Received: 20 May 2022 | Revised: 1 September 2022 | Accepted: 6 September 2022

DOI: 10.1002/hyp.14698

SCIENTIFIC BRIEFING

HP TODAY WILEY

# On the urgent need for standardization in isotope-based ecohydrological investigations

Cody Millar[1] | Kim Janzen[1] | Magali F. Nehemy[1,2] | Geoff Koehler[3] | Pedro Hervé-Fernández[1,4] | Hongxiu Wang[1] | Natalie Orlowski[5] | Adrià Barbeta[6,7] | Jeffrey J. McDonnell[1,8]

[1]Global Institute for Water Security, University of Saskatchewan, Saskatoon, Canada

[2]Trent School of the Environment, Trent University, Peterborough, Canada

[3]NHRC Stable Isotope Laboratory, Environment and Climate Change Canada, Saskatoon, Canada

[4]Laboratorio de Monitoreo de Pastizales, Departamento de Ciencias Agropecuarias y Acuícolas, Facultad de Ciencias, Universidad de Magallanes, Punta Arenas, Chile

[5]Department of Hydrology, University of Freiburg, Freiburg im Breisgau, Germany

[6]INRAE, Bordeaux Sciences Agro, ISPA, Villenave d'Ornon, France

[7]BEECA, Universitat de Barcelona, Barcelona, Spain

[8]School of Geography, Earth & Environmental Sciences, University of Birmingham, Birmingham, UK

**Correspondence**
Cody Millar, Global Institute for Water Security, School of Environment and Sustainability, University of Saskatchewan, 11 Innovation Boulevard, Saskatoon, SK S7N 3H5, Canada.
Email: cody.millar@usask.ca

**Funding information**
Natural Sciences and Engineering Research Council of Canada

## Abstract

Ecohydrological investigations commonly use the stable isotopes of water (hydrogen and oxygen) as conservative ecosystem tracers. This approach requires accessing and analysing water from plant and soil matrices. Generally, there are six steps involved to retrieve hydrogen and oxygen isotope values from these matrices: (1) sampling, (2) sample storage and transport, (3) extraction, (4) pre-analysis processing, (5) isotopic analysis, and (6) post-processing and correction. At each step, cumulative errors can be introduced which sum to non-trivial magnitudes. These can impact subsequent interpretations about water cycling and partitioning through the soil–plant-atmosphere continuum. At each of these steps, there are multiple possible options to select from resulting in tens of thousands of possible combinations used by researchers to go from plant and soil samples to isotopic data. In a newly emerging field, so many options can create interpretive confusion and major issues with data comparability. This points to the need for development of shared standardized approaches. Here we critically examine the state of the process chain, reflecting on the issues associated with each step, and provide suggestions to move our community towards standardization. Assessing this shared 'process chain' will help us see the problem in its entirety and facilitate community action towards agreed upon standardized approaches.

**KEYWORDS**
ecohydrology, plant water extraction, plant water uptake, soil water extraction, stable isotopes of hydrogen and oxygen, standard operating procedure

## 1 | INTRODUCTION

Stable isotope values of hydrogen and oxygen ($\delta^2H$, $\delta^{18}O$, and less so $\delta^{17}O$) have become standard tools for investigating the water cycle (Barbeta et al., 2022; Cernusak et al., 2016; Halbritter et al., 2020; Jasechko et al., 2013; Sprenger, Tetzlaff, & Soulsby, 2017). But, unlike isotope hydrology investigations that rely simply on sampled liquid water, ecohydrological studies tracing plant and soil water require extraction of that water before isotope analysis. Reviews of the literature raise such questions as: What plant/ soil water extraction approaches and parameters were used (e.g., temperature, pressure, etc.)? What were the associated errors and extraction efficiencies? Were corrections for extraction biases or organic contamination made to the isotopic data, how were these corrections made, and are they reported? Are the methods (i.e., extraction approach) described in detail or just references to the literature? Were extracted samples tested for organic contamination and did this inform isotopic analysis approach selection? Answers to these questions are rarely obvious,

  

and more problematic, the methods we all use are anything but standardized.

Generally, there are six steps followed to retrieve stable isotope values from plant and soil matrices: (1) sampling, (2) sample storage and transport, (3) extraction, (4) pre-analysis processing, (5) isotopic analysis, and (6) post-processing and correction (collectively the 'process chain'). For steps 1–5 errors can be introduced through evaporative water loss. These cumulative errors may sum to ±24‰ for $\delta^2H$ and ±9.1‰ for $\delta^{18}O$ (Fischer et al., 2019). Such errors are not trivial when determining plant water sources nor in ecohydrological modelling, see Allen and Kirchner (2022). While in situ and in-line methods that reduce the number of steps are seeing increased use (Kühnhammer et al., 2022), destructive analyte extraction, specifically cryogenic vacuum distillation (CVD), is still the most commonly applied in plant ecohydrological research.

But for each discrete step, there are multiple possible options to select from resulting in tens of thousands of combinations. This can create interpretive confusion and major issues with data comparability, and points to the need for the development of standardized approaches.

Here we critically examine the state of the process chain used in ecohydrological investigations, reflecting on the issues associated with each step. We hope this might help us see the current problem in its entirety and facilitate community action towards development of common standardized approaches. This would allow future studies to be directly comparable, which is not possible now (excluding data generated from the same site under the same conditions). We suggest improvements for each step with a focus on community development of best practices and standardized approaches for isotopic ecohydrological investigations.

## 2 | THE ISSUES

For each process chain step (1–6) ecohydrologists select from multiple options, summarized below. We use a plus symbol (+) to indicate that a larger number of options may exist (classification dependent). We conservatively simplify to the fewest options for each process chain step.

For sampling, plant water sources are collected from soils, precipitation/irrigation, groundwater, streams, or other water pools available to plants (resulting in 5+ options). For sources of plant extracted analytes researchers collect leaves; root components (fine roots, taproots, etc.); stem cores containing heartwood, sapwood, inner bark, outer bark, or specific components; whole branches or twigs with or without inner and/ or outer bark; free flowing sap water; and vapour sampled in situ from vegetation (conservatively ~6+ options). Ecohydrologists often refer to the liquids collected from samples as *water*. However, the term analyte is more appropriate, especially in the case of plant, clay soil, and organic soil extracts as there are often organic compounds co-extracted with the water. Those compounds can cause errors during isotopic analyses (Brand et al., 2009; Martín-Gómez et al., 2015; Millar et al., 2021; West et al., 2010). Differentiation between the terms 'water' and 'analyte' is essential, as 'water' implies no co-extracted organics, leading to mistreatment further down the process chain. Even *pure water* is an *analyte*; but our point here is about using terminology that differentiates between pure and organic compound contaminated water. For pre-extraction and analysis sample storage, one could choose plastic bags; plastic or glass vials; or aluminium-laminated bags (4+ options). After collection, samples are transported to a laboratory, often undergoing further storage. During transport and storage samples could be in a frozen, cooled, or non-cooled setting (3 options).

Next the plant and soil matrix constrained analytes are accessed, typically via extraction. We are aware of eight common methods (8+ options): CVD (Jusserand, 1980; Koeniger et al., 2011; Orlowski et al., 2013), microwave in-line distillation (Munksgaard et al., 2014), chemical distillation approaches (Revesz & Woods, 1990), vapour equilibration (in-line) approaches (Hendry et al., 2011; Kulmatiski & Forero, 2021; Magh et al., 2022; Scrimgeour, 1995), centrifugation approaches (Barbeta et al., 2022; Peters & Yakir, 2008), high pressure mechanical squeezing (Bottcher et al., 1997), Scholander-type pressure chamber (plants only) (Geißler et al., 2019; Magh et al., 2020; Zuecco et al., 2022), suction or passive lysimeters (soil only) (Weihermüller et al., 2005, 2007). We exclude in situ approaches (Magh et al., 2022; Marshall et al., 2020; Rothfuss et al., 2013; Volkmann et al., 2016; Wang et al., 2012) as these collect, instead of extract, analytes via natural processes (i.e., plant internal vapours).

Before isotopic analysis, collected analytes can be processed to remove co-extracted organic compounds. Charcoal filters can adsorb contaminants (Martín-Gómez et al., 2015), in-line combustion modules burn off contaminants (Cui et al., 2021), or no pre-processing occurs (3 options). Options for isotopic analysis are isotope ratio mass spectrometry (IRMS) or isotope ratio infrared spectroscopy (IRIS). IRMS methods include high temperature thermal conversion, and $CO_2$ and $H_2$ equilibration, while IRIS methods (liquid or vapour modes) are off axis-integrated cavity output spectroscopy (OA-ICOS) and cavity ring down spectroscopy (CRDS) (6 options) (Epstein & Mayeda, 1953; Martín-Gómez et al., 2015; Morrison et al., 2001; West et al., 2010). During isotopic analysis, water standards are required to calibrate and validate generated data. The frequency of standard measurements during analyses affects the precision of the calibrated isotope values (Wassenaar et al., 2021). Subsequently, post-processing correction and/or contamination identification approaches can be applied to correct organic compound induced spectral contamination errors, surface fractionation, or other introduced biases (i.e., those potentially introduced by CVD) (Chen et al., 2020; Leen et al., 2012; Millar et al., 2021; Nehemy et al., 2019; Schmidt et al., 2010). Spectral contamination induced errors occur during IRIS analyses only (Brand et al., 2009; Martín-Gómez et al., 2015; Millar et al., 2021; West et al., 2010). Many kinds of corrections exist, so we simplify to the binary choice: applied correction(s) or no correction(s) (2 options).

To summarize, the number of possible option combinations to go from plant and soil samples to isotopic data is between 38 880 and

103 680 combinations! (Table 1). This exemplifies the urgent need for standardization, community discussion, and action. We now elaborate on the process chain issues to define and review the status quo, after which we outline possible routes towards standardization.

## 2.1 | Step 1: Sampling approaches

### 2.1.1 | Step 1a: Plant sampling

Sampling approach covers the parts of the plant/soil matrix sampled and how samples are processed. During plant sampling, several temporal and spatial considerations are made. Decisions about ecosystem-representative species can be difficult and may require local expertise. Various plant components can be selected and processed in multiple ways. One can choose from suberized or non-suberized twigs, or bark can be left on/removed. Cores can include xylem and phloem, or those components can be separated. Leaf veins can be separated from whole leaf tissue. Samples can be macerated or left intact. Duration of field sampling and local environmental conditions (temperature, humidity, etc.) can impact evaporation rates from samples.

When plant samples are collected, clipped edges will lose water through evaporation while other tissues keep transpiring. The latter affects the analyte's isotopic values through evaporative fractionation (Fischer et al., 2019). Analyte source choices (e.g., xylem only vs. whole tissue) can impact which pools of plant water we access, impacting our measured isotope values and subsequent interpretations (Zhao et al., 2016). Previous research showed significant $\delta^2H$ differences of up to 24‰ between xylem water and whole tissue water from roots, stem cores and twigs taken from *Populus euphratica* (Zhao et al., 2016). In other research, root, stem, leaf, and flower analytes showed differences of up to 51‰ for $\delta^2H$ and 14‰ for $\delta^{18}O$ in a herbaceous species (Millar et al., 2018). Barbeta et al. (2022) showed distinct differences between the $\delta^2H$ of whole stem samples and those extracted from xylem sap only. Furthermore, vegetation transpiration has daily and seasonal cycles. Thus, temporal sampling choices are important as these impact which plant water sources are reflected in extracted plant analytes (Nehemy et al., 2022).

Plants contain different cells and organs which participate in water and organic compound transport and storage. These pools of water (hydrogen and oxygen), which are not entirely separated and may exchange within vegetation, are the apoplastic 'xylem sap' water; the symplastic phloem water; the symplastic radial and axial parenchyma water; the intercellular 'capillary' water; and the 'fibre' or cell wall and organelle bound water (Barbeta et al., 2022). Xylem can contain actively flowing apoplastic sap and parenchyma and fibre cells that are not actively transporting water. Plants also contain organic compounds such as methanol, ethanol, carbohydrates, cellulose, terpenes, and so on, which themselves contain hydrogen and oxygen that might exchange with water in the plant at variable rates (Chen et al., 2020; Fogel & Cifuentes, 1993), in specific cells sites, and in the presence of specific enzymes (Gessler et al., 2013). The composition and amounts of these compounds change depending on the plant's phenological state. These various pools of plant bound hydrogen and oxygen can contribute to the isotopic values of the extracted analytes and are particularly relevant in cacti and other succulents given the way those species compartmentalize and constrain water (Hultine et al., 2019). There are currently large uncertainties and debates regarding how these plant-bound hydrogen and oxygen pools contribute to the analyte's overall isotopic composition during bulk extraction (i.e., with CVD, see step 3 below) (Barbeta et al., 2022; Zhao et al., 2016). The literature has shown that analytes held in different plant components can have different stable isotope values. We reiterate this to highlight the importance of sampling considerations on access to unique pools of plant matrix-constrained hydrogen and oxygen.

### 2.1.2 | Step 1b: Plant water source(s) sampling: soils, groundwater, streams, precipitation, irrigation

Plant water sources have fewer sampling options, relative to plant sampling options, given the nature of the material. Additionally, temporal sampling choices (snapshot vs. continuous in situ monitoring) affect how/if we detect changes in the soil water isotope values over time. The latter also relates to water uptake timing in plants, that is, how long does a change in the isotope values of vegetation water sources take to be reflected in that vegetation's water isotope signals? See Seeger and Weiler (2021) for discussion. As with plants there are isotopically unique pools of soil water (Bowers et al., 2020; Brooks, 2015; Brooks et al., 2010; Oerter & Bowen, 2017; Sprenger et al., 2015). For soils, the analyte extraction method determines which water pools are extracted from a given sample (Bowers et al., 2020). Extraction approaches for soil are mentioned here due to overlap between sampling considerations and choice of extraction method. Soil particle size and matric tensions partially partition soil water pools. Extraction approach access of these partitioned pools is addressed in Bowers et al. (2020), Oerter and Bowen (2017), Orlowski and Breuer (2020), Sprenger et al. (2015).

Typically, boreholes and pit excavation approaches are used when collecting soil samples near vegetation of interest. The number of samples and the ways in which they are grouped (i.e., arbitrary depth groupings) have implications for how hydrological source end members are defined. An original underpinning assumption of using stable isotope values to determine plant water sources is that 'The isotopic composition of the soil water is laterally homogeneous within the rooting area of the tree'. (Brunel et al., 1995), although the authors noted that was unlikely to be true. Modern research confirms that soil water isotope values are highly heterogeneous (Berry et al., 2017; Brodersen et al., 2000; Gaj, Kaufhold, Koeniger, et al., 2017; Oerter & Bowen, 2019; Sprenger, Leistert, et al., 2016; Sprenger, Seeger, et al., 2016; Sprenger, Tetzlaff, Tunaley, et al., 2017). Increasing the number of soil samples collected and the time frame over which sampling occurs could improve the resolution of soil stable isotope values; as could in situ continuous monitoring techniques. Still, both options

**TABLE 1** Summary of the isotope based ecohydrological process chain steps, options at each step, lower and upper bounds for the number of options at each step, and error contributions via water loss at each step (±X‰ VSMOW)

| Process chain step: | | (1) Sampling | (2) Storage and transport | (3) Extraction approaches | (4) Pre-analysis processing | (5) Isotopic analysis | (6) Post-processing correction | Total number of combinations (#) and total error contributions via water loss (‰) |
|---|---|---|---|---|---|---|---|---|
| Process chain options: | | *Water sources for plants*:<br>1. Precipitation<br>2. Soil water<br>3. Ground water<br>4. Streams<br>5. Other sources<br>*Plant analyte sources*:<br>1. Leaves<br>2. Root components<br>3. Stem core variants<br>4. Branch/twig variants<br>5. Free flowing sap water<br>6. In situ collected vapour | *Storage*:<br>1. Zip-seal plastic bags<br>2. Aluminium-plastic bags<br>3. Plastic vials<br>4. Glass vials<br>*Transport*:<br>1. Frozen (<0°C)<br>2. Cooled (~5°C)<br>3. Ambient temp (≥23°C)<br>Exposure to pressure changes? | 1. Lysimeter variants (soil only)<br>2. Scholander pressure chamber variants (plant only)<br>3. Centrifugation variants<br>4. HPMS<br>5. Chemical distillation variants<br>6. CVD variants<br>7. Microwave in-line distillation<br>8. Vapour in-line (and in situ variants | 1. Charcoal filtration<br>2. In-line combustion<br>3. No pre-processing | *IRMS*:<br>1. High temperature thermal conversion<br>2. $CO_2$ and $H_2$ equilibration<br>*IRIS*:<br>1. OA-ICOS liquid-mode<br>2. OA-ICOS vapour-mode<br>3. CRDS liquid-mode<br>4. 4CRDS vapour-mode | 1. Applied corrections[a]<br>2. No applied corrections | |
| Options (#): | Lower: | 5+, 3+ | 4+, 3+ | 6+ | 3 | 6 | 2 | 38 880 |
| | Upper | 5+, 6+ | 4+, 3+ | 8+ | 3 | 6 | 2 | 103 680 |
| Error contributions (via water loss): | $\delta^2H$: | ±10‰ | ±10‰ | ±3‰ | no data | ±1‰ | no data | 24‰ |
| | $\delta^{18}O$: | ±3‰ | ±3‰ | ±3‰ | no data | ±0.1‰ | no data | 9.1‰ |

*Note*: The lower bound for the number of options at each step uses only sampling and extraction approaches specifically applied to xylem analyte investigations. The upper bound includes all options. For the sampling approach lower bounds, we exclude leaves and in situ collected vapour (typically used for transpiration investigations), and root components. Recent research used leaf transpiration and vapour data to calculate xylem isotopic ratios. Root components may also be used as a xylem water source, typically only with herbaceous species. A plus symbol (+) is listed after the number of options to indicate that a larger number may exist depending on classification approach. Error contributions via water loss are from Fischer et al. (2019), which likely vary when comparing between different methods (i.e., CVD vs. HPMS, or sample storage media types). There is currently no complete direct comparison of those potential differences in the literature.

[a]There are many types of corrections and reasons to apply them. For simplicity we use the binary choice: correct or do not correct.

increase costs and technical expertise for sampling. Since in situ methods tend to non-destructively access plant/ soil constrained analytes, they could be considered within the sampling step. Recent research has assessed these techniques and their uncertainties (Kühnhammer et al., 2022).

## 2.2 | Step 2: Sample storage and transport

### 2.2.1 | Step 2a: Storage media choices can affect sample isotopic integrity

Sample storage considerations are rarely reported in published research, likely because it was assumed they did not impact isotopic results. However, these are important as samples will continue to evaporate within their storage containers. Thus, it is critical that storage containers do not leak. Storage media choices are plentiful, ranging from glass vials, plastic vials of various composition (e.g., high- and low-density polyethylene; HDPE and LDPE respectively), zip-sealable freezer bags by various manufacturers, and zip-sealed or heat-sealed aluminium laminated bags. Bags are typically used with vapour approaches while vials are used for CVD and others. Typical bag volumes are ~1 L with vials being between ~12 ml to 1+ L. Vials are sealed with plastic caps which are solid or contain plastic or rubber septa. Another issue is the temperatures used during sample transport and pre-extraction storage: are they frozen (<0°C), kept in a cool setting (~5°C), or kept at ambient room temperature (≥23°C)? Recent research has suggested a correction method for storage effects on sample isotopic compositions (Magh et al., 2022), but study specific storage correction coefficients should be developed and applied.

Storage media materials can affect sample stable isotope values. Isotopic fractionation effects can occur via diffusion across membranes (Chmielewski et al., 1991) and during storage in polymer vials (Böttcher & Schmiedinger, 2021; Spangenberg, 2012; Spangenberg & Vennemann, 2008). Research showed isotope fractionation up to 4‰ and 5‰ for $\delta^2H$ and 0.7‰ and 2‰ for $\delta^{18}O$, over a ~1-year storage period for water in polymer vials (Böttcher & Schmiedinger, 2021; Spangenberg, 2012; Spangenberg & Vennemann, 2008). These effects were connected to the container size, type of organic polymer, and wall thickness. Glass vials with polypropylene (PP) screw caps, thick-walled HDPE bottles, and perfluoroalkoxy-teflon (PFA) containers all preserved the original sample isotope values (Böttcher & Schmiedinger, 2021; Spangenberg, 2012). Other research assessed storage media choices and equilibration times for the direct vapour equilibration-IRIS approach (DVE-IRIS) (Gralher et al., 2021). They tested 10 different inflatable bags made of differing materials and volumes (0.8–2.5 L) for sample loss during equilibration time periods up to 71 days. They found significant water loss over time from plastic freezer bags while heat-sealed aluminium (Al) laminated bags were water-loss free. As illustration, we provide $O_2$ transmission rates through common storage media materials: LDPE, PET (Polyethyleneterephthalate) and PP plastics (commonly used in zip seal bags), as compared to Al-laminated plastics. At 23°C and 0% relative humidity 117 μm thick LDPE and PET coated papers showed $O_2$ permeation values of 768 and 78 $cm^3/m^2$/day, respectively. Al-laminated LDPE and Al-laminated PET papers had values of 277 and 18 $cm^3/m^2$/day, respectively (Johansson et al., 2019). Further, a 77 μm thick multi-layer Al-laminated plastic membrane (composed of PP-Al/ PET-Al/ LDPE) at 23°C and 50% relative humidity, had $O_2$ transmission rates of 0.07 $cm^3/m^2$/day (Schwab et al., 2016).

To highlight storage media material issues, Figure 1 shows new data from our pilot testing of the diffusion effects of sample containers used in the DVE-IRIS approach. Four storage media were tested: two kinds of zip-seal plastic bags (PB1 and PB2) (~1 L), aluminium lined mylar bags (Al-mylar) (~1 L), and glass canning jars (0.25 L). Four sample volumes were assessed: 2, 5, 10, and 20 ml ($n = 6$ per volume and container type). Results showed that both types of zip-seal plastic bags (PB1, PB2) are unacceptable containers for sample storage or equilibration greater than 24 h (Figure 1). For example, isotopic values for the PB1 24-h equilibration 10 ml samples had an absolute difference from the reference water of 3.67‰ ($\delta^2H$) and 1.23‰ ($\delta^{18}O$), exceeding our lab accepted error for vapour analyses (±2.2‰ for $\delta^2H$ and ±0.4‰ for $\delta^{18}O$). Both the Al-mylar bags and the glass jars showed acceptable results for all water volumes at 24-h equilibration time. After 5 days of equilibration the Al-mylar bags and glass jars outperformed the two zip-seal plastic bags at all water volumes. The Al-mylar bags had the smallest variability (±1.30‰ ($\delta^2H$) and ±0.2‰ ($\delta^{18}O$)) ($n = 24$) for all volumes at 5 days equilibration. The latter variabilities were below our accepted error for vapour analyses. These results confirm previous findings (Gralher et al., 2021) and demonstrates the perils of storing samples (especially low water volume samples) in zip-sealed plastic bags for even short periods. Clearly storage media choices matter.

### 2.2.2 | Step 2b: Temperature and pressure effects during storage and transport

Temperature and pressure variables are interrelated with the sampling step, and are not often reported (i.e., temperatures during suction lysimeter sampling). Microbial reproduction and respiration can continue during sample storage (depending on ambient conditions), until samples are placed in a state which limits these processes (e.g., cooled storage) (Fischer et al., 2019). Freezing or cooling samples during transport and storage is thought to mitigate temperature related fractionation effects, limit sample decomposition, limit generation of reducing environments, and reduce microbial activity (typically an issue in soils) which thereby limits $CO_2$ buildup (Fischer et al., 2019; Gralher et al., 2021). For IRIS analysers, changes in the gas matrix (i.e., caused by $CO_2$ build up), can affect measured isotopic values (Gralher et al., 2016, 2021). However, freezing samples may destroy soil microstructures (Gralher et al., 2021), and causes bursting of cell walls in plant samples (Fischer et al., 2019; Millar et al., 2018). Thawing of frozen samples stored in plastic bags can result in sample water loss up to 10%–20% (Fischer et al., 2019). Plant cell wall bursting could enhance the release of organic compounds and mixing of plant

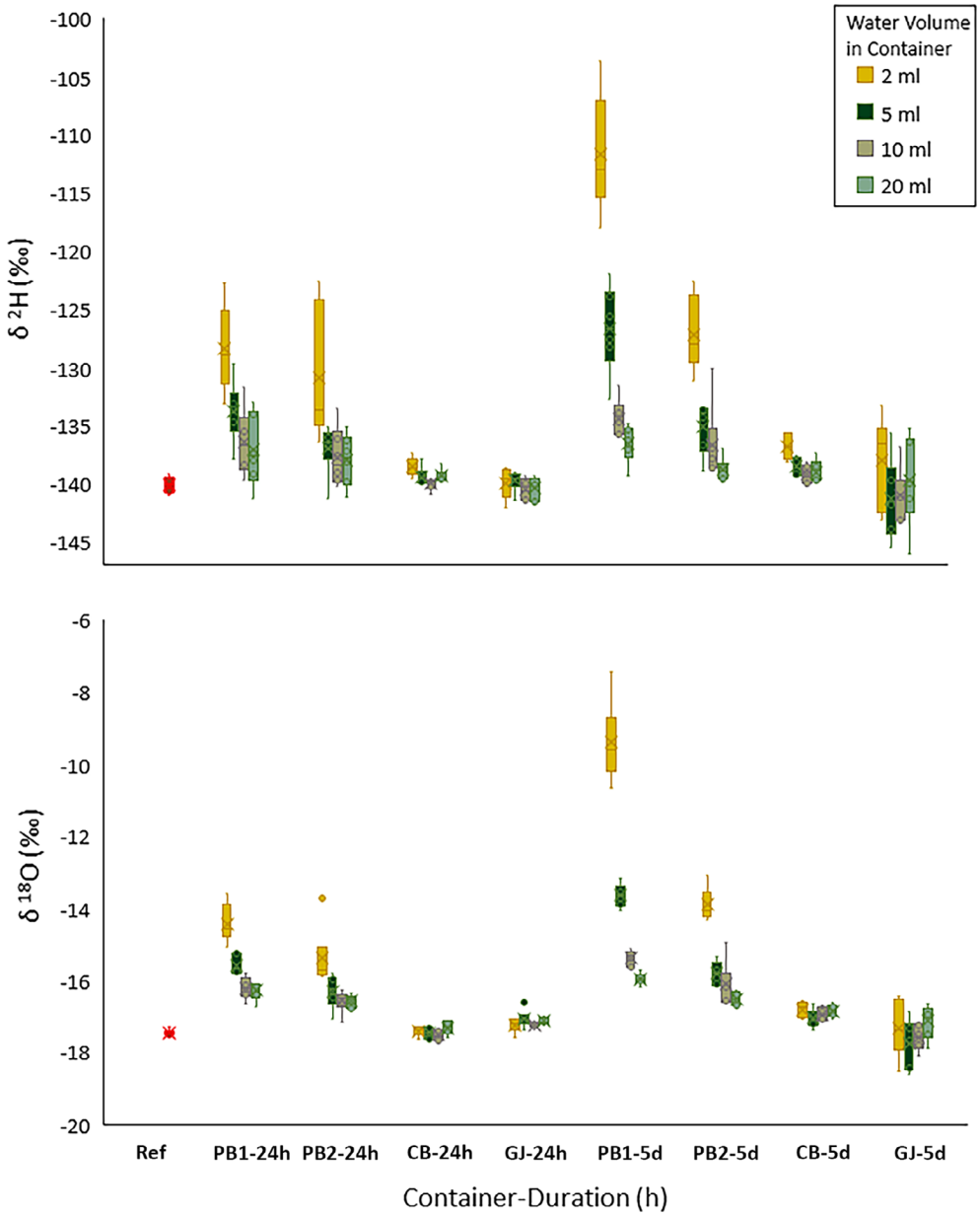


**FIGURE 1** Box plots of $\delta^2H$ and $\delta^{18}O$ values for water equilibrated in various storage media; measured via DVE-IRIS on a Los Gatos Research (LGR; Los Gatos research Inc., San Jose, CA, United States) OA-ICOS system. Tested storage media were: Zip-seal plastic freezer bag 1 (PB1), zip-seal plastic freezer bag 2 (PB2) both 1-L volumes; aluminium-lined mylar bags (CB) (1-L); and glass canning jars (GJ) (0.25-L). Each replicate was measured at equilibration times of 24 h and again at 5 days (5d). Sampling locations on each container were resealed between the two measurement events. Replicate isotopic values were compared against known references. reference water was the same source water used in preparation of all samples. The reference, analysed as liquid samples via OA-ICOS, had $\delta^2H$ of −140.56‰ (±0.7), and $\delta^{18}O$ of −17.44‰ (±0.09), $n = 6$. Our lab's 2-sigma uncertainties for liquid water analyses are 2‰ ($\delta^2H$), 0.8‰ ($\delta^{18}O$). For liquid water analyses our lab's reproducibility is ±1.0‰ ($\delta^2H$), ±0.2‰ ($\delta^{18}O$). Generally, our lab error for the DVE-IRIS method is greater with 2-sigma uncertainties of 4.0‰ ($\delta^2H$), 0.7‰ ($\delta^{18}O$); and a reproducibility of ±2.2 ($\delta^2H$), ±0.4 ($\delta^{18}O$), $n = 58$ for $\delta^2H$ and $\delta^{18}O$. All values are reported as parts per thousand (‰) according to the Vienna Standard Mean Ocean Water-Standard Light Antarctic Precipitation (VSMOW-SLAP) scales

bound hydrogen and oxygen pools within storage containers. For bulk plant analyte extraction methods (e.g., CVD), this *water pool mixing* may be less of an issue as the extraction step is thought to collect the bulk analyte isotopic composition anyways. This hypothesis requires further testing to confirm; however, with newer extraction methods aimed at collecting specific pools of plant analytes (Barbeta et al., 2022; Zuecco et al., 2022), potential mixing of plant water pools within storage media is a concern.

Samples transported by airplane can be exposed to conditions that could compromise storage media integrity. Samples could undergo freezing and thawing unless stored in temperature-controlled conditions; and are exposed to pressure changes that can result in

leakage. Additionally, research materials are commonly held up to months in customs under variable and uncontrolled conditions. Storage temperature may induce fractionation effects in water samples stored in LDPE containers (Böttcher & Schmiedinger, 2021). Over a 1.5-year storage period at temperatures of 4–10°C, they found no significant sample diffusion occurred through container walls, but at higher temperatures (23 and 60°C) diffusion resulted in hydrogen and oxygen fractionation. At 23°C isotope values increased by +0.5‰ ($\delta^2$H) +0.1‰ ($\delta^{17}$O), and +0.2‰ ($\delta^{18}$O), while at a 60°C storage temperature isotope values had increased by +28‰ ($\delta^2$H), +5‰ ($\delta^{17}$O), +10 ‰ ($\delta^{18}$O). Although, 60°C storage conditions are uncommon, this indicates the risks of storing samples at room or higher temperatures over long periods in plastic containers.

## 2.3 | Step 3: Extraction of analytes

The extraction step involves analyte removal from plant and soil samples. Beyond potential extraction induced errors (Chen et al., 2020; Fischer et al., 2019), we suggest that the greatest concern is the wide variety of extraction approaches being utilized and the lack of unified standard operating procedures (SOP) to guide their use (see Gralher et al., 2021; Orlowski et al., 2018). Research showed that significant differences were found in the isotopic composition of analytes extracted by various tested extraction methods (Barbeta et al., 2022; Kelln et al., 2001; Mennekes et al., 2021; Millar et al., 2018, 2019; Orlowski et al., 2018; Orlowski, Pratt, & McDonnell, 2016; Zuecco et al., 2022). See Table 2 for extraction method error ranges.

Even the well-established and commonly applied CVD approach (Koeniger et al., 2011; Orlowski, Pratt, & McDonnell, 2016; Song & Barbour, 2016) lacks a unified SOP and suffers from data incomparability (Orlowski et al., 2018). The lack of extraction method SOPs (for all methods) is troubling given the commonality of American Society for Testing and Materials (ASTM) and International Atomic Energy Agency (IAEA) standards for many laboratory procedures, and given the widespread use and cross comparison of data generated by these methods (e.g., Evaristo et al., 2015). Here we centre our discussion around the CVD method due to its common use. However, our statements could also be applied to other analyte extraction methods. CVD is one of the most extreme in terms of its extraction of metabolic process waters in addition to our typical water pool of interest: transpiration waters.

The physical characteristics of an extraction system can be unique between laboratories. CVD designs vary between large manifold style systems (Orlowski et al., 2013; West et al., 2006) to smaller single chamber systems (Koeniger et al., 2011). However, barring extreme cases like air leaks, these design differences are not as relevant to isotopic measurement outcomes as the extraction conditions are. CVD extraction conditions of concern are temperature, time, and pressure, Variations in which can affect isotopic results (MeiBner et al., 2014; Orlowski et al., 2013; Orlowski, Breuer, & McDonnell, 2016). Published extraction temperatures range between 80 and 200°C (Koeniger et al., 2011; Orlowski, Pratt, & McDonnell, 2016; West et al., 2006); extraction times are between 15 min (Koeniger et al., 2011; Millar et al., 2018) and 360 min (Mora & Jahren, 2003; Orlowski, Breuer, & McDonnell, 2016); and extraction pressures are between 0.13 and 13 Pa (Orlowski et al., 2013). CVD utilizes pressure and temperature changes to induce a phase change in the matrix bound water. Extraction of the lighter isotopes occurs first, followed by the heavier ones as extraction progresses.

Extraction temperatures affect the complete recovery of water from a given sample and incomplete recovery causes differences in the isotope values of extracted analytes (Kelln et al., 2001; Orlowski et al., 2018; Orlowski, Breuer, & McDonnell, 2016; Orlowski et al., 2013). CVD extraction times depend on the design of the system, and more importantly on sample size and water content. Extraction temperature and time effects on an analytes' isotopic results are interrelated with sample water contents. For instance, CVD $\delta^2$H extraction biases can be exacerbated by plant sample water contents (smaller effects with high water contents) (Chen et al., 2020). An incomplete extraction (<98%) results in an analyte whose isotopic composition is fractionated relative to the sample's original composition (Araguás-Araguás et al., 1995). While a variety of extraction conditions are published for CVD, the critical concern relating to analyte fractionation is extraction efficiency. This information is often not reported. Gravimetric extraction efficiency tests (see Hervé-Fernández et al., 2016) should be applied to all samples undergoing phase-change-driven analyte extraction. For those extraction approaches, any samples with extraction efficiency below 98% are considered failed extractions (Araguás-Araguás et al., 1995). This is a crucial check for data integrity.

Newly developed extraction methods are often validated against the CVD approach (Munksgaard et al., 2014; Peters & Yakir, 2008; Scrimgeour, 1995; Volkmann et al., 2016). However a worldwide inter-laboratory comparison of CVD systems showed data reproducibility issues between the participating laboratories with substantial differences in extracted analytes of +18.1‰ to −108.4‰ for $\delta^2$H and +11.8‰ to −14.9‰ for $\delta^{18}$O relative to a known reference (Orlowski et al., 2018). These differences were linked to soil type, water content, extraction efficiency, and laboratory internal accuracy.

Beyond the issue of CVD generated analyte's dissimilarity, another problem was that inter-lab differences were not linked to internal lab extraction conditions nor to the standardized extraction conditions set out in that trial (Orlowski et al., 2018). This implies that developing a unified SOP for CVD (or other extraction approaches) may be difficult. Orlowski et al. (2018) suggested labs could internally test their extraction systems against a variety of spiked soils with differing physicochemical properties to develop system specific transfer functions. However, discussion of the validity of soil spiking experiments pertaining to analyte extraction and analysis are ongoing since soil properties can impact analyte isotope composition (Gaj, Kaufhold, & McDonnell, 2017; Thielemann et al., 2019; Wen et al., 2021). One such issue is spike water retention via hydration of clay minerals and other soil cations (Gaj, Kaufhold, & McDonnell, 2017; Kelln et al., 2001; Lin et al., 2018; Lin & Horita, 2016; Oerter et al., 2014; Wen et al., 2021). Another is that soil carbonate induced biases in $\delta^{18}$O

**TABLE 2** Accuracies of various analyte extraction methods reported in the literature

| Extraction approaches | Analyte source material | Method accuracy as SD (‰ VSMOW) $\delta^2H$: | Method accuracy as SD (‰ VSMOW) $\delta^{18}O$: | Literature source |
|---|---|---|---|---|
| Scholander pressure chamber variants | Plant only | 3.1–6.3 (a) | 0.49–0.83 (a) | (a) Zuecco et al., 2022 |
| Centrifugation variants | Soil | 0.3 (b), 0.36–3.35 (c) | 0.025 (b), 0.01–0.23 (c) | (b) Sprenger et al., 2015, (c) Orlowski, Pratt, & McDonnell, 2016 |
| | Plant | 0.88–6.78 (d), (e), 0.27–2.25 (f) | 0.3–1.45 (d), 0.5 (e), 0.2–0.38, (f) | (d) Millar et al., 2018, (e) Peters & Yakir, 2008, (f) Barbeta et al., 2022 |
| HPMS | Soil | 0.27–2.51 (c) | 0.1–0.47 (c) | (c) Orlowski, Pratt, & McDonnell, 2016 |
| | Plant | 4.2–6.9 (d) | 0.58–0.95 (d) | (d) Millar et al., 2018 |
| Chemical distillation variants | Soil | 0.2–3 (b), 2 (g) | 0.2–0.5 (b), 0.2 (g) | (b) Sprenger et al., 2015, (g) Revesz and Woods (1990) |
| | Plant | 0.8–3.3 (k) | 0.15–0.66 (k) | (h) Walker and Richardson (1991) |
| CVD variants | Soil | 0.49–3 (b), 0.53–6.57 (c), 4.5–13.3 (i) | 0.1–0.4 (b), 0.09–0.88 (c), 0.7–2.3 (i) | (b) Sprenger et al., 2015, (c) Orlowski, Pratt, & McDonnell, 2016, (i) Orlowski et al., 2018 |
| | Plant | 3.7 (j), 0.86–3.24 (d) | 1.9 (j), 0.34–4.39 (d) | (j) Millar et al., 2019, (d) Millar et al., 2018 |
| Microwave in-line distillation | Soil | 1.47–13.71 (c), 2 (k) | 0.41–1.95 (c), 0.3 (k) | (c) Orlowski, Pratt, & McDonnell, 2016, (k) Munksgaard et al., 2014 |
| | Plant | 2 (k), 5.5–13.7 (d) | 0.3 (k), 1.3–2.9 (d) | (k) Munksgaard et al., 2014, (d) Millar et al., 2018 |
| Vapour in-line + in situ variants | Soil | 0.7–3.06 (b), 0.94–6.04 (c) | 0.2–0.46 (b), 0.41–1.43 (c) | (b) Sprenger et al., 2015, (c) Orlowski, Pratt, & McDonnell, 2016 |
| | Plant | 1.5–6.9 (f), 6.7 (j), 2.8 (l) | 0.64–3.41 (f), 1.2 (j), 0.33 (l) | (f) Millar et al., 2018, (j) Millar et al., 2019, (l) Volkmann et al., 2016 |

*Note*: Method accuracy is shown as the SD of isotopic data in ‰ VSMOW. In some case a method error *range* is provided given the effects of different variables in the studies. SD data for the Scholander pressure chamber were from four different tree species under unique land uses and climates (Zuecco et al., 2022). SD data from Orlowski, Pratt, and McDonnell (2016) were dependent on soil type and water content. SD data from Millar et al. (2018) were dependent upon plant part sampled from. SD ranges from Barbeta et al. (2022) were from different tree species sampled at different times during the season. SD data from Orlowski et al. (2018) were calculated from the standardized approach dataset. SD data from Barbeta et al. (2022), Orlowski et al. (2018), and Zuecco et al. (2022) were provided in personal correspondence.

values of extracted analytes (Meißner et al., 2014). Finally, non-equal equilibrium isotope fractionation was found between two systems commonly present in vapour analysis scenarios: the first being a silica-adsorbed soil pore water–water vapour system, and the second a bulk liquid water–water vapour system (Lin & Horita, 2016). The latter has implications for vapour methods (in situ, in-line, DVE-IRIS) used on soils rich in silica, alumina, and other clay minerals. The differences noted in Orlowski et al. (2018) could be related to spiking treatments, soil properties, or to some as of yet unknown biases (Allen & Kirchner, 2022).

A further issue is the lack of comparability between the various types of extraction approaches. Extraction method intercomparisons utilizing soils spiked with waters of known isotope values, natural bulk soils, and plants grown in controlled and natural conditions showed significant differences in the isotopic values of analytes extracted by the tested methods (Barbeta et al., 2020; Kelln et al., 2001; Kübert et al., 2020; Mennekes et al., 2021; Millar et al., 2018, 2019; Orlowski et al., 2019; Orlowski, Pratt, & McDonnell, 2016; Zuecco et al., 2022). In the case of soils, the research found some extraction approaches were closer to their 'truth value' than others or that isotope values of extracted analytes were statistically different between methods. However, analytes extracted from soils by different methods (such as suction lysimeters, squeezing or centrifugation) might also represent distinct soil water pools, relative to analytes obtained by CVD (Bowers et al., 2020; Sprenger et al., 2015). For plant analyte extraction method intercomparisons, where 'truth' (stable isotope reference) values were difficult to discern, the extraction systems produced results that were notably/statistically different from one another (Mennekes et al., 2021; Millar et al., 2018). However, other recent research showed that isotopically similar results were generated between CVD extraction and in situ analysis of plant analytes (Kühnhammer et al., 2022).

In situ plant vapour analyses techniques combined with IRIS analyses (Marshall et al., 2020; Volkmann et al., 2016) have shown promise at generating reliable isotopic data for plants. However, currently unresolved issues related to organic contamination driven spectral interference (Millar et al., 2021; Nehemy et al., 2019; Volkmann et al., 2016), which may be enhanced in vapour equilibration approaches, including in situ measurements (Millar et al., 2021), suggests such methods be used with caution. These vapour analyses may be more prone to organic compound driven spectral interference errors due to the potential for contaminants concentrating at higher levels in the vapour phase headspace (relative to their original concentrations in the liquid phase).

## 2.4 | Step 4, 5, and 6: Pre-processing, isotope analyses, and post analysis corrections

Isotopic analysis of plant and soil extracted analytes takes place on IRMS or IRIS systems. IRIS system advantages include lower operation costs, higher sample throughput, and generation of results as accurate as IRMS approaches for pure water (Schultz et al., 2011). Therefore IRIS systems have become widely adopted as analytical methods for hydrological and ecohydrological research. Between the 2016 and 2020 IAEA water stable isotope inter-comparisons (WICO), there was a 23% decline in submissions from laboratories using IRMS systems (Wassenaar et al., 2018, 2021). 68.9% of labs submitting data to WICO2020 used IRIS systems. In ecohydrology the shift from IRMS to IRIS systems increases the need to resolve spectral contamination driven error issues, and inter-laboratory data consistency for IRIS systems. Steps 4–6 are interrelated and thus discussed together here.

Pre-processing of analytes can be used to address the issue of co-extracted organic compounds. Spectral contamination induced errors during IRIS analyses of plant and soil extracted analytes is well-studied, the sources of which are understood (Brand et al., 2009; Martín-Gómez et al., 2015; West et al., 2010; Zhao et al., 2011). Techniques have been developed for removal of organic compounds before analysis such as pre-processing filtration (West et al., 2010), combustion (Gazquez et al., 2015; Martín-Gómez et al., 2015), or solid-phase extraction (SPE) (Chang et al., 2016). Filtration was less useful than combustion or SPE at removal of organic contaminants (Chang et al., 2016; West et al., 2010). While these techniques have utility they are not a catch-all; commercially available combustion techniques increase errors in ethanol contaminated samples due to the production of alcohol primary oxidation products (Chang et al., 2016). Of the two IRIS system manufacturers (Picarro and LGR), only Picarro makes a proprietary combustion device, whereas no such device currently exists from LGR.

If pre-processing options are not available, or effective, IRIS users have access to software that can detect spectral contamination *during* liquid-mode analyses (Leen et al., 2012; Schultz et al., 2011; West et al., 2011): LGR's Liquid Water Isotope Analyser-Spectral Contamination Identifier (LWIA-SCI) software, and Picarro's ChemCorrect organic interference identification software. The LWIA-SCI software allows for user modification of standard deviation (SD) settings which controls the flagging of contaminated samples. Settings modification by users can thus obscure contamination detection by this software. LGR recommends a SD setting of 3 and 5 for narrow band and broad band spectral contamination metrics, respectively, for proper and complete contamination detection (LGR, 2017). However, LGR's software currently only functions during liquid-mode analyses. Nevertheless, post analysis detection of organic contamination is possible for vapour-mode analyses via IRIS by utilizing $^{17}O$-excess values or a $CH_4$ metric (Kühnhammer et al., 2022; Millar et al., 2021; Nehemy et al., 2019). Contamination detection software usable during vapour-mode analyses is sorely needed given the enhanced risks that contaminants present specifically to vapour methods; see Millar et al. (2021).

While both methodologies (IRMS/IRIS) are well-established, 17% of participating laboratories in the WICO2020 report produced results that did not match to the known water sample isotope values (Wassenaar et al., 2021). Even more concerning, 49% of laboratories could not replicate results within their lab's stated error ranges for a set of blind duplicate samples! Promisingly, considering the proliferation of IRIS systems, the top performing laboratories for $\delta^2H$ and $\delta^{18}O$ categories utilized IRIS systems. However, in the $\delta^{17}O$ category IRMS methodologies outperform IRIS systems. All labs utilizing IRMS for

$\delta^{17}O$ analyses produced exceptional results, whereas only a few IRIS labs produced acceptable $\delta^{17}O$ results within the ±0.03‰ precisions required by WICO2020. Seventy percent of the WICO2020 IRIS participants could not replicate $\delta^{17}O$ data within their lab's stated error ranges for the blind duplicate samples (Wassenaar et al., 2021). To generate accurate $\delta^{17}O$ data from water with IRIS instruments, specialized procedures (high injection replication and comprehensive post processing) are needed to achieve the required precisions of ±0.01‰ (Berman et al., 2013; Wassenaar et al., 2021). However, utilizing $^{17}O$-excess values as a contaminant detection tool requires lower precisions (see Millar et al., 2021). Wassenaar et al. (2021) note the following issues regarding IRIS generated $\delta^{17}O$ data: (i) there is a lack of $^{17}O$-certified primary reference waters for standard calibration; VSMOW2 being the only one with a certified $\delta^{17}O$ value; (ii) new SOPs and post processing procedures are required to overcome IRIS memory and drift issues; (iii) SLAP2 (Standard Light Antarctic Precipitation 2) has multiple published $\delta^{17}O_{VSMOW}$ values (−29.2000‰ to −29.7090‰) which are used during data normalization (Wassenaar et al., 2021).

The choice of unique SLAP2 $\delta^{17}O_{VSMOW}$ normalization values affects the final $\delta^{17}O$ isotope value data. Following the WICO2020 findings we internally tested the commonly used SLAP2 $\delta^{17}O_{VSMOW}$ value of −29.6986‰ (83% of WICO2020 participants use this $\delta^{17}O$ value for SLAP2) and a less widely used SLAP2 $\delta^{17}O_{VSMOW}$ value of −29.2000‰. We re-analysed the WICO2020 sample OH27 using both SLAP2 $\delta^{17}O_{VSMOW}$ values during separate normalization procedures. We found that while using the more common SLAP2 $\delta^{17}O_{VSMOW}$ value of −29.6986‰, measured $\delta^{17}O$ values would fall within the WICO2020 acceptable ranges. But, when using the less common SLAP2 $\delta^{17}O_{VSMOW}$ value of −29.2000‰, measured $\delta^{17}O$ values were unacceptable. Therefore, research using $\delta^{17}O$ data should report which SLAP2 $\delta^{17}O_{VSMOW}$ normalization value is used to increase transparency and data comparability.

After isotopic analysis, $\delta^{2}H$, $\delta^{17}O$, and $\delta^{18}O$ data can undergo mathematical corrections and other post processing analyses (i.e., the $^{17}O$ contamination detection method). Corrections can be applied given detection of contamination, other analysis interferences such as 'surface fractionation' effects, or extraction induced biases (Chen et al., 2020; Gralher et al., 2016; Hendry et al., 2011; Leen et al., 2012; Martín-Gómez et al., 2015; Song et al., 2021). Song et al. (2021) explicitly state their correction approach was a test, and not a proposed method per se. Corrections have also been carried out on ecohydrological data (Barbeta et al., 2022; Duvert et al., 2021) concerning newly studied 'surface fractionation' effects (Chen et al., 2016). Research notes that organic contamination correction methods should be based on device-specific calibrations (Leen et al., 2012; Schultz et al., 2011). Allen and Kirchner (2022) note that there is no universal correction factor, and that development of soil type-specific and plant species-specific correction factors should be advanced due to phenological changes in the amounts and composition of organics in samples throughout the seasons (e.g., soil with/without litter layers, plants with high sugar contents).

Development of organic contamination correction approaches requires detailed knowledge of the contaminants present and their concentrations. Yet, few studies have published these analyte contaminant concentration data alongside isotopic data (see Millar et al., 2018 for one example). This may be due to increased sample requirements and costs involved in measuring contaminant concentrations in extracted analytes. Analyses such as proton transfer reaction-time of flight-mass spectrometry (PTR-ToF-MS) (Vivaldo et al., 2017) and gas chromatography-flame ionization detection (GC-FID) (Millar et al., 2018) can be utilized to determine organic compound concentrations in plant and soil extracted analytes. However, those are costly and require larger volumes of water than are used in typical isotope analyses (i.e., 1–4 ml), thereby requiring greater amounts of analyte source materials.

## 3 | A VISION FOR SOPs GUIDING ISOTOPE TRACING IN ECOHYDROLOGY

We hope that we have shown the staggering range of issues affecting stable isotope-based ecohydrological research. So now, what to do about it? It seems clear that the way forward is standardization. Indeed, a staple of the scientific method is reproducibility through the use of unified SOPs and methods. Standardization results in data being collected the same way by all and dramatically improves our confidence in the comparability of data sets. Best practices guidelines are common in science (see Bond & Hobson, 2012 or Szpak et al., 2017), as is terminology standardization (McMillan, 2022). But currently there are no SOPs guiding stable isotope-based ecohydrological investigations. The WATSON-COST initiative (https://watson-cost.eu/about/) is an internationally collaborative effort between critical zone researchers currently working to develop such standardized protocols for our shared process chain. To standardize our methods, we collectively need to reduce the number of process chain combinations. We suggest several options, amplifying voices of other researchers, that could lead our field towards standardization.

### 3.1 | Regarding sampling considerations

- There is a need for a deeper understanding of and transparency in reporting for sampling approaches. The parts of the plant we sample and how we do so can affect our isotopic data and thus subsequent sourcing and modelling interpretations. Our various research questions provide many reasons to sample different plant components (Halbritter et al., 2020). Therefore, we hesitate to suggest a standard approach as there is yet to be a community consensus. Data are currently limited regarding how various sampling approaches affects sample isotope composition. A too rigid unified SOP at this stage would not be helpful.
- As with WATSON-COST, we suggest that defining best practices may be a way forward. A starting point would involve a global survey that compiles and assesses best practices guiding plant and soil

sampling. Follow up experiments could test sampling technique effects on isotopic results. The WATSON-COST initiative and others are actively addressing this issue.

### 3.2 | For storage and transport

- Sample storage best practices should use glass vials with polymer screw caps (Böttcher & Schmiedinger, 2021; Spangenberg, 2012; Spangenberg & Vennemann, 2008), thick walled HDPE and PFA containers (Spangenberg, 2012) and/or Al-laminated bags (Gralher et al., 2016). Reviewers should demand these best practices during manuscript review.
- Specifically for users of vapour-bag methods, samples should be stored in heat-sealed Al-laminated bags of 1-L volume, utilizing equilibration times of no less than 2 days. Samples should contain ≥2-ml of water (Gralher et al., 2021). This last consideration requires pre sampling testing of collected materials to ensure appropriate water contents. The approach by Magh et al. (2022) shows promise for storage of unfractionated vapour samples.
- Samples should be stored as soon as possible in cooled conditions between 0 and 10°C to limit the destructive effects of freezing on soil microstructures and plant cell walls while simultaneously decreasing the risk of diffusive fractionation (Böttcher & Schmiedinger, 2021; Fischer et al., 2019; Gralher et al., 2016).

Agreement on and implementation of these practices appears straightforward.

### 3.3 | For analyte extraction

- There are currently unresolved issues with data reproducibility across extraction systems of the same type, (e.g., CVD; Orlowski et al., 2018) and across different extraction approaches (Barbeta et al., 2022; Millar et al., 2018; Orlowski et al., 2019; Orlowski, Pratt, & McDonnell, 2016; Zuecco et al., 2022). This is highly problematic for comparability and meta-analyses of isotopic data. Others state that it may not be possible to develop a unified SOP for a given extraction approach (Jiang et al., 2021; Orlowski et al., 2019). However, we suggest a unification of system designs could be required before attempted implementation of standardized extraction procedure. Much more research is urgently needed here.
- Allen and Kirchner (2022) suggest research is needed to fully define extraction system induced biases, and the interrelated biases induced by soil properties and specific plant species. Given the wide variety of system designs, soil property combinations, and plant species, this will take time. But we agree that it is urgently needed! As a first step, soil property analysis and plant phenological parameters should be reported alongside isotopic data.
- Orlowski et al. (2018) suggest that in-house testing of spiked soils with different properties may be helpful as a lab-based calibration approach. Corrections generated from these calibrations must also be reported for transparency. We acknowledge the current debate on soil-spiking experiments but advocate more research on this topic to inform future SOP discussions.
- At minimum, we advocate that all publications utilizing extractions report their extraction conditions, and more importantly, extraction efficiencies as a crucial internal check for data integrity and to increase transparency.
- Water-to-water extraction tests (as per Orlowski et al., 2013) should be carried out along all extraction systems capable of doing them as a proof of concept and reported in publications. This may not be possible for some extraction systems (i.e., centrifugation and HPMS), due to design constraints.
- Method intercomparisons suggest selecting approaches based on soil and plant water pools of interest. We emphasize that the various soil analyte extraction approaches may be accessing unique pools of water relative to those extracted with CVD (Bowers et al., 2020). For plant samples, the cavitron and Scholander pressure-bomb methodology shows promise for selective extraction of sap analytes (Barbeta et al., 2022; Zuecco et al., 2022). Those methods are dependent on the size of the sampled twig/branch and the resulting amount of analyte sampled. More research is needed to define extraction approach biases and their access to plant and soil water pools to inform future SOPs.
- A worldwide survey of laboratories' extraction system methodologies assessing extraction conditions and system design would be a helpful starting point for the development of standardized design, best practices documents, and finally, a unified SOP for individual extraction approaches.
- Vapour innovations such as in situ and in-line techniques can bypass some parts of the process chain. While currently costly and labour intensive, these techniques may be a way of simplifying the number of steps in these investigations. However, noted issues with these systems must be addressed first (see below).

### 3.4 | For isotope analysis and correction approaches

- Following on from the last point: due to enhanced issues with organic contamination during vapour analyses (Millar et al., 2021), these approaches require further refining before full adoption. Further research focused on addressing organic contaminant vapour phase concentration issues during vapour method analyses should be prioritized.
- Increased transparency and collaboration could be pursued with manufacturers of IRIS systems to better understand the mathematical fitting functions used by these systems to produce isotopic data and contamination flagging.
- Techniques like GC-FID and PTR-ToF-MS should be utilized to determine the types and quantities of organic contaminants

present in extracted analytes. Simplification of these techniques would decrease costs and significantly improve our ability to develop and report on contaminant-specific correction approaches. Regardless of costs these techniques should be utilized, and results reported in publications suggesting correction methods focused on organic compound contaminated analytes.

- Development of internationally certified water standards for $\delta^{17}O$ values is needed (Wassenaar et al., 2021) and improved SOPs and post-processing techniques should be advanced to enhance $\delta^{17}O$ measurement accuracy with IRIS systems. Until that is accomplished, researchers reporting $\delta^{17}O$ data should also report which SLAP2 values they used for $\delta^{17}O$ normalization (SLAP2 example in section 2.4). Labs using IRIS methods can calibrate their own reference water $^{17}O$ values (see Schoenemann et al., 2013).
- Accurate and transparent reporting of error propagation (Pierchala et al., 2019; Wassenaar et al., 2021), applied correction factors, and correction techniques should occur in any publications using corrections.
- A database of biases induced by specific plant species and soil properties could assist in development of a future internationally agreed upon set of correction factors (Allen & Kirchner, 2022).
- Finally, we suggest that developing correction approaches for isotopic data, while valuable and necessary, should proceed cautiously due to the large number of potential errors and contributing factors detailed in our process chain discussion. Specifically, corrections based on *surface fractionation* effects and *extraction induced biases* should be preceded by further research to better understand the mechanisms responsible for these effects. It may be that some of the errors introduced along the process chain will propagate in ways that impact correction approaches. The latter is not to say that the development of correction approaches should halt, but rather that these potential confounding problems should be accounted for beforehand or be folded into correction calculations and clearly reported in publications.

## 4 | SUMMARY

To achieve best practices in ecohydrology, unified SOPs for ecohydrological isotope investigations will be needed. This will require community willpower, which starts with seeing the problem in its entirety. This Briefing has attempted to outline the problem as a whole, detailing the confounding issues at each process chain step. We hope to spur debate, discussion, community response, and—most importantly—action! In the end, standardization will come from concerted community efforts, through cooperation to study and address the problems noted here, and through mutual efforts to increase transparency across isotope-based ecohydrological investigations.

## ACKNOWLEDGEMENTS

We thank our fellow researchers who have been involved with some of these local analyses: (Dave Schneider, Dyan Pratt, Marcel Gaj, Adriana Vega). We thank Giulia Zuecco for sharing data on the Scholander pressure chamber extraction approach and other useful conversations. We thank Scott Allen for discussions on field sampling practices for xylem. We also thank others for more general discussion of the issues along the way (Suzanne Jacobs, Christine Stumpp, Michael Stockinger, Benjamin Fischer, Daniele Penna, Maren Dubbert, Marco Lehmann, Christiane Werner, Matthias Beyer).

## DATA AVAILABILITY STATEMENT

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

## ORCID

*Cody Millar* https://orcid.org/0000-0002-2171-581X
*Magali F. Nehemy* https://orcid.org/0000-0002-2212-3592
*Natalie Orlowski* https://orcid.org/0000-0003-4368-4580

## REFERENCES

Allen, S. T., & Kirchner, J. W. (2022). Potential effects of cryogenic extraction biases on plant water source partitioning inferred from xylem-water isotope ratios. *Hydrological Processes*, *36*(2). https://doi.org/10.1002/hyp.14483

Araguás-Araguás, L., Rozanski, K., Gonfiantini, R., & Louvat, D. (1995). Isotope effects accompanying vacuum extraction of soil water for stable isotope analysis. *Journal of Hydrology*, *168*(1–4), 159–171. https://doi.org/10.1016/0022-1694(94)02636-P

Barbeta, A., Burlett, R., Martín-Gómez, P., Fréjaville, B., Devert, N., Wingate, L., Domec, J. C., & Ogée, J. (2022). Evidence for distinct isotopic compositions of sap and tissue water in tree stems: Consequences for plant water source identification. *The New Phytologist*, *233*, 1121–1132. https://doi.org/10.1111/nph.17857

Barbeta, A., Gimeno, T. E., Clavé, L., Fréjaville, B., Jones, S. P., Delvigne, C., Wingate, L., & Ogée, J. (2020). An explanation for the isotopic offset between soil and stem water in a temperate tree species. *The New Phytologist*, *227*(3), 766–779. https://doi.org/10.1111/nph.16564

Berman, E. S., Levin, N. E., Landais, A., Li, S., & Owano, T. (2013). Measurement of delta18O, delta17O, and 17O-excess in water by off-axis integrated cavity output spectroscopy and isotope ratio mass spectrometry. *Analytical Chemistry*, *85*(21), 10392–10398. https://doi.org/10.1021/ac402366t

Berry, Z. C., Evaristo, J., Moore, G., Poca, M., Steppe, K., Verrot, L., Asbjornsen, H., Borma, L. S., Bretfeld, M., Hervé-Fernández, P., Seyfried, M., Schwendenmann, L., Sinacore, K., de Wispelaere, L., & McDonnell, J. (2017). The two water worlds hypothesis: Addressing multiple working hypotheses and proposing a way forward. *Ecohydrology*, *11*, e1843. https://doi.org/10.1002/eco.1843

Bond, A. L., & Hobson, K. (2012). Reporting stable-isotope ratios in ecology: Recommended terminology, guidelines and best practices. *Waterbirds*, *35*(2), 324–331.

Bottcher, G., Brumsack, H. J., Heinrichs, H., & Pohlmann, M. (1997). A new high pressure squeezing technique for pore fluid extraction from terrestrial soils. *Water, Air, & Soil Pollution*, *94*(3), 289–296. https://doi.org/10.1023/A:1026499319006

Böttcher, M., & Schmiedinger, I. (2021). The impact of temperature on the water isotope (2H/1H, 17O/16O, 18O/16O) fractionation upon transport through a low-density polyethylene membrane. *Isotopes in Environmental and Health Studies*, *57*(2), 183–192. https://doi.org/10.1080/10256016.2020.1845668

Bowers, W. H., Mercer, J. J., Pleasants, M. S., & Williams, D. G. (2020). A combination of soil water extraction methods quantifies the isotopic mixing of waters held at separate tensions in soil. *Hydrology and Earth System Sciences*, *24*(8), 4045–4060. https://doi.org/10.5194/hess-24-4045-2020

Brand, W. A., Geilmann, H., Crosson, E. R., & Rella, C. W. (2009). Cavity ring-down spectroscopy versus high-temperature conversion isotope ratio mass spectrometry; a case study on $\delta^2H$ and $\delta^{18}O$ of pure water samples and alcohol/water mixtures. *Rapid Communications in Mass Spectrometry*, *23*(12), 1879–1884. https://doi.org/10.1002/rcm.4083

Brodersen, C., Pohl, S., Lindenlaub, M., Leibundgut, C., & von Wilpert, K. (2000). Influence of vegetation structure on isotope content of throughfall and soil water. *Hydrological Processes*, *14*(8), 1439–1448. https://doi.org/10.1002/1099-1085(20000615)14:8<1439::AID-HYP985>3.0.CO;2-3

Brooks, J. (2015). Hydrology. Water, bound and mobile. *Science*, *349*(6244), 138–139. https://doi.org/10.1126/science.aac4742

Brooks, J., Barnard, H., Coulombe, R., & McDonnell, J. (2010). Ecohydrologic separation of water between trees and streams in a Mediterranean climate. *Nature Geoscience*, *3*(2), 100–104. https://doi.org/10.1038/ngeo722

Brunel, J. P., Walker, G. R., & Kennett-Smith, A. K. (1995). Field validation of isotopic procedures for determining sources of water used by plants in a semi-arid environment. *Jounal of Hydrology*, *167*(1–4), 351–368. https://doi.org/10.1016/0022-1694(94)02575-V

Cernusak, L. A., Barbour, M. M., Arndt, S. K., Cheesman, A. W., English, N. B., Feild, T. S., Helliker, B. R., Holloway-Phillips, M. M., Holtum, J. A. M., Kahmen, A., McInerney, F. A., Munksgaard, N. C., Simonin, K. A., Song, X., Stuart-Williams, H., West, J. B., & Farquhar, G. D. (2016). Stable isotopes in leaf water of terrestrial plants. *Plant, Cell & Environment*, *39*(5), 1087–1102. https://doi.org/10.1111/pce.12703

Chang, E., Wolf, A., Gerlein-Safdi, C., & Caylor, K. K. (2016). Improved removal of volatile organic compounds for laser-based spectroscopy of water isotopes. *Rapid Communications in Mass Spectrometry*, *30*(6), 784–790. https://doi.org/10.1002/rcm.7497

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

Chen, Y., Helliker, B., Tang, X., Li, F., Zhou, Y., & Song, X. (2020). Stem water cryogenic extraction biases estimation in deuterium isotope composition of plant source water. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(52), 33345–33350. https://doi.org/10.1073/pnas.2014422117

Chmielewski, A. G., Zakrzewska-Trznadel, G., Miljevic, N. R., & Van Hook, W. A. (1991). 16O/18O and HD seperation factors for liquid-vapour permation of water through an hydrophobic membrane. *Journal of Membrane Science*, *60*, 319–329. https://doi.org/10.1016/S0376-7388(00)81543-4

Cui, J., Lide, T., & Yu, W. (2021). Organic contamination in online laser-based plant stem and leaf water isotope measurements for pre-extracted samples. *Isotopes in Environmental and Health Studies*, *57*(3), 262–270. https://doi.org/10.1080/10256016.2021.1883010

Duvert, C., Canham, C. A., Barbeta, A., Cortes, D. A., Chandler, L., Harford, A. J., Leggett, A., Setterfield, S. A., Humphrey, C. L., & Hutley, L. B. (2021). Deuterium depletion in xylem water and soil isotopic effects complicate the assessment of riparian tree water sources in the seasonal tropics. *Ecohydrology*, *15*. https://doi.org/10.1002/eco.2383

Epstein, S., & Mayeda, T. (1953). Variation of O18 content of waters from natural sources. *Geochimica et Cosmochimica Acta*, *4*(5), 213–224. https://doi.org/10.1016/0016-7037(53)90051-9

Evaristo, J., Jasechko, S., & McDonnell, J. J. (2015). Global separation of plant transpiration from groundwater and streamflow. *Nature*, *525*(7567), 91–94. https://doi.org/10.1038/nature14983

Fischer, B. M. C., Frentress, J., Manzoni, S., Cousins, S. A. O., Hugelius, G., Greger, M., Smittenberg, R. H., & Lyon, S. W. (2019). Mojito, anyone? An exploration of low-tech plant water extraction methods for isotopic analysis using locally-sourced materials. *Frontiers in Earth Science*, *7*, 1–11. https://doi.org/10.3389/feart.2019.00150

Fogel, M. L., & Cifuentes, L. A. (1993). Isotope fractionation during primary production. In M. H. Engel & S. A. Macko (Eds.), *Organic geochemistry Topics in Geobiology* (Vol. 11). Springer.

Gaj, M., Kaufhold, S., Koeniger, P., Beyer, M., Weiler, M., & Himmelsbach, T. (2017). Mineral mediated isotope fractionation of soil water. *Rapid Communications in Mass Spectrometry*, *31*(3), 269–280. https://doi.org/10.1002/rcm.7787

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

Gazquez, F., Mather, I., Rolfe, J., Evans, N. P., Herwartz, D., Staubwasser, M., & Hodell, D. A. (2015). Simultaneous analysis of (17) O/(16) O, (18) O/(16) O and (2) H/(1) H of gypsum hydration water by cavity ring-down laser spectroscopy. *Rapid Communications in Mass Spectrometry*, *29*(21), 1997–2006. https://doi.org/10.1002/rcm.7312

Geißler, K., Heblack, J., Uugulu, S., Wanke, H., & Blaum, N. (2019). Partitioning of water between differently sized shrubs and potential groundwater recharge in a semiarid savanna in Namibia. *Frontiers in Plant Science*, *10*, 10. https://doi.org/10.3389/fpls.2019.01411

Gessler, A., Brandes, E., Keitel, C., Boda, S., Kayler, Z. E., Granier, A., Barbour, M., Farquhar, G. D., & Treydte, K. (2013). The oxygen isotope enrichment of leaf-exported assimilates—Does it always reflect lamina leaf water enrichment? *The New Phytologist*, *200*(1), 144–157. https://doi.org/10.1111/nph.12359

Gralher, B., Herbstritt, B., & Weiler, M. (2021). Technical note: Unresolved aspects of the direct vapor equilibration method for stable isotope analysis ($\delta^{18}O$, $\delta^2H$) of matrix-bound water: Unifying protocols through empirical and mathematical scrutiny. *Hydrology and Earth System Sciences*, *25*(9), 5219–5235. https://doi.org/10.5194/hess-25-5219-2021

Gralher, B., Herbstritt, B., Weiler, M., Wassenaar, L. I., & Stumpp, C. (2016). Correcting laser-based water stable isotope readings biased by carrier gas changes. *Environmental Science & Technology*, *50*(13), 7074–7081. https://doi.org/10.1021/acs.est.6b01124

Halbritter, A. H., De Boeck, H. J., Eycott, A. E., Reinsch, S., Robinson, D. A., Vicca, S., Berauer, B., Christiansen, C. T., Estiarte, M., Grünzweig, J. M., Gya, R., Hansen, K., Jentsch, A., Lee, H., Linder, S., Marshall, J., Peñuelas, J., Schmidt, I. K., Stuart-Haëntjens, E., … Vandvik, V. (2020). The handbook for standardized field and laboratory measurements in terrestrial climate change experiments and observational studies (ClimEx). *Methods in Ecology and Evolution*, *11*(1), 22–37. https://doi.org/10.1111/2041-210X.13331

Hendry, M. J., Richman, B., & Wassenaar, L. I. (2011). Correcting for methane interferences on $\delta^2H$ and $\delta^{18}O$ measurements in pore water using $H_2O$ liquid-$H_2O$ vapor equilibration laser spectroscopy. *Analytical Chemistry*, *83*(14), 5789–5796. https://doi.org/10.1021/ac201341p

Hervé-Fernández, P., Oyarzún, C., Brumbt, C., Huygens, D., Bodé, S., Verhoest, N. E. C., & Boeckx, P. (2016). Assessing the 'two water worlds' hypothesis and water sources for native and exotic evergreen species in south-Central Chile. *Hydrological Processes*, *30*(23), 4227–4241. https://doi.org/10.1002/hyp.10984

Hultine, K. R., Dettman, D. L., English, N. B., & Williams, D. G. (2019). Giant cacti: Isotopic recorders of climate variation in warm deserts of the Americas. *Journal of Experimental Botany*, *70*(22), 6509–6519. https://doi.org/10.1093/jxb/erz320

Jasechko, S., Sharp, Z. D., Gibson, J. J., Birks, S. J., Yi, Y., & Fawcett, P. J. (2013). Terrestrial water fluxes dominated by transpiration. *Nature*, *496*(7445), 347–350. https://doi.org/10.1038/nature11983

Jiang, S., Rao, W., & Han, L. (2021). Determining the stable isotope composition of porewater using low temperature multi-step extraction for low water content soils. *Journal of Hydrology*, *596*, 126079. https://doi.org/10.1016/j.jhydrol.2021.126079

Johansson, P., Lahti, J., Vihinen, J., & Kuusipalo, J. (2019). Permeability of oxygen and carbon dioxide through pinholes in barrier coatings. *Journal of Applied Packaging Research*, *11*(2), 4.

Jusserand, C. (1980). Extraction de l'eau interstitielle des sédiments et des sols—Comparaison des valeurs de l'oxygène 18 par différentes méthodes, premiers résultats. *Catena*, *7*(1), 87–96. https://doi.org/10.1016/S0341-8162(80)80006-3

Kelln, C. J., Wassenaar, L. I., & Hendry, M. J. (2001). Stable isotopes ($\delta^{18}O$, $\delta^{2}H$) of pore waters in clay-rich aquitards: A comparison and evaluation of measurement techniques. *Groundwater Monitoring and Remediation*, *21*(2), 108–116. https://doi.org/10.1111/j.1745-6592.2001.tb00306.x

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

Kübert, A., Paulus, S., Dahlmann, A., Werner, C., Rothfuss, Y., Orlowski, N., & Dubbert, M. (2020). Water stable isotopes in ecohydrological field research: Comparison between in situ and destructive monitoring methods to determine soil water isotopic signatures. *Frontiers in Plant Science*, *11*, 387. https://doi.org/10.3389/fpls.2020.00387

Kühnhammer, K., Dahlmann, A., Iraheta, A., Gerchow, M., Birkel, C., Marshall, J. D., & Beyer, M. (2022). Continuous in situ measurements of water stable isotopes in soils, tree trunk and root xylem: Field approval. *Rapid Communications in Mass Spectrometry*, *36*(5), e9232. https://doi.org/10.1002/rcm.9232

Kulmatiski, A., & Forero, L. E. (2021). Bagging: A cheaper, faster, non-destructive transpiration water sampling method for tracer studies. *Plant and Soil*, *462*(1–2), 603–611. https://doi.org/10.1007/s11104-021-04844-w

Leen, J. B., Berman, E. S. F., Liebson, L., & Gupta, M. (2012). Spectral contaminant identifier for off-axis integrated cavity output spectroscopy measurements of liquid water isotopes. *The Review of Scientific Instruments*, *83*(4), 044305. https://doi.org/10.1063/1.4704843

LGR. (2017). LWIA post analysis software user manual.

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

Lin, Y., Horita, J., & Abe, O. (2018). Adsorption isotope effects of water on mesoporous silica and alumina with implications for the land-vegetation-atmosphere system. *Geochimica et Cosmochimica Acta*, *223*, 520–536. https://doi.org/10.1016/j.gca.2017.12.021

Magh, R.-K., Eiferle, C., Burzlaff, T., Dannenmann, M., Rennenberg, H., & Dubbert, M. (2020). Competition for water rather than facilitation in mixed beech-fir forests after drying-wetting cycle. *Journal of Hydrology*, *587*, 124944. https://doi.org/10.1016/j.jhydrol.2020.124944

Magh, R.-K., Gralher, B., Herbstritt, B., Kübert, A., Lim, H., Lundmark, T., & Marshall, J. (2022). Technical note: Conservative storage of water vapour—Practical in situ sampling of stable isotopes in tree stems. *Hydrology and Earth System Sciences*, *26*(13), 3573–3587. https://doi.org/10.5194/hess-26-3573-2022

Marshall, J. D., Cuntz, M., Beyer, M., Dubbert, M., & Kühnhammer, K. (2020). Borehole equilibration: Testing a new method to monitor the isotopic composition of tree xylem water in situ. *Frontiers in Plant Science*, *11*, 358. https://doi.org/10.3389/fpls.2020.00358

Martín-Gómez, P., Barbeta, A., Voltas, J., Peñuelas, J., Dennis, K., Palacio, S., Dawson, T. E., & Ferrio, J. P. (2015). Isotope-ratio infrared spectroscopy: A reliable tool for the investigation of plant-water sources? *The New Phytologist*, *207*(3), 914–927. https://doi.org/10.1111/nph.13376

McMillan, H. (2022). A taxonomy of hydrological processes and watershed function. *Hydrological Processes*, *36*(3). https://doi.org/10.1002/hyp.14537

MeiBner, M., Kohler, M., Schwendenmann, L., Holscher, D., & Dycksmans, J. (2014). Soil water uptake by trees using water stable isotopes ($\delta^{2}H$ and $\delta^{18}O$)—A method test regarding soil moisture, texture and carbonate. *Plant and Soil*, *376*(1), 327–335. https://doi.org/10.1007/s11104-013-1970-z

Meißner, M., Köhler, M., Schwendenmann, L., Hölscher, D., & Dyckmans, J. (2014). Soil water uptake by trees using water stable isotopes ($\delta^{2}H$ and $\delta^{18}O$)—A method test regarding soil moisture, texture and carbonate. *Plant and Soil*, *376*(1), 327–335. https://doi.org/10.1007/s11104-013-1970-z

Mennekes, D., Rinderer, M., Seeger, S., & Orlowski, N. (2021). Ecohydrological travel times derived from in situ stable water isotope measurements in trees during a semi-controlled pot experiment. *Hydrology and Earth System Sciences*, *25*(8), 4513–4530. https://doi.org/10.5194/hess-25-4513-2021

Millar, C., Janzen, K., Nehemy, M. F., Koehler, G., Hervé-Fernández, P., & McDonnell, J. J. (2021). Organic contamination detection for isotopic analysis of water by laser spectroscopy. *Rapid Communications in Mass Spectrometry*, *35*, e9118. https://doi.org/10.1002/rcm.9118

Millar, C., Pratt, D., Schneider, D. J., Koehler, G., & McDonnell, J. J. (2019). Further experiments comparing direct vapor equilibration and cryogenic vacuum distillation for plant water stable isotope analysis. *Rapid Communications in Mass Spectrometry*, *33*, 1850–1854. https://doi.org/10.1002/rcm.8530

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

Mora, G., & Jahren, A. H. (2003). Isotopic evidence for the role of plant development on transpiration in deciduous forests of southern United States. *Global Biogeochemical Cycles*, *17*(2). https://doi.org/10.1029/2002gb001981

Morrison, J., Brockwell, T., Merren, T., Fourel, F., & Phillips, A. M. (2001). On-line high precision stable hydrogen isotopic analyses on nanoliter water samples. *Analytical Chemistry*, *73*(15), 3570–3575. https://doi.org/10.1021/ac001447t

Munksgaard, N. C., Cheesman, A. W., Wurster, C. M., Cernusak, L. A., & Bird, M. I. (2014). Microwave extraction-isotope ratio infrared spectroscopy (ME-IRIS): A novel technique for rapid extraction and in-line analysis of delta18O and delta2H values of water in plants, soils and insects. *Rapid Communications in Mass Spectrometry*, *28*(20), 2151–2161. https://doi.org/10.1002/rcm.7005

Nehemy, M., Maillet, J., Perron, N., Pappas, C., Sonnentag, O., Baltzer, J., Laroque, C. P., & McDonnell, J. (2022). Snowmelt water use at transpiration onset: Phenology, isotope tracing and tree water transit time. *Water Resources Research*, *58*. https://doi.org/10.1029/2022WR032344

Nehemy, M., Millar, C., Janzen, K., Gaj, M., Pratt, D., Laroque, C., & McDonnell, J. (2019). 17O- excess as a detector for co-extracted organics in vapor analyses of plant isotope signatures. *Rapid Communications in Mass Spectrometry*, *33*, 1301–1310. https://doi.org/10.1002/rcm.8470

Oerter, E., & Bowen, G. (2017). In situ monitoring of H and O stable isotopes in soil water reveals ecohydrologic dynamics in managed soil systems. *Ecohydrology*, *10*(4), e1841. https://doi.org/10.1002/eco.1841

Oerter, E., & Bowen, G. (2019). Spatio-temporal heterogeneity in soil water stable isotopic composition and its ecohydrologic implications in semiarid ecosystems. *Hydrological Processes*, *33*(12), 1724–1738. https://doi.org/10.1002/hyp.13434

Oerter, E., Finstad, K., Schaefer, J., Goldsmith, G., Dawson, T., & Amundson, R. (2014). Oxygen isotope fractionation effects in soil water via interaction with cations (Mg, Ca, K, Na) adsorbed to phyllosilicate clay minerals. *Journal of Hydrology*, *515*, 1–9. https://doi.org/10.1016/j.jhydrol.2014.04.029

Orlowski, N., & Breuer, L. (2020). Sampling soil water along the pF curve for $\delta^2$H and $\delta^{18}$O analysis. *Hydrological Processes*, *34*(25), 4959–4972. https://doi.org/10.1002/hyp.13916

Orlowski, N., Breuer, L., Angeli, N., Boeckx, P., Brumbt, C., Cook, C., Dubbert, M., Dyckmans, J., Gallagher, B., Gralher, B., Herbstritt, B., Hervé-Fernández, P., Hissler, C., Koeniger, P., Legout, A., Macdonald, C. J., Oyarzún, C., Redelstein, R., Seidler, C., … McDonnell, J. J. (2018). Inter-laboratory comparison of cryogenic water extraction systems for stable isotope analysis of soil water. *Hydrology and Earth System Sciences Discussions*, *22*, 1–36. https://doi.org/10.5194/hess-2018-128

Orlowski, N., Breuer, L., & McDonnell, J. J. (2016). Critical issues with cryogenic extraction of soil water for stable isotope analysis. *Ecohydrology*, *9*(1), 1–5. https://doi.org/10.1002/eco.1722

Orlowski, N., Frede, H. G., Brüggemann, N., & Breuer, L. (2013). Validation and application of a cryogenic vacuum extraction system for soil and plant water extraction for isotope analysis. *Journal of Sensors*, *2*(2), 179–193. https://doi.org/10.5194/jsss-2-179-2013

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

Orlowski, N., Pratt, D. L., & McDonnell, J. J. (2019). Intercomparison of soil pore water extraction methods for stable isotope analysis and interpretation of hillslope runoff sources. *Hydrological Processes*, *33*(22), 2939–2954. https://doi.org/10.1002/hyp.13539

Peters, L. I., & Yakir, D. (2008). A direct and rapid leaf water extraction method for isotopic analysis. *Rapid Communications in Mass Spectrometry*, *22*(18), 2929–2936. https://doi.org/10.1002/rcm.3692

Pierchala, A., Rozanski, K., Dulinski, M., Gorczyca, Z., Marzec, M., & Czub, R. (2019). High-precision measurements of $\delta^2$H, $\delta^{18}$O and $\delta^{17}$O in water with the aid of cavity ring-down laser spectroscopy. *Isotopes in Environmental and Health Studies*, *55*(3), 290–307. https://doi.org/10.1080/10256016.2019.1609959

Revesz, K., & Woods, P. H. (1990). A method to extract soil water for stable isotope analysis. *Journal of Hydrology*, *115*(1–4), 397–406. https://doi.org/10.1016/0022-1694(90)90217-L

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

Schmidt, M., Maseyk, K., Lett, C., Biron, P., Richard, P., Bariac, T., & Seibt, U. (2010). Concentration effects on laser-based $\delta^{18}$O and $\delta^2$H measurements and implications for the calibration of vapour measurements with liquid standards. *Rapid Communications in Mass Spectrometry*, *24*(24), 3553–3561. https://doi.org/10.1002/rcm.4813

Schoenemann, S. W., Schauer, A. J., & Steig, E. J. (2013). Measurement of SLAP2 and GISP delta17O and proposed VSMOW-SLAP normalization for delta17O and 17O(excess). *Rapid Communications in Mass Spectrometry*, *27*(5), 582–590. https://doi.org/10.1002/rcm.6486

Schultz, N. M., Griffis, T. J., Lee, X., & Baker, J. M. (2011). Identification and correction of spectral contamination in 2H/1H and 18O/16O measured in leaf, stem, and soil water. *Rapid Communications in Mass Spectrometry*, *25*(21), 3360–3368. https://doi.org/10.1002/rcm.5236

Schwab, H., Heinemann, U., Beck, A., Ebert, H.-P., & Fricke, J. (2016). Permeation of different gases through foils used as envelopes for vacuum insulation panels. *Journal of Thermal Envelope and Building Science*, *28*(4), 293–317. https://doi.org/10.1177/1097196305051791

Scrimgeour, C. M. (1995). Measurement of plant and soil water isotope composition by direct equilibration methods. *Journal of Hydrology*, *172*(1–4), 261–274. https://doi.org/10.1016/0022-1694(95)02716-3

Seeger, S., & Weiler, M. (2021). Temporal dynamics of tree xylem water isotopes: In situ monitoring and modelling. *Biogeosciences*, *18*, 4603–4627. https://doi.org/10.5194/bg-18-4603-2021

Song, X., & Barbour, M. M. (2016). Leaf water oxygen isotope measurement by direct equilibration. *The New Phytologist*, *211*(3), 1120–1128. https://doi.org/10.1111/nph.13962

Song, X., Chen, Y., Helliker, B. R., Tang, X., Li, F., & Zhou, Y. (2021). Reply to Evaristo et al.: Strong evidence for the need of correcting extraction bias in an early study of ecohydrological separation. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(17). https://doi.org/10.1073/pnas.2103604118

Spangenberg, J. (2012). Caution on the storage of waters and aqueous solutions in plastic containers for hydrogen and oxygen stable isotope analysis. *Rapid Communications in Mass Spectrometry*, *26*(22), 2627–2636. https://doi.org/10.1002/rcm.6386

Spangenberg, J., & Vennemann, T. (2008). The stable hydrogen and oxygen isotope variation of water stored in polyethylene terephthalate (PET) bottles. *Rapid Communications in Mass Spectrometry*, *22*(5), 672–676. https://doi.org/10.1002/rcm.3415

Sprenger, M., Herbstritt, B., & Weiler, M. (2015). Established methods and new opportunities for pore water stable isotope analysis. *Hydrological Processes*, *29*(25), 5174–5192. https://doi.org/10.1002/hyp.10643

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

Sprenger, M., Seeger, S., Blume, T., & Weiler, M. (2016). Travel times in the vadose zone: Variability in space and time. *Water Resources Research*, *52*(8), 5727–5754. https://doi.org/10.1002/2015WR018077

Sprenger, M., Tetzlaff, D., & Soulsby, C. (2017). Soil water stable isotopes reveal evaporation dynamics at the soil–plant–atmosphere interface of the critical zone. *Hydrology and Earth System Sciences*, *21*(7), 3839–3858. https://doi.org/10.5194/hess-21-3839-2017

Sprenger, M., Tetzlaff, D., Tunaley, C., Dick, J., & Soulsby, C. (2017). Evaporation fractionation in a peatland drainage network affects stream water isotope composition. *Water Resources Research*, *53*(1), 851–866. https://doi.org/10.1002/2016wr019258

Szpak, P., Metcalfe, J. Z., & Macdonald, R. A. (2017). Best practices for calibrating and reporting stable isotope measurements in archaeology. *Journal of Archaeological Science: Reports*, *13*, 609–616. https://doi.org/10.1016/j.jasrep.2017.05.007

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

Vivaldo, G., Masi, E., Taiti, C., Caldarelli, G., & Mancuso, S. (2017). The network of plants volatile organic compounds. *Scientific Reports*, *7*(1), 11050. https://doi.org/10.1038/s41598-017-10975-x

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

Walker, C. D., & Richardson, S. B. (1991). The use of stable isotopes of water in characterising the source of water in vegetation. *Chemical Geology*, *94*(2), 145–158.

Wang, L., Good, S. P., Caylor, K. K., & Cernusak, L. A. (2012). Direct quantification of leaf transpiration isotopic composition. *Agricultural and Forest Meteorology*, *154-155*, 127–135. https://doi.org/10.1016/j.agrformet.2011.10.018

Wassenaar, L. I., Terzer-Wassmuth, S., & Douence, C. (2021). Progress and challenges in dual- and triple-isotope ($\delta^{18}$O, $\delta^2$H, $\Delta^{17}$O) analyses of environmental waters: An international assessment of laboratory performance. *Rapid Communications in Mass Spectrometry*, *35*, e9193. https://doi.org/10.1002/rcm.9193

Wassenaar, L. I., Terzer-Wassmuth, S., Douence, C., Araguas-Araguas, L., Aggarwal, P. K., & Coplen, T. B. (2018). Seeking excellence: An evaluation of 235 international laboratories conducting water isotope

analyses by isotope-ratio and laser-absorption spectrometry. *Rapid Communications in Mass Spectrometry*, *32*(5), 393–406. https://doi.org/10.1002/rcm.8052

Weihermüller, L., Kasteel, R., Vanderborght, J., Pütz, T., & Vereecken, H. (2005). Soil water extraction with a suction cup. *Vadose Zone Journal*, *4*(4), 889–907. https://doi.org/10.2136/vzj2004.0156

Weihermüller, L., Siemens, J., Deurer, M., Knoblauch, S., Rupp, H., Göttlein, A., & Pütz, T. (2007). In situ soil water extraction: A review. *Journal of Environmental Quality*, *36*(6), 1735–1748. https://doi.org/10.2134/jeq2007.0218

Wen, M., Si, B., Lu, Y., & Wang, H. (2021). Water recovery rate and isotopic signature of cryogenic vacuum extracted spiked soil water following oven-drying at different temperatures. *Hydrological Processes*, *35*. https://doi.org/10.1002/hyp.14248

West, A., Goldsmith, G., Brooks, P., & Dawson, T. (2010). Discrepancies between isotope ratio infrared spectroscopy and isotope ratio mass spectrometry for the stable isotope analysis of plant and soil waters. *Rapid Communications in Mass Spectrometry*, *24*(14), 1948–1954. https://doi.org/10.1002/rcm.4597

West, A., Goldsmith, G., Matimati, I., & Dawson, T. (2011). Spectral analysis software improves confidence in plant and soil water stable isotope analyses performed by isotope ratio infrared spectroscopy (IRIS). *Rapid Communications in Mass Spectrometry*, *25*(16), 2268–2274. https://doi.org/10.1002/rcm.5126

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

Zhao, L., Wang, L., Cernusak, L. A., Liu, X., Xiao, H., Zhou, M., & Zhang, S. (2016). Significant difference in hydrogen isotope composition between xylem and tissue water in *Populus euphratica*. *Plant, Cell & Environment*, *39*(8), 1848–1857. https://doi.org/10.1111/pce.12753

Zhao, L., Xiao, H., Zhou, J., Wang, L., Cheng, G., Zhou, M., Yin, L., & McCabe, M. F. (2011). Detailed assessment of isotope ratio infrared spectroscopy and isotope ratio mass spectrometry for the stable isotope analysis of plant and soil waters. *Rapid Communications in Mass Spectrometry*, *25*(20), 3071–3082. https://doi.org/10.1002/rcm.5204

Zuecco, G., Amin, A., Frentress, J., Engel, M., Marchina, C., Anfodillo, T., Borga, M., Carraro, V., Scandellari, F., Tagliavini, M., Zanotelli, D., Comiti, F., & Penna, D. (2022). A comparative study of plant water extraction methods for isotopic analyses: Scholander-type pressure chamber vs. cryogenic vacuum distillation. *Hydrology and Earth System Sciences*, *26*(13), 3673–3689. https://doi.org/10.5194/hess-26-3673-2022

**How to cite this article:** Millar, C., Janzen, K., Nehemy, M. F., Koehler, G., Hervé-Fernández, P., Wang, H., Orlowski, N., Barbeta, A., & McDonnell, J. J. (2022). On the urgent need for standardization in isotope-based ecohydrological investigations. *Hydrological Processes*, *36*(10), e14698. https://doi.org/10.1002/hyp.14698