Does back-flow of leaf water introduce a discrepancy in plant water source tracing through stable isotopes?

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Abstract. Plant water source tracing studies often rely on differences in stable isotope composition of different water sources.
10 However, an increasing number of studies has indicated a discrepancy between the isotopic signature of plant xylem water and the water sources assumed to be used by plants. Based on a meta-analysis we have reconfirmed this discrepancy between plant xylem water and groundwater and suggest back-flow of leaf water (BFLW), defined as a combination of (i) the Péclet effect, (ii) foliar water uptake (FWU) and (iii) hydraulic redistribution of leaf water, as a possible explanation for these observations. Using the average 2.21‰ ^{18}O enrichment of xylem water compared to groundwater in our meta-analysis, we modelled the
15 potential of BFLW to result in this observed isotopic discrepancy. With a low flow velocity of 0.052 m.h^{-1} and an effective path length of 2 m, the Péclet effect alone was able to account for the average offset between xylem water and groundwater. When including a realistic fraction of 5-10% xylem water originating from FWU and tissue dehydration, 60-100% of the average observed enrichment can be explained. By combining the Péclet effect with FWU and tissue dehydration, some of the more extreme offsets in our meta-analysis can be elucidated. These large effects are more probable during dry conditions when
20 drought stress lowers transpiration rates, leading to a larger Péclet effect, more tissue dehydration, and a potential greater contribution of FWU.

25 ## 1 Introduction

Determining the stable isotopic composition of H (^{2}H/^{1}H) and O (^{18}O/^{16}O) has become a useful tool to trace which water pools are being returned to the atmosphere by transpiring plants (Ehleringer and Dawson, 1992). Studies using stable isotopes for tracing plant water sources assume that: (i) root water uptake is a fraction-free process, i.e. no change in isotopic

composition occurs during this uptake (Dawson and Ehleringer, 1991; Zimmermann et al., 1967); and (ii) there is no
30 fractionation during sap transport through the xylem until it reaches the leaves and is transpired into the atmosphere (Dawson
and Ehleringer, 1993). However, over recent decades, an increasing amount of stable isotope research has shown that: (i)
fractionation during root water uptake can occur, mainly, but not exclusively (Vargas et al., 2017; Poca et al., 2019), in
xerophytic and halophytic species (Lin and Sternberg, 1993; Ellsworth and Williams, 2007; Zhao et al., 2016; Barbeta et al.,
2019); and (ii) a range of processes can affect the isotopic composition during sap transport in the xylem, such as xylem-
35 phloem water exchange (Cernusak et al., 2005; Nehemy et al., 2022) and absorption and evaporation through bark (Ellsworth
and Sternberg, 2015; Gimeno et al., 2022).

A dual isotope approach (δ^2H and δ^{18}O) is often used in water source tracing studies (Berry et al., 2017). In the
absence of fractionation, xylem, soil, ground, and stream water samples plot on the Local Meteoric Water Line (LMWL; linear
relationship between the δ^2H and δ^{18}O composition of local rainfall samples) on a δ^2H - δ^{18}O biplot. Findings by Brooks et
40 al. (2010), however, showed that xylem water plotted below ground and stream water, indicating a relative enrichment in ^{18}O
compared to ^{2}H in the xylem water, which has been confirmed for numerous species (Evaristo et al., 2015). This observed
divergence could be exacerbated by methodical errors (Chen et al., 2020), but does not solely result from extraction artefacts
(Song et al., 2021; Evaristo et al., 2021). To explain these observations, Brooks et al. (2010) hypothesized that '…*plants are
able to take up tightly bound soil water when mobile water is not available…*', suggesting the use of an alternative water source
45 by plants when it is available. While other mechanisms have been suggested to explain this observed enrichment, e.g., mixing
of soil water and rainwater during percolation (Sprenger et al., 2016), spatio-temporal dynamics between soil and plant vs
groundwater based on evaporative enrichment (Dubbert et al., 2019), and fractionation by roots during uptake (Barbeta et al.,
2019; Ellsworth and Williams, 2007; Lin and Sternberg, 1993; Zhao et al., 2016), which could be enhanced by mycorrhizae
(Poca et al., 2019), there could be yet another water source at play. One potential source that could influence isotopic
50 measurements of stem xylem water is back-flow of leaf water (BFLW). BFLW consists of a combination of (i) the Péclet
effect (Farquhar and Lloyd, 1993), (ii) foliar water uptake (FWU) (Berry et al., 2019; Schreel and Steppe, 2020) and (iii)
hydraulic redistribution of leaf water towards lower plant parts, e.g. during tissue dehydration (Nadezhdina et al., 2010).

The Péclet effect, as described by Farquhar and Lloyd (1993), represents the diffusion of isotopically enriched leaf water
-originating from fractionation during transpiration- in the opposite direction to bulk flow. While this effect predominantly
55 results in an isotopic gradient within the leaves themselves, the isotopic effect could extend past the leaves and into small
branches and stems when transpiration rates are low (Farquhar and Lloyd, 1993). Even though countercurrent flow from leaves
to twigs and branches has been recently observed (Lehmann et al., 2018; Wang et al., 2021), the magnitude of the Péclet effect
can significantly vary, both within a single leaf and between leaves and different plant species, due to heterogeneity in stomatal
conductance (Farquhar and Lloyd, 1993) and anatomical and physiological traits affecting mean leaf water residence time
60 (Farquhar and Cernusak, 2005; Simonin et al., 2013; Lehmann et al., 2020) and diffusion (Lehmann et al., 2020).

Globally, precipitation inputs leading to leaf wetting events occur on more than 100 days per year (Dawson and
Goldsmith, 2018). These leaf wetting events diminish or stop transpiration by forming a liquid water film on the leaf surface,

which can be absorbed (FWU); however, the amount of water absorbed by FWU and its occurrence depends on species and environment (Limm et al., 2009; Limm and Dawson, 2010; Berry et al., 2019; Schreel and Steppe, 2020). When water is

65 absorbed by leaves, it results in an increased leaf water content and leaf water potential (Burgess and Dawson, 2004; Simonin et al., 2009; Schreel et al., 2019; Pan et al., 2021). If the leaf water potential increases enough to exceed the stem water potential, water may flow bi-directionally from both the leaves and the roots towards the stem (Goldsmith, 2013; Boanares et al., 2020). This reverse flow of water from leaves to the stem can be large enough in volume to replenish the internal water reserves and lead to irreversible or turgor-driven stem diameter growth (Steppe et al., 2018; Schreel et al., 2019). Even though

70 some species are unable to absorb water through their leaves (Limm et al., 2009), Kim and Lee (2011) found that dew water could contribute up to 72% of leaf water in multiple crops after a 16 h dew exposure and Kagawa (2022) observed that about 55% of hydrogen and oxygen in branch cellulose of 2-year-old *Cryptomeria japonica* saplings originated from FWU after seven leaf wetting events during three consecutive days. The effect of leaf wetting on leaf and branch isotopic composition can be substantial and can be measurable at the leaf level up to a day after the leaf wetting event (Lehmann et al., 2018). Even

75 in the absence of FWU it has been indicated that the isotopic composition of foliar water can be altered due to the exchange of isotopically distinct water between leaves and the atmosphere (Farquhar and Cernusak, 2005; Goldsmith et al., 2017). While the number of leaf wetting events, their duration and the exchange of isotopically distinct water is contextual, these observations indicate the large potential of FWU to influence the isotopic composition of stem xylem water and water reserves located in living woody tissues.

80 Hydraulic redistribution of leaf water towards lower plant parts can occur during FWU and varying degrees of tissue dehydration (Nadezhdina et al., 2010). Not only the water absorbed by FWU, but also the water already present in the leaves can be transported downward when the water potential gradient is favourable. Bulk leaf water is heavily enriched in $\delta^{18}O$ compared to stem xylem water leading to isotopic differences of over 25‰ (Berkelhammer et al., 2013). As a result, the redistribution of bulk leaf water can have a measurable effect on the isotopic composition of stem xylem water.

85 The effect of each of the three individual types of BFLW may be small, but their compounded effect on measured isotope ratios might be a source of considerable bias. In this paper we discuss the potential of BFLW to alter the isotopic composition of plant water by analysing BFLW's individual components. First, we compiled data from a meta-analysis on the relative enrichment of xylem water compared to groundwater. Second, we modelled the Péclet effect to estimate how much this would influence the isotopic composition of stem xylem water based on the levels of enrichment observed in our meta-analysis during

90 low flow velocities. Third, these data were used to model how enriched an alternative stem water source (e.g., hydraulic redistribution originating from FWU and/or tissue dehydration) would need to be to account for the observed enrichment.

2 Materials and Methods

2.1 Meta-analysis

Isotopic composition of H and O in water samples are expressed in the delta notation (δ^2H and δ^{18}O, Eq. 1), i.e. the deviation of the isotopic ratio from VSMOW (Vienna Standard Mean Ocean Water), the primary measurement standard (Gonfiantini, 1978).

$$\delta_{sample} = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \cdot 1000 \tag{1}$$

with R_{sample} and $R_{standard}$ representing the heavy to light isotope ratios (^{2}H/^{1}H or ^{18}O/^{16}O) measured in the sample and standard, respectively.

The contribution of groundwater to a plant's water budget significantly increases during dry conditions, an occurrence which is not restricted to arid and seasonally dry ecosystems (Barbeta and Peñuelas, 2017; Evaristo and McDonnell, 2017). During these same dry conditions, the effect of BFLW on a plant's isotopic water composition is expected to be larger due to tissue dehydration and low flow velocities resulting in a stronger Péclet effect (see below). To estimate the offset between plant xylem water and groundwater needed for further modelling, we performed a meta-analysis using a keyword-based search of published data for stable isotopes in ecology and ecohydrology. Data were extracted from papers by using an online plot digitizer (https://automeris.io/WebPlotDigitizer/). Consulted papers can be found in the supplement. Relative enrichment in oxygen-18 composition was assessed by a weighted linear regression:

$$\delta^{18}O_G = m \cdot \delta^{18}O_X + b \tag{2}$$

where $\delta^{18}O_X$ (‰) and $\delta^{18}O_G$ (‰) represent the isotopic ^{18}O compositions of xylem water and groundwater, respectively. m and b represent the slope and intercept of the linear relationship, respectively. Weights were calculated as the sum of the multiplicative inverse δ^{18}O standard errors for xylem water and groundwater. All statistical analyses were performed in R (R Core Team, 2020).

2.2 Péclet effect: modelling the counterflow diffusion in xylem water based on bulk foliar enrichment

The Péclet effect is the diffusion of evaporatively enriched leaf water in the opposite direction of the bulk water flow. This back-diffusion of enriched water can influence leaf and twig xylem water (Cernusak et al., 2016; Gan et al., 2002; Lehmann et al., 2018), suggesting that the isotopic composition of small branches and stems could be altered by back-diffusion of enriched water. The effect on branches and stems could be particularly pronounced when flow velocities rates are low (Farquhar and Lloyd, 1993). Assuming an isotopic steady state, ^{18}O enrichment of the xylem compared to groundwater absorbed by the roots ($\Delta^{18}O_{XG}$; ‰) can be described as (Farquhar and Lloyd, 1993):

$$\Delta^{18}O_{XG} = \frac{\Delta^{18}O_{LG} \cdot (1 - e^{-\wp})}{\wp} \tag{3}$$

where $\Delta^{18}O_{LG}$ (‰) is the ^{18}O enrichment of the bulk leaf water compared to the groundwater absorbed by the roots and $\wp$ (-) is the Péclet number. The Péclet number is defined as:

$$\wp = \frac{V \cdot L}{D} \tag{4}$$

where V is the flow velocity (m.s^{-1}), L is the effective path length (m) and D is the diffusivity of H$_2$^{18}O in water

125 (2.29 10^{-9} m^2 s^{-1}), which is defined relative to leaf temperature (T$_L$; set to 25 °C in our calculations) as (Cuntz et al., 2007):

$$D = 119 \cdot 10^{-9} \cdot e^{-637/((T_L + 273.15) - 137)} \tag{5}$$

The Péclet number quantifies the degree to which counterflow diffusion is overtaken by the advective flow (Cernusak et al., 2016). The effective path length (L) is the product of the distance between the point of measurement (e.g., the bulk lamina or the twig) and the evaporation site and a scaling factor that accounts for tortuosity in the liquid water path (Cernusak

130 et al., 2016). In our calculations, the effective path length was defined as the distance between the leaves and the point of measurement in the stem xylem, including the tortuosity of this pathway.

Rearranging Eq. 3 to enable the calculation of $\Delta^{18}O_{LG}$ as a function of L and V results in:

$$\Delta^{18}O_{LG} = \frac{\Delta^{18}O_{XG} \cdot \wp}{(1 - e^{-\wp})} \tag{6}$$

where $\Delta^{18}O_{XG}$ is the enrichment of stem water above groundwater that was obtained from our meta-analysis (mean

135 of 2.21‰; Fig. S1). $\Delta^{18}O_{LG}$ is, therefore, the leaf water enrichment that influences the enrichment of stem water ($\Delta^{18}O_{XG}$) due to the Péclet effect. However, the influence of the Péclet effect on the isotopic signature of the xylem water not only is a function of the isotopic signature of leaf water, but also is constrained by the volume of leaf water that flows into the xylem. Using a mass balance, this constraint can be assessed in terms of the fractional volume of water (rather than the absolute volume) in each pool:

140 $$X_G \cdot \delta^{18}O_G + X_L \cdot \delta^{18}O_L = \delta^{18}O_X \tag{7}$$

where X_G and X_L are the volumetric fractions (%) of stem xylem water that come from the ground and from leaf water, respectively, and $\delta^{18}O_L$ is the ^{18}O signatures of leaf water (‰). As xylem water is assumed to be the sum of ground and leaf water, the algebraic sum of their fractions equals 1:

$$X_G + X_L = 1 \tag{8}$$

145 Substituting Eq. 8 into Eq. 7 results in a linear two-end-member mixing model:

$$\Delta^{18}O_{XG} = X_L \cdot \Delta^{18}O_{LG} \tag{9}$$

By combining Eq. 9 and Eq. 3 we can calculate the volume fraction of water found in the stem xylem coming from groundwater and from foliar water:

$$X_L = 1 - X_S = \frac{(1 - e^{-\wp})}{\wp} \tag{10}$$

150 As such, it becomes apparent that the proportional difference (f) used by Song et al. (2013) to assess the Péclet effect within a leaf equals the volume fraction of water in the leaf that comes from the stem. Based on Eq. 6 and 10 we were able to model a combination of enrichments ($\Delta^{18}O_{LG}$) and volume fractions (X_L) resulting from the Péclet effect as a function of V and L. All calculations and modelling were performed in RStudio (The R Project for Statistical Computing, 2018) and plotted using the '*fields*' package.

2.3 Hydraulic redistribution: modelling the effect of an enriched alternative water source on stem xylem water isotopic composition

To test whether BFLW could affect stem xylem water isotope ratios, we considered the situation in which there are only two water sources: groundwater and an alternative water source, e.g., BFLW. Because ^{18}O is relatively more enriched than ^2H in xylem water compared to groundwater (Fig. S1 and S2), $\delta^{18}O$ values were used in our calculations. This two-source scenario can be modelled using a two-end-member mixing model:

$$\delta^{18}O_X = X_A \cdot \delta^{18}O_A + (1 - X_A) \cdot \delta^{18}O_G \tag{11}$$

where $\delta^{18}O_A$ (‰) represents the isotopic ^{18}O compositions of an alternative water source. X_A (%) and $(1-X_A)$ represents the fraction of water in the xylem originating from an alternative water source and groundwater, respectively. This model can resolve the contributions of two distinct water sources to the total water pool in the stem xylem, based on the specific isotopic ^{18}O signatures of each source.

Eq. 11 can be rearranged to solve for the isotopic ^{18}O composition of the alternative water source ($\delta^{18}O_A$) as a function of the fraction of water originating from this alternative water source found in the xylem (X_A):

$$\delta^{18}O_A = \frac{\delta^{18}O_X - (1 - X_A) \cdot \delta^{18}O_G}{X_A} \tag{12}$$

Using Eq. 12 we can assess how the isotopic composition of an alternative water source and its fractional contribution (X_A) together influence the ^{18}O enrichment in xylem water compared to groundwater. Based on Eq. 12 and the linear relationship between $\delta^{18}O_X$ and $\delta^{18}O_G$ found in our meta-analysis (Fig. S1), all possible combinations of $\delta^{18}O_A$ and X_A resulting in this observed difference can be modelled. All modelling was performed in RStudio (The R Project for Statistical Computing, 2018) and plotted using the '*fields*' package.

3 Results and Discussion

The discrepancy between the isotopic signatures of xylem water and below ground and stream water observed by Brooks et al. (2010) was reconfirmed in our meta-analysis for groundwater (p < 0.01; Fig. 1) and could have numerous explanations, e.g., fractionation by roots during uptake (Barbeta et al., 2019; Ellsworth and Williams, 2007; Lin and Sternberg, 1993; Zhao et al., 2016), which could be enhanced by mycorrhizae (Poca et al., 2019), xylem-phloem water exchange (Cernusak et al., 2005; Nehemy et al., 2022) and absorption and evaporation through bark (Ellsworth and Sternberg, 2015; Gimeno et al., 2022), including an alternative water source, as we hypothesise. While it is important to note that 3 out of the 25 datasets used in our meta-analysis indicate enrichment in groundwater rather than xylem water, the overall combined dataset indicates the opposite, with an average xylem isotopic $\delta^{18}O$ enrichment of 2.21‰ compared to groundwater (Fig. S1).

There are two parameters that would determine how much an alternative water source could influence xylem water: its isotopic composition and its proportional contribution to the total xylem water pool. Because xylem water is more enriched than groundwater, the alternative water source mixing with xylem water must be more enriched than xylem water. If BFLW

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is this alternative water source, then the combinations of $\delta^{18}O_A$ and X_A needed to enrich $\delta^{18}O_X$ relative to $\delta^{18}O_G$ must be realistic. As stated before, BFLW has three distinct mechanisms that could each contribute to the overall observed discrepancy between $\delta^{18}O_X$ and $\delta^{18}O_G$: (i) the Péclet effect (Farquhar and Lloyd, 1993), (ii) foliar water uptake (FWU) (Berry et al., 2019; Schreel and Steppe, 2020) and (iii) hydraulic redistribution of leaf water towards lower plant parts (Nadezhdina et al., 2010).

190 The former is discussed in Sect 3.1, the latter two are discussed in Sect. 3.2.

3.1 Can the Péclet effect alter the isotopic composition of xylem water?

The Péclet effect indicates that leaf water diffuses down an isotopic concentration gradient in the opposite direction of the mass flow for transpiration. When transpiration rates become low, e.g. at night, back-diffusion is more probable (Loucos et al., 2015; Roden et al., 2015; Song et al., 2013), reaching twigs (Wang et al., 2021), branches (Lehmann et al., 2018) and

195 possibly stem xylem sap (Farquhar and Lloyd, 1993). However, at higher transpiration rates (Cernusak et al., 2016), when the air is dryer and warmer (Dongmann et al., 1974) and has a higher vapour pressure deficit (Flanagan et al., 1991; Harwood et al., 1998; Ripullone et al., 2008), leaf ^{18}O is more enriched. Thus, isotopic enrichment of stem xylem water as a result of the Péclet effect depends on the interplay between more foliar enrichment due to higher transpiration rate and a longer backflow distance due to a lower transpiration rate. While the former is a function of timing (temporal) and environment, as the

200 transpiration rate and foliar ^{18}O enrichment fluctuate throughout the day (Förstel, 1978; Harwood et al., 1998) as a function of light availability and vapour pressure deficit, the latter is a function of environment, time, and distance between the point of stem xylem sampling and the leaves (temporospatial). With a 2.21‰ difference between $\delta^{18}O_X$ and $\delta^{18}O_G$ –the average obtained from our meta-analysis ($\Delta^{18}O_{XG}$; Fig. S1)–, a low flow velocity of 0.052 m.h^{-1} (V) and an effective path length of 4 m (L) from the leaves to the sampling site in the xylem, the difference between $\delta^{18}O_L$ and $\delta^{18}O_G$ ($\Delta^{18}O_{LG}$) should be about

205 56‰ (Fig. 2a) with volumetric fractions of 4% of stem xylem water that comes from leaf water (X_L; Fig. 2b), while 96% comes from groundwater (X_G). The biological relevance of these values depends on the species and plant growth form. While a high X_L might be realistic for an epiphytic orchid, it is not for a full-grown tree. However, as tree saplings are regularly used to assess the isotopic discrepancy between xylem and irrigation water (e.g., Poca et al., 2019), leaves can be closer than 4 m to the sampling site in the xylem, or herbaceous plant species can be used in this assessment. Effective path length, flow velocity,

210 $\Delta^{18}O_{LG}$ and X_L are interrelated (Eq. 4, 6 and 10). When reducing the effective path length to 2 m, given a flow velocity of 0.052 m.h^{-1}, X_L increases to 8% and $\Delta^{18}O_{LG}$ decreases to about 28‰ (Fig. 2).

      This theoretical approach suggests that the Péclet effect cannot be responsible for the mean observed $\Delta^{18}O_{XG}$ (2.21‰) in full grown trees for distant foliage (L ≥ 4 m) as $\Delta^{18}O_{LG}$ would need to be unrealistically large. However, when L is reduced to 2 m or less, an $\Delta^{18}O_{XG}$ of 2.21‰ can be fully explained by the Péclet effect. Furthermore, when L is large (e.g., 4 m), the

215 smallest observed enrichment ($\Delta^{18}O_{XG}$) of 0.22‰ can still be fully explained by the Péclet effect as X_L remains 4% and $\Delta^{18}O_{LG}$ needs to be only about 6‰ for the same value of V (0.052 m.h^{-1}). As a result, the Péclet effect could (partially) explain, the isotopic enrichment in ^{18}O of 8‰ in oak (*Quercus faginea*) saplings observed by Martín-Gómez et al. (2016) during a 3-day cycle of reduced transpiration.

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Important to note is that diffusion is a slow process. Based on simple diffusion time in the absence of transpiration, it would take water enriched in ^{18}O about 27.7 years to travel a distance of 2m when there is no mass flow driving its movement. However, two points are worth considering about the BFLW. First, diffusion from leaves to the stem is an ongoing process that occurs throughout the lifespan of the plant, such that the isotopic signal of slow back diffusion would accumulate over time. Second, mass flow of water can promote the movement of heavy isotopologues away from leaves and towards the stem. This mass flow occurs when there is FWU, HR, or even during normal cycling of water due to water exchange between the xylem and phloem. Thus, while diffusion alone is slow, prior evidence suggests that diffusion due to the Péclet effect can be significant enough to have measurable effects on leaf water, and we argue that mass flow can reinforce this effect by transporting enriched leaf water into the stem.

3.2 FWU and hydraulic redistribution as alternative water source

As expected, our model showed that when the alternative water source, e.g., BFLW, is more enriched a smaller proportion of it is needed to produce a given offset of xylem water from water absorbed by the roots (Fig. 3). In trees, an estimated 5-10% of stem xylem water could originate from FWU (Binks et al., 2019; Schreel et al., 2019). Assuming that an alternative water source comprises 5-10% of the stem xylem water (Binks et al., 2019; Schreel et al., 2019) and that stem xylem water is enriched above groundwater by 2.21‰ ($\delta^{18}O_X$ - $\delta^{18}O_G$; Fig. S1), our model shows that water from this alternative water source would need to be about 20-42‰ more enriched than xylem water ($\delta^{18}O_A$ - $\delta^{18}O_X$) (Eq. 12 and Fig. 3). These values are realistic as transpiration can lead to bulk leaf water becoming over 25‰ more enriched in $\delta^{18}O$ than stem xylem water (Berkelhammer et al., 2013). This observation indicates that, on average, about 60 (25 out of 42‰) to 100% (25 out of 25‰) of the observed discrepancy in isotopic signatures could result from BFLW, if the water absorbed by leaves has the same isotopic signature as bulk leaf water. However, a relative enrichment in ^{18}O not only results from transpiration, but also occurs when a water film surrounding the leaf evaporates, especially during a relative humidity below 100% (Fig. 4a). During such events, lighter water isotopologues (i.e., isotopically lighter water molecules), such as $^{1}H_2^{16}O$ and $^{1}H^{2}H^{16}O$, preferably evaporate from the water film surrounding the leaf, thus leaving a water layer on the leaf surface enriched in $^{1}H_2^{18}O$. Under these conditions water can be absorbed through FWU (Vesala et al., 2017) or exchanged with the leaf with no net increase in foliar water content (Goldsmith et al., 2017) and redistributed within the plant resulting in ^{18}O enrichment in the xylem. We hypothesize that this type of leaf wetting event can occur in three distinct ways. First, following an atmospheric precipitation event, leaves remain wet for some time allowing both fractionation on the leaf surface and FWU. Second, dew formation causes heavy isotopologues to precipitate more easily, resulting in an enriched water layer on the leaf surface. Furthermore, lighter isotopologues will evaporate preferentially once the atmosphere is again unsaturated, amplifying the enrichment of this water layer. Third, microscopic leaf wetness can form a continuous layer on the leaf surface of less than 1 μm (Burkhardt and Hunsche, 2013). Burkhardt and Hunsche (2013) hypothesized that microscopic leaf wetness could occur on almost all plants worldwide. When relative humidity reaches 100%, the water absorbed by leaves would still be depleted of lighter isotopologues ($^{1}H_2^{16}O$); however, as both $^{1}H^{2}H^{16}O$ and $^{1}H_2^{18}O$ are still available for FWU the ratio $\delta^{18}O$ to $\delta^{2}H$ within the plant would change less

significantly compared to FWU during events with a RH below 100% (Fig. 4). In other words, the degree of enrichment in ^{18}O water absorbed by leaves primarily depends on environmental conditions, such as relative humidity and temperature.

255   Enrichment of xylem water resulting from hydraulic redistribution of leaf water, including FWU and tissue dehydration, could explain some observations of xylem water enrichment in literature. For example, the smallest enrichment of $\delta^{18}O_X$ compared to $\delta^{18}O_G$ of 0.22‰ observed in our meta-analysis requires an enrichment of hydraulically redistributed water of only about 2-4‰ (Eq. 12), which falls within the biologically realistic range of BFLW. In contrast, the largest observed discrepancy of 4.5‰ cannot be explained by this mechanism as it would require a foliar enrichment of 41-86‰ in redistributed water (Eq. 12), assuming BFLW contributes 5-10% to total xylem water (X_A), which is far outside the range of enrichment

260   reported in literature. However, when compounding the Péclet effect with the effects of FWU and hydraulic redistribution, BFLW can introduce and/or explain a considerable discrepancy in xylem isotopic composition.

3.3 BFLW during drought

During drought, the soil water potential declines, thus making soil water less available for plants. In response, xylem water potential declines, allowing soil water uptake by roots as long as soil and roots are hydraulically connected (Körner, 2019).

265   Under dry conditions, deep-rooted trees typically use deeper groundwater sources (Barbeta and Peñuelas, 2017).

    When drought occurs, trees will close their stomata to reduce transpirational water loss. This process allows for diffusion of water from the leaves towards other plants parts during the absence of transpiration. If drought persists, the water potential of the soil and roots can drop below the water potential of the leaves and stem, potentially forcing water to flow from the leaves towards the stem and the roots (Nadezhdina et al., 2010). Furthermore, a low xylem water potential can ultimately

270   lead to embolism formation and hydraulic failure (Choat et al., 2018), starting with embolism formation in the leaves (Johnson et al., 2016). Water released by foliar embolism contributes to its capacitance (Vergeynst et al., 2015) and can be redistributed throughout the plant after hydraulic failure. The occurrence of hydraulic failure is a result of tissue dehydration (Körner, 2019) -one of the possible causes for BFLW- and is based on the interplay of two properties: (i) the physical limits of the vascular system of the plant, and (ii) the capacity of the plant to maintain its xylem water potential within functional limits (Choat et

275   al., 2018), which, in turn, is influenced by hydraulic capacitance (Körner, 2019; Vergeynst et al., 2015; Steppe et al., 2015; Epila et al., 2017). In other words, both a plant's usage of groundwater (Barbeta and Peñuelas, 2017) and BFLW are more pronounced during drought. This co-occurrence explains why a decrease in leaf water potential -an indicator for dry conditions-correlates well with an enrichment in twig or stem xylem water (Kurz-Besson et al., 2006; Jackson et al., 1995; Filella and Peñuelas, 2003). Furthermore, Jackson et al. (1995) observed a similar increase in stem xylem water enrichment when

280   transpiration decreases. During low transpiration rates, the Péclet effect becomes more pronounced (Fig. 2) which could amplify these observations. The amplified effect of BFLW during drought also illuminates why xylem water is more enriched during the dry season when plants are stressed, and not during the wet season (Hervé-Fernández et al., 2016; Mensforth et al., 1994).

Surprisingly, despite the effects we demonstrated, de la Casa et al. (2022) found that the offset between bulk stem

285   water and water source was largest in cold and wet climates based on δ^2H signatures, while we observed no overall significant

difference in δ^2H between xylem water and groundwater (Fig. S2). While the absence of stress during dry conditions could

have reduced the effect of BFLW due to the absence of embolism and tissue dehydration, it does not explain the offset in cold

and wet climates. Based on our observations of ^{18}O and the strong link between BFLW and L we do agree with the conclusion

reached by de la Casa et al. (2022): using bulk stem water to assess plant water source contributions may lead to erroneous

290   conclusions.

During dry conditions, foliar water potential decreases, creating a larger gradient to drive FWU when leaves are wet

(Eller et al., 2013; Schreel and Steppe, 2019). FWU can cause the foliar water potential to increase above the water potential

of the stem, allowing for BFLW. This flow resulting from FWU could increase the path length of hydraulic redistribution

resulting from tissue dehydration (Nadezhdina et al., 2010). Furthermore, V is generally lower during droughts, implying that

295   the Péclet effect would become more relevant (Lehmann et al., 2018). As such, the influence of BFLW would be stronger

during drought, allowing BFLW to at least partially explain the observed divergence between the isotopic compositions of

groundwater and plant xylem water.

4 Concluding remarks and future perspectives

Because many studies have indicated a discrepancy between the isotopic signature of plant xylem water and the water sources

300   assumed to be used by plants, we investigated the potential of BFLW to contribute to the isotopic signatures of stem xylem

water. Using the average 2.21‰ ^{18}O enrichment of xylem water compared to groundwater in our meta-analysis, the Péclet

effect alone was able to account for the average observed enrichment during low flow velocity of 0.052 m.h^{-1} and assuming

an effective path length of 2 m. When using the same isotopic enrichment and a linear two-end-member mixing model, we

were able to pinpoint realistic $\delta^{18}O$ values for BFLW resulting from FWU and hydraulic redistribution. On average, 60 to

305   100% of the observed discrepancy in isotopic signatures could potentially be explained by this type of BFLW. These large

effects are more probable during dry conditions when a larger Péclet effect, more tissue dehydration, and more fractionation

during FWU occur.

In addition to the processes explored here, other factors could also result in isotopic discrepancies: enhanced

fractionation at the root level by mycorrhizae and biochemical fractionation along the sap flow pathway. Both of these

310   processes could affect the fractionation patterns resulting in the observed discrepancy between the isotopic composition of

groundwater and plant xylem water.

Data availability

The dataset generated and used in this publication can be accessed at http://doi.org/10.6084/m9.figshare.21940955 (Schreel et al., 2023)

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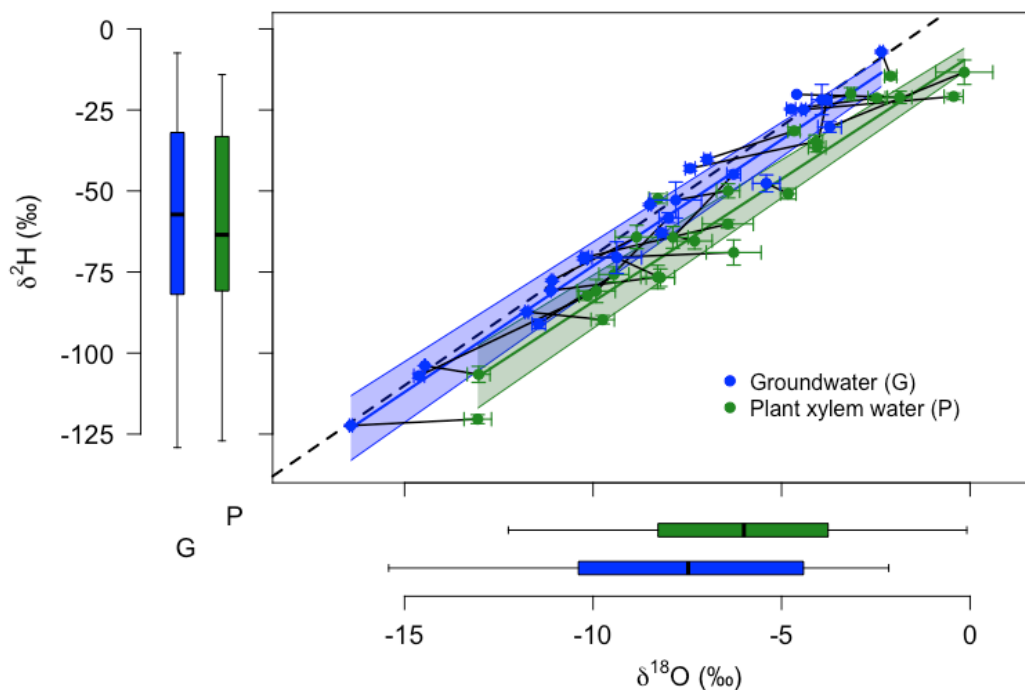
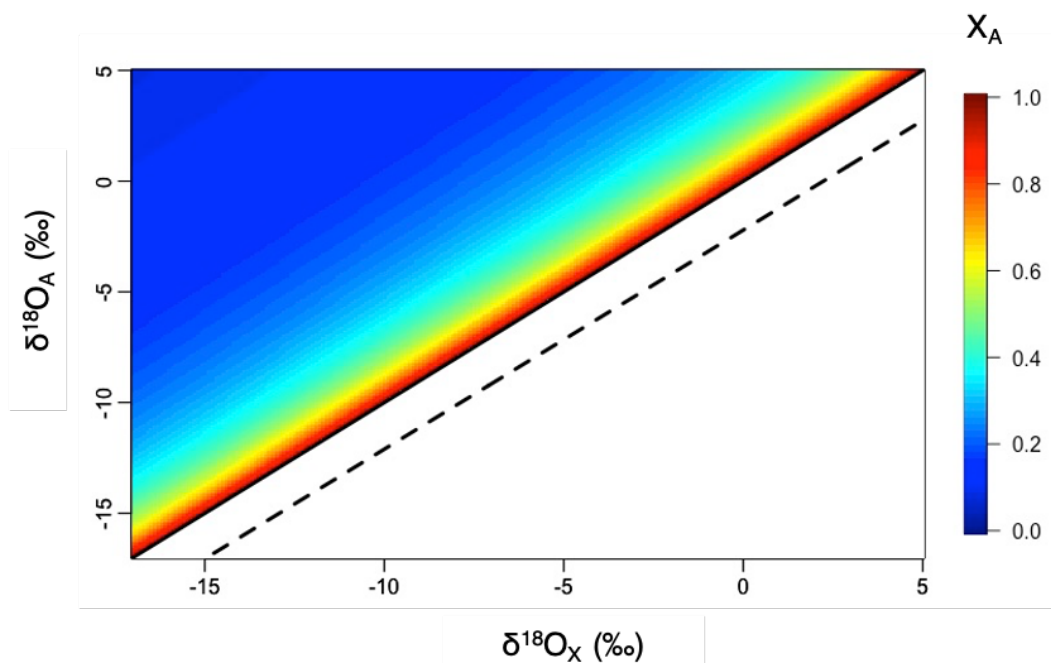**Figures**

Figure 1: δ¹⁸O and δ²H biplot based on a global meta-analysis. Blue and green dots represent the average values of groundwater and plant xylem water per paper, respectively. Error bars indicate the mean ± standard error. Black dashed line indicates the global
520 **meteoric water line, while blue and green dashed lines indicate the trend for groundwater and plant xylem water, respectively. Shaded areas indicate the standard error for the trend in groundwater and plant xylem water. Black lines connect groundwater and plant xylem water from the same study.**
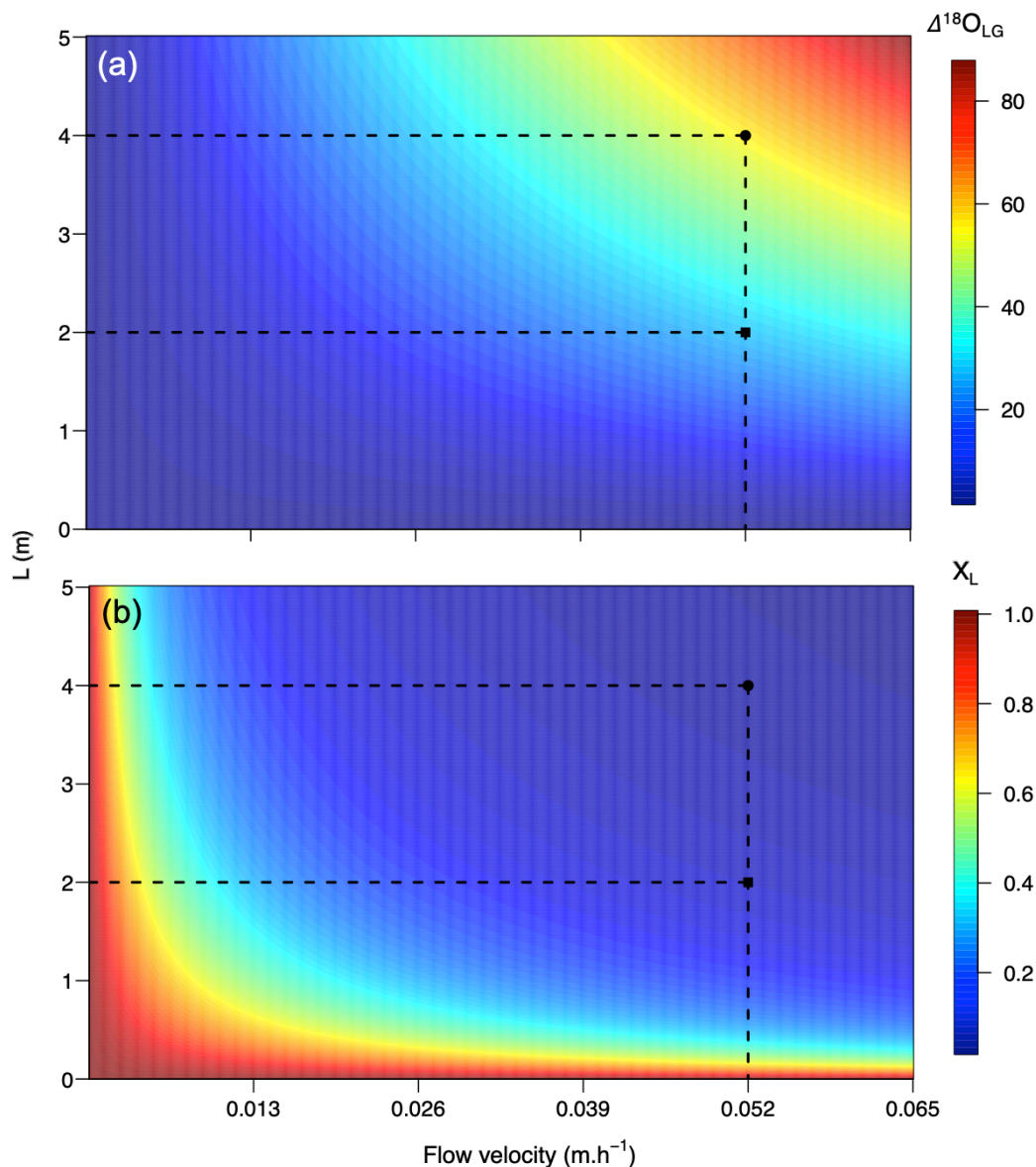
Figure 2: (a) Isotopic $\delta^{18}O$ enrichment of the bulk leaf water compared to water absorbed by the plant from groundwater ($\Delta^{18}O_{LG}$) and (b) the volume fraction of water found in the xylem coming from leaf water (X_L) as a function of flow velocity (V) and effective path length (L) based on an average observed difference in Isotopic $\delta^{18}O$ signature between xylem water and groundwater of 2.21‰ (Fig. S1). Example illustrated by dashed line: for a flow velocity of 0.052 m.h⁻¹ and a path length of 4 m, $\Delta^{18}O_{LG}$ should be 56‰ and X_L 4% to result in the observed difference of 2.21‰ ($\Delta^{18}O_{XG}$). When reducing the effective path length to 2 m, X_L increases to 8% and $\Delta^{18}O_{LG}$ decreases to 28‰.

Figure 3: Isotopic $\delta^{18}O$ composition of an alternative water source ($\delta^{18}O_A$) and the proportion in xylem water originating from this alternative water source (X_A) as a function of the difference between the isotopic $\delta^{18}O$ signature of xylem water ($\delta^{18}O_X$; full line) and groundwater (dashed line) based on the meta-analysis indicating $\delta^{18}O_G = 0.99\ \delta^{18}O_X - 2.21$ (Fig. S1).
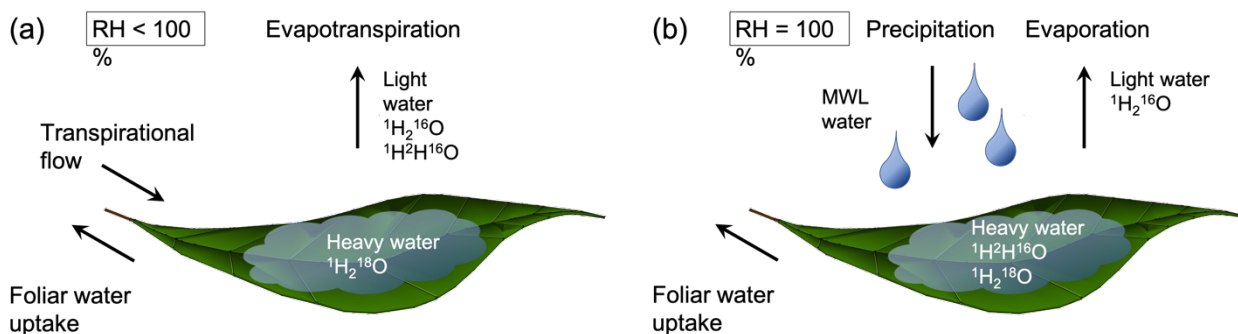

Figure 4: Isotopic fractionation of water molecules on a wet leaf surface, during a relative humidity (RH) (a) below and (b) equal to 100 %.

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