldo and the ECHO laboratory at EPFL for hosting the lysimeter experiment that provided the data used in this study and Kim Janzen for assistance with isotopic analysis. PB thanks ENAC school at EPFL for financial support. The authors wish to thank Scott Allen, Daniele Penna and Charlotte Grossiord for useful discussion along the way, and Gabe Bowen and one reviewer for their constructive comments.

DATA AVAILABILITY STATEMENT

Data is available as part of the SPIKE II tracer experiment dataset (Nehemy et al., 2020) at doi.org/10.5281/zenodo.4037240.

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SUPPORTING INFORMATION

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

How to cite this article: Benettin P, Nehemy MF, Cernusak LA, Kahmen A, McDonnell JJ. On the use of leaf water to determine plant water source: A proof of concept. *Hydrological Processes*. 2021;35:e14073. https://doi.org/10.1002/hyp.14073