

Potential Effects of Cryogenic Extraction Biases on Plant Water Source Partitioning Inferred from Xylem Water Isotope Ratios

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1 INTRODUCTION

Recent studies show that cryogenic extraction artifacts may bias determinations of $\delta^{18}\text{O}$
 and $\delta^2\text{H}$ isotope ratios of plant and soil waters (Barbeta et al., 2020; Chen et al., 2020; Fischer et
 al., 2019; Gaj & McDonnell, 2019; Newberry et al., 2017; Orlowski, Breuer, et al., 2018;
 Orlowski, Winkler, et al., 2018). Such insights are important for interpreting the resulting data
 and for developing correction factors to compensate for extraction biases. But do these
 extraction biases invalidate the use of xylem-water isotopes to infer plant water sources, as some
 (e.g., Chen et al., 2020) have argued?

Our view is less absolute. All analytical work entails uncertainties and biases; the key
 question is whether they are large or small compared to the environmental signals we seek to
 detect. Uncertainties and biases that are small compared to the signals of interest will have small
 effects on the inferences drawn from them. Conversely, uncertainties and biases that are not
 small compared to the signals of interest can fundamentally alter the inferences we draw. In this
 contribution, we (a) show how biases and uncertainties propagate differently through isotope
 mixing model calculations, (b) briefly review the current understanding of soil and xylem water
 cryogenic extraction biases, and (c) illustrate, using hypothetical calculations based on several
 published studies, the range of potential effects that xylem water cryogenic extraction biases may
 have.

2 PROPAGATION OF BIASES AND RANDOM UNCERTAINTIES IN END-MEMBER MIXING

Random uncertainties and systematic biases are different and need to be handled differently. Random uncertainties, whether they arise from random sampling variability or random measurement errors, can be handled by conventional error propagation, with standard errors shrinking by the square root of the sample size because random errors tend to average out. Systematic biases, by contrast, do not average out because they do not vary randomly from sample to sample (so more sampling will not help). If they are well enough constrained, they can simply be subtracted from the measured values. But in practice the magnitude of the bias itself will usually be uncertain, and that uncertainty must also be taken into account. Formulas and software are widely available for propagating random uncertainties through mixing models (Genereux, 1998; Kirchner & Allen, 2020; Phillips et al., 2005; Phillips & Gregg, 2001; Rothfuss & Javaux, 2017), but no such guidance has been available for quantifying the effects of (potentially uncertain) extraction biases.

Consider a mixing model that partitions a xylem-water sample M (with an isotope value of δ_M) among two end-members A and B with isotope values of δ_A and δ_B , respectively. The fractional contribution f_A from end-member A can be inferred from the conventional mixing equation,

$$f_A = \frac{\delta_M - \delta_B}{\delta_A - \delta_B} \quad (1)$$

To understand how bias and uncertainty in δ_M , δ_A , and δ_B propagate to create bias and uncertainty in f_A , it is useful to define its partial derivatives, which approximate the dependence of f_A on δ_M , δ_A , and δ_B :

$$\begin{aligned} \frac{\partial f_A}{\partial \delta_M} &= \frac{1}{\delta_A - \delta_B} \\ \frac{\partial f_A}{\partial \delta_A} &= -\frac{\delta_M - \delta_B}{(\delta_A - \delta_B)^2} = \frac{-f_A}{\delta_A - \delta_B} \\ \frac{\partial f_A}{\partial \delta_B} &= \frac{\delta_M - \delta_A}{(\delta_A - \delta_B)^2} = \frac{f_A - 1}{\delta_A - \delta_B} = \frac{-f_B}{\delta_A - \delta_B} \end{aligned} \quad (2)$$

2.1 Bias propagation

Any bias in δ_M , δ_A , and δ_B will bias f_A proportionally to the corresponding partial derivatives:

$$\text{BIAS}(f_A) \approx \frac{\partial f_A}{\partial \delta_M} \text{BIAS}(\delta_M) + \frac{\partial f_A}{\partial \delta_A} \text{BIAS}(\delta_A) + \frac{\partial f_A}{\partial \delta_B} \text{BIAS}(\delta_B) \quad , \quad (3)$$

or, using the expressions from Eq. (2):

$$\text{BIAS}(f_A) \approx \frac{\text{BIAS}(\delta_M)}{\delta_A - \delta_B} + \frac{-f_A \text{BIAS}(\delta_A)}{\delta_A - \delta_B} + \frac{(f_A - 1)\text{BIAS}(\delta_B)}{\delta_A - \delta_B} \quad , \quad (4)$$

or equivalently

$$\text{BIAS}(f_A) \approx \frac{\text{BIAS}(\delta_M)}{\delta_A - \delta_B} - \frac{\text{BIAS}(\delta_B)}{\delta_A - \delta_B} + f_A \frac{\text{BIAS}(\delta_B) - \text{BIAS}(\delta_A)}{\delta_A - \delta_B} \quad . \quad (5)$$

Note that the biases in Eqs. (3-5) are signed quantities. These equations are not exact because the partial derivatives are linear approximations to the nonlinear dependence of f_A on δ_A and δ_B .

In the special case that the two end-members share the same bias, Eqs. (3-5) take on the particularly simple form

$$\text{BIAS}(f_A) = \frac{\text{BIAS}(\delta_M) - \text{BIAS}(\delta_{AB})}{\delta_A - \delta_B} \quad , \quad (6)$$

where $\text{BIAS}(\delta_{AB})$ denotes the bias in both δ_A and δ_B . Unlike Eqs. (3-5), Eq. (6) is exact, because the nonlinearities in the dependence of f_A on δ_A and δ_B cancel one another out. Equation (6) is also exact in a second sense: whereas Eqs. (3-5) are expressed in terms of the un-biased values of δ_M , δ_A , and δ_B (which will only be known if the biases are known), if the biases in δ_A and δ_B are equal, the right-hand side of Eq. (6) will be the same for either biased or unbiased values of δ_A and δ_B . The form of Eq. (6) also simplifies correcting for known biases: the bias-corrected value of f_A is simply the biased value, minus the differences $\text{BIAS}(\delta_M) - \text{BIAS}(\delta_{AB})$ divided by $\delta_A - \delta_B$. Equations (4-6) demonstrate that the potential importance of a bias depends on its magnitude relative to the difference between δ_A and δ_B .

2.2 Uncertainty propagation

First-order, second-moment error propagation yields the following formula for the standard error (SE) of the mixing fraction f_A :

$$\text{SE}(f_A) \approx \sqrt{\sum_{\substack{i=A,B,M \\ j=A,B,M}} \frac{\partial f_A}{\partial \delta_i} \frac{\partial f_A}{\partial \delta_j} \rho_{\delta_i, \delta_j} \text{SE}(\delta_i) \text{SE}(\delta_j)} \quad , \quad (7)$$

79 which uses partial derivatives to linearly approximate the nonlinear dependence of f_A on δ_A and
80 δ_B . In the special case that all of the cross-correlations $\rho_{\delta_i, \delta_j}$ among the uncertainties are zero for
81 $i \neq j$, Eq. (7) reduces to the well-known Gaussian error propagation formula (Gauss, 1823):

$$82 \quad SE(f_A) \approx \sqrt{\left(\frac{\partial f_A}{\partial \delta_M} SE(\delta_M)\right)^2 + \left(\frac{\partial f_A}{\partial \delta_A} SE(\delta_A)\right)^2 + \left(\frac{\partial f_A}{\partial \delta_B} SE(\delta_B)\right)^2} \quad (8)$$

83 Uncertainty in the tracer concentrations δ_i ($i = A, B, M$) may arise not only from random sampling
84 variability and measurement error, but also from uncertainty in extraction-bias magnitudes. The
85 extraction bias will be an imprecisely known constant, and thus its uncertainty will not be reduced
86 by increasing n . Assuming that the sampling error and measurement variability are uncorrelated
87 with the extraction bias (which must true if the bias is constant), their combined effects can be
88 expressed as

$$89 \quad SE(\delta_i) = \sqrt{\frac{\text{var}(\delta_i)}{n_i} + SE_{\text{BIAS}}(\delta_i)^2} \quad , \quad (9)$$

90 where $SE_{\text{BIAS}}(\delta_i)$ denotes the uncertainty in the bias in the measurements of δ_i , and $\text{var}(\delta_i)$ and
91 n_i denote the variances and numbers of samples and measurements used to estimate the mean δ_i .
92 Combining Eqs. (8), (9), and (2), we obtain:

$$93 \quad SE(f_A) \approx \frac{1}{|\delta_A - \delta_B|} \sqrt{\frac{\text{var}(\delta_M)}{n_M} + f_A^2 \frac{\text{var}(\delta_A)}{n_A} + (1 - f_A)^2 \frac{\text{var}(\delta_B)}{n_B} + SE_{\text{BIAS}}(\delta_M)^2 + f_A^2 SE_{\text{BIAS}}(\delta_A)^2 + (1 - f_A)^2 SE_{\text{BIAS}}(\delta_B)^2} \quad (10)$$

94 In the special case that the bias in the two end-members is the same, the uncertainty in f_A must be
95 estimated from Eq. (7) instead of Eq. (8) (because the terms $SE_{\text{BIAS}}(\delta_A)$ and $SE_{\text{BIAS}}(\delta_B)$ will be
96 perfectly correlated, instead of uncorrelated as assumed in Eq. 8), yielding:

$$97 \quad SE(f_A) \approx \frac{1}{|\delta_A - \delta_B|} \sqrt{\frac{\text{var}(\delta_M)}{n_M} + f_A^2 \frac{\text{var}(\delta_A)}{n_A} + (1 - f_A)^2 \frac{\text{var}(\delta_B)}{n_B} + SE_{\text{BIAS}}(\delta_M)^2 + SE_{\text{BIAS}}(\delta_{AB})^2} \quad , \quad (11)$$

98 where $SE_{\text{BIAS}}(\delta_{AB})^2$ denotes the uncertainty in the bias in both δ_A and δ_B . In the further special
99 case that the measurement bias in both end-members and the mixture is the same, the bias terms
100 disappear completely, because the value of f_A in Eq. (1) remains unchanged. Equations (10) and

(11) again demonstrate that the impacts of uncertainties depend on their magnitudes relative to the difference between δ_A and δ_B .

Readers should note that $SE_{BIAS}(\delta_i)$ should also be added (in quadrature) to conventional standard errors for many other types of analyses, such as t-tests comparing xylem waters and groundwaters or comparing xylem waters among species. Readers should also note that the major uncertainties in $BIAS(\delta_M)$, $BIAS(\delta_A)$, and $BIAS(\delta_B)$ may arise from uncertainty in whether the experiments used to estimate these biases are relevant to the particular situations where those estimates are employed. This highlights the importance of the ongoing efforts to determine how extraction biases vary inter-specifically, intra-specifically, and among different extraction procedures.

3 CRYOGENIC EXTRACTION BIASES IN SOILWATER AND PLANT-WATER ISOTOPE RATIOS

Among five recent studies that quantified xylem-water extraction biases (Barbeta et al., 2020; Chen et al., 2020; Newberry et al., 2017; Poca et al., 2019; L. Zhao et al., 2016), all but one (Newberry et al., 2017) found that extracted xylem water was significantly lower than its source in δ^2H . Another key study, Ellsworth and Williams (2007), found δ^2H extraction biases ranging from -9.2 to -0.8 in 16 species. Together, the mean extraction bias reported in these six studies (representing 30 species) was -6.1 ‰ δ^2H , with a standard deviation of 3.4 ‰ among by-species values (Goldsmith and Allen, 2021). The origins of species-to-species differences and difference among extraction procedures remain unclear (Song et al., 2021; Y. Zhao, 2021). Consequently, correction factors for specific groups of samples will remain uncertain for the foreseeable future, even in species for which bias magnitudes have previously been reported. We suggest that it is reasonable to adopt the across-study average of -6.1 ± 3.4 ‰ as a general indication of the likely bias in cryogenically extracted xylem-water δ^2H , but any bias-correction factor must be used cautiously.

In contrast to δ^2H , cryogenic extraction biases in xylem-water $\delta^{18}O$ are often unmeasurably small. Of the six previously discussed studies that quantified differences between xylem-water and source-water δ^2H , only Poca et al. (2019) found significant $\delta^{18}O$ differences, in

only a subset of their samples. The mean bias value (\pm standard deviation) across all species included in these past studies was -0.3 ± 0.5 ‰ $\delta^{18}\text{O}$.

Soilwater cryogenic-extraction biases vary substantially among different soils and extraction procedures. In the most extensive evaluation to date, 18 laboratories applied a reference water to oven-dried soils (Orlowski 2018). By digitizing Figure 3 in that study, we estimated the mean biases (\pm standard deviations) to be approximately -12 ± 8 , -10 ± 5 , -5 ± 4 , and -4 ± 3 ‰ $\delta^2\text{H}$, respectively for the 8%-water-content clayey loam, 20%-water-content clayey loam, 8%-water-content silty sand, and 20%-water-content silty sand; waters extracted from coarser, wetter soils were less clearly biased. Orlowski et al. reported smaller biases in $\delta^{18}\text{O}$, averaging -1.1 ± 2.8 , -0.4 ± 1.0 , -0.5 ± 0.6 , and -0.4 ± 0.4 ‰, respectively, for the four treatments listed above. Thielemann et al. (2019) offer a countering perspective: rewetting experiments, such as that used by Orlowski et al. (2018), are undermined by residual water left behind by the oven-drying procedure, but extraction still introduces biases because cryogenic extraction is not 100% efficient. Regardless, the considerable inter-laboratory variability in Orlowski et al. (2018) should not be ignored because it reflects uncertainty due to unknown bias. Given the inconsistent sign and magnitude of soil-water extraction biases, we suggest that until they are better quantified, they should be treated as having a mean of 0 and a standard error approximately ± 6 ‰ for $\delta^2\text{H}$ and ± 1.5 ‰ for $\delta^{18}\text{O}$ (the standard deviations across all by-study mean values in Orlowski et al., 2018), but again, we advise that such values be used cautiously (because, for example, carbonates in soils can introduce biases of 1.3 ‰ $\delta^{18}\text{O}$; Meißner et al., 2014).

4 ILLUSTRATIONS OF POTENTIAL EFFECTS OF EXTRACTION BIASES

Here, we illustrate how cryogenic extraction biases could potentially have affected four studies that exhibit a range of sensitivities to biases in the xylem water $\delta^2\text{H}$ measurements that were to identify plant water sources. As a rough correction for the presumed extraction bias, we increased the xylem-water $\delta^2\text{H}$ values reported in these previous studies by $+6.1 \pm 3.4$ ‰ (see Section 3) for purposes of illustration. We stress that the actual bias for any given species and any given laboratory's extraction procedures could differ substantially from this illustrative number. We simplified the original data and analyses since we had insufficient information to fully replicate the original calculations, so our results should be taken as only rough indications.

We also illustrate the potential consequences of bias uncertainties introduced by cryogenic extraction of soilwater ($SE_{BIAS}=6\text{ ‰ } \delta^2H$; Section 3). We focus on δ^2H values because available evidence (Section 3) suggests that plant-water $\delta^{18}O$ biases introduced by cryogenic extraction are small.

Case A: Water sources of riparian trees

Dawson and Ehleringer (1991) compared δ^2H values of tree xylem water with soilwater, groundwater, and nearby streamwater. The xylem-water δ^2H of large streamside trees was -133.3 ‰ , which was lighter than streamwater δ^2H (-121.4 ‰), suggesting that the trees instead used groundwater (-132.3 ‰). However, the groundwater and streamwater were separated by only 11 ‰ , so correcting the xylem-water δ^2H for a possible cryogenic extraction bias of -6.1 ‰ and a two-standard-error uncertainty yields a confidence interval of -134 to -120.4 ‰ , overlapping with either groundwater or streamwater. By contrast, the xylem-water δ^2H of younger, non-streamside trees ($-89.6 \pm 4.3\text{ ‰}$) was sufficiently different that neither groundwater or streamwater could be a plausible primary source, and thus conclusions that those trees used soilwater (approximately -120 to -30 ‰) were robust regardless of cryogenic extraction biases (Fig. 1A).

Case B: Seasonal water-uptake depths in a semi-arid forest

Kerhoulas et al. (2013) quantified how water sources of Ponderosa pine trees varied across seasons, including the dry season of Spring 2009 and the monsoon season of Summer 2010. In the dry season, soilwater δ^2H exhibited a strong gradient, from -37 ‰ at 0-2 cm depth to -81 and -91 ‰ at depths of 19-21 cm and 39-41 cm, respectively. The average xylem-water δ^2H of -92 ‰ suggested use of deeper soilwater. A bias correction of $+6.1 \pm 3.4\text{ ‰}$ would suggest somewhat less uptake from the deepest soils (Fig. 1B) but still exclude the possibility of shallow soilwater being the primary source; expanding the end-member bounds to account for soilwater cryogenic extraction uncertainties would minimally affect these inferences (Fig. 1B). The soilwater gradient was weaker in the monsoon season; δ^2H was -84 , -79 , and -95 ‰ at depths of 0-2 cm, 19-21 cm, and 39-41 cm, respectively. Shifting the reported xylem-water δ^2H of -95 ‰ by $+6.1$ resulted in it best matching the shallowest soilwater rather than the deepest. Accounting for uncertainties in soilwater δ^2H introduced by cryogenic extraction resulted in

overlap among the end-member values. This example illustrates how source attribution may be more robust during dry periods, when evaporative fractionation can yield strong soilwater $\delta^2\text{H}$ gradients (Muñoz-Villers et al., 2018), and more unclear during rainy periods, when such gradients can be erased by infiltrating precipitation (Sprenger et al., 2016).

Case C: Depth of soil-water uptake by tropical-forest trees

Xylem-water $\delta^2\text{H}$ in six tree species (Goldsmith et al., 2012) suggested their relative use of shallow soilwaters in a seasonally dry Mexican tropical forest. Here we re-analyze xylem-water values and soilwater profiles for four species. Soilwater $\delta^2\text{H}$ mostly decreased with depth, ranging from approximately -12 to -35 ‰ at 5 cm, -23 to -28 ‰ at 15 cm, -29 to -39 ‰ at 30 cm, -40 to -50 ‰ at 50 cm, -49 to -75 ‰ at 70 cm, and -61 to -79 ‰ at 100 cm. Xylem-water $\delta^2\text{H}$ in *Q. lanceifolia*, *C. macrophylla*, and *M. glaberrima* varied between -31 ‰ and -28 ‰, consistent with soilwaters from depths of roughly 5-30 cm (regardless of whether soilwater-extraction uncertainties are accounted for or xylem-water values are shifted by $+6.1$ ‰). However, if extraction biases differ by species (e.g., from -5 to -11 ‰; Chen et al. 2020), the high-to-low $\delta^2\text{H}$ ranking across these three species may change. The xylem-water $\delta^2\text{H}$ of *A. latifolia* was distinctly lower (-48 ‰), matching soilwater from 50 cm; with a $+6.1$ ‰ shift, it would overlap with soilwaters at 50 cm and shallower depths (Fig. 1C), especially if the by-depth soilwater ranges were widened by ± 6 ‰ to account for uncertainties introduced by cryogenic extraction. The originally reported findings remain somewhat robust because of the large soilwater gradient (50 ‰ $\delta^2\text{H}$) and the coarseness of the inferences (i.e., xylem water was qualitatively matched with soilwaters rather than being used to solve for fractional uptake from different depths, as was done in other studies).

Case D: Use of summer versus winter precipitation across sites and species

Allen et al. (2019) analyzed xylem water from three tree species across 182 Swiss forest sites as a mixture of summer-precipitation and winter-precipitation end-members, which averaged -43 ‰ and -103 ‰ $\delta^2\text{H}$, respectively. Measured xylem-water $\delta^2\text{H}$ was roughly intermediate to those potential sources, and compensation for presumed xylem-water extraction biases is relatively small compared to the 60 ‰ difference between the end-members (Fig. 1D). However, the analysis estimated the original composition of the source precipitation by

compensating for the effects of evaporative fractionation in dual-isotope space, which makes the results more vulnerable to cryogenic-extraction biases, as described in the next section.

5 POTENTIAL EFFECTS OF $\delta^2\text{H}$ BIASES IN DUAL-ISOTOPE APPLICATIONS

Many water-isotope studies measure both $\delta^2\text{H}$ and $\delta^{18}\text{O}$. If the two isotopes yield similar results after accounting for their respective biases and uncertainties, they provide a consistency check; conversely, if they suggest different interpretations, the reason for the discrepancy should be investigated. Other applications, such as quantifying evaporative fractionation, require both isotopes together. Problematically, extraction biases alone could cause xylem waters derived from precipitation to plot well below the meteoric water line (MWL) if $\delta^{18}\text{O}$ biases are trivial. Thus, cryogenic-extraction biases could lead researchers to infer evaporative fractionation where none has actually occurred (Fig. 2A).

Dual-isotope approaches are used to correct for the putative effects of evaporation, to infer the isotopic composition of original precipitation sources by extrapolating back along a hypothesized evaporation line to its intersection with the MWL (Allen et al., 2019; Benettin et al., 2018; Bowen et al., 2018; Evaristo et al., 2015). Cryogenic extraction biases could affect the inferred isotopic compositions of such pre-evaporation source waters. If a biased sample (*sensu* Figure 2A) is extrapolated back along an evaporation line, it will intersect the MWL far below the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of its true source water (Fig. 2B). For evaporation slopes ranging from 2.5 to 5.0 and a MWL with a slope of 8.0, a cryogenic-extraction bias of -6.1‰ $\delta^2\text{H}$ will shift the inferred pre-evaporation source waters by -1.1 to -2.0‰ $\delta^{18}\text{O}$ and -8.9 to -16.3‰ $\delta^2\text{H}$, amplifying the bias in $\delta^2\text{H}$ while also introducing a bias in $\delta^{18}\text{O}$ (Fig. 2B). If xylem samples are affected by extraction biases, correctly estimating their original source waters requires *first correcting* for the extraction bias, *before extrapolating* the bias-corrected values back along the putative evaporation line to the MWL (Fig. 2C).

6 CONCLUSION

Our analysis shows that reported cryogenic extraction biases in xylem-water $\delta^2\text{H}$ can be large enough to substantially affect inferences about plant water sources. Alternatively, their consequences can be minimal if the isotopic differences among the potential end-members are

sufficiently large. Thus, conclusions drawn from cryogenically extracted xylem waters are neither generally valid nor generally invalid; what matters is the size of the potential extraction biases in relation to the isotopic signals in the data.

Further work on extraction artifacts is urgently needed because evidence suggests there is no single universal correction factor (although we used one here for illustrative purposes). Nonetheless, as demonstrated here, sensitivities to suspected biases can be assessed, even if their exact magnitudes remain unknown. Thus, even imperfect measurements of plant and soil stable isotopes can yield useful inferences, if studies are designed to be sufficiently robust against their expected biases and uncertainties.

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Figure 1 Xylem-water $\delta^2\text{H}$ values from four field studies, with ("Bias-corrected") and without ("Reported") a $+6.1 \pm 3.4$ ‰ adjustment to compensate for presumed xylem-water cryogenic-extraction biases (filled and open circles, respectively), overlaid on $\delta^2\text{H}$ values of potential source waters (colored bars). The meanings of the colored bar are study-specific, reflecting the following: ± 1 standard deviation in stream and groundwater and the range of observations in soilwater (1A); ± 1 standard error (1B); the range in soilwater values at 70-100 cm (deep), 50 cm (mid-depth), and 5-30 cm (shallow) (1C); 95% prediction intervals (1D). To account for uncertainties due to the unknown biases introduced by soilwater extraction, soilwater ranges were expanded by ± 6 ‰ where ranges were reported, whereas ± 6 ‰ was added in quadrature to standard errors due to sampling where soilwater $\delta^2\text{H}$ standard errors were reported. The data are from Figure 1, Table 1, and the text of Dawson and Ehleringer (1991), Table 3 of Kerhoulas et al. (2013), Figure 3A, C, E and G of Goldsmith et al. (2013), and the original dataset of Allen et al. (2019); see also (Allen & Kirchner, 2021)

Figure 2 Implications of $\delta^2\text{H}$ biases in dual-isotope space. A) Measurements of xylem water that would otherwise lie on the meteoric water line (implying no evaporative fractionation since that water fell as precipitation) will instead fall below the line if extraction introduces a negative bias in $\delta^2\text{H}$. This bias creates an apparent evaporation signal in dual-isotope space, and also artifactually suggests that the pre-evaporation source waters are lower in both $\delta^2\text{H}$ and $\delta^{18}\text{O}$ than the actual sources are. B) Biases in $\delta^2\text{H}$ will be amplified if they are translated to inferred pre-evaporation source waters. For example, an initial precipitation source with values of -5.5 ‰ $\delta^{18}\text{O}$ and -34 ‰ $\delta^2\text{H}$ (the black X) could evaporate, becoming enriched in heavy isotopes along an evaporation line with a slope of 4 (the solid red arrow), resulting in xylem water with isotope values of -2.5 ‰ $\delta^{18}\text{O}$ and -22 ‰ $\delta^2\text{H}$ (the black dot). If the measured $\delta^2\text{H}$ includes an extraction bias of -6.1 ‰ (and thus is -28 ‰ $\delta^2\text{H}$ rather than the true value of -22 ‰), the apparent isotopic signature of the xylem water (the open dot) will suggest substantially more evaporation than actually occurred. Attempting to correct for this apparent evaporation, by extrapolating back to the meteoric water line along a slope of 4 (the dashed red arrow), will yield an apparent pre-evaporation source at -7.0 ‰ $\delta^{18}\text{O}$ and -46 ‰ $\delta^2\text{H}$ (the open X). Thus the resulting bias in the apparent pre-evaporation source will be -1.5 ‰ $\delta^{18}\text{O}$ and -12 ‰ $\delta^2\text{H}$, relative to the true pre-evaporation source. C) If the size of the extraction bias is known, the measured xylem water can be corrected for this bias (black arrow), yielding a better estimate of the true xylem-water isotope signature (the black dot). Then, by evaporation-compensating the bias-corrected value (the dashed red arrow), the true pre-evaporation source water (the black X) can be retrieved.

Accounting for extraction uncertainties and biases in soilwater and plant-water $\delta^2\text{H}$

A) Dawson & Ehleringer, 1991

B) Kerhoulas et al., 2013

C) Goldsmith et al., 2012

D) Allen et al., 2019



