

## ***Interactive comment on “Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest” by Adrià Barbeta et al.***

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Comment on Barbeta et al.

Barbeta et al. argue that fractionation could have occurred upon uptake or within plants because they often observed that xylem water samples were lower in  $\delta^2\text{H}$  than any of the potential sources they measured (rock water, stream water, fog, soils from 70-80 cm, and soils from 0-10 cm). They consider a few possible explanations (e.g., “separation between mobile and bound” and “compartmentalization between vessel water and other stem water pools”), but mostly they “argue that an isotopic fractionation in the unsaturated zone and/or within the plant tissues could underlie” their observations.

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Of course evaporation causes fractionation in the unsaturated zone, which they show clear evidence of, but they are arguing that there may be an unexplained fractionation that occurs in stems or upon root uptake (similar to that which is sometimes observed in halophytes and xerophytes). Such an argument may be valid if a reasonably comprehensive set of potential explanations have been considered and rejected. However, they did not sample highly likely water sources, and thus there are very probable explanations for their results that were not considered.

In the introduction, the authors state “if H1 is true [i.e., that there is no fractionation upon root uptake], the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  of xylem water should always lie within the range of values of all water sources.” Thus, to test H1, all source waters should be sampled. Although sampling all source waters is an infeasible task, even highly likely sources were not measured (e.g., soils between 10 and 70 cm depth). Thus, the rejection of H1 is not a logical extension of this study’s findings, and it is unclear why the authors focus on attributing their findings to fractionation upon uptake or during within-plant transport.

Isotopic fractionation during plant root uptake can be most accurately tested in controlled settings where the “true” value is predictable, not in ambient field conditions where there are many un-controlled complicating factors. Controlled experiments have shown that xylem water accurately reflects soil water isotope values (e.g., Newberry et al 2017); consequently, challenging those findings requires a robust, well-constrained experiment. In the present study, it is not clear that the observed differences between the sampled end members and xylem water samples are due to fractionation during uptake or within the plant, as opposed to numerous other likely explanations. Several of these are listed below.

1) No soil water samples were collected at depths where roots are often found (10 to 70 cm). Thus, the authors cannot exclude the possibility that the trees’ apparent source waters occurred between their shallow (0-10 cm) and deep (>70 cm) samples. If these profiles were only affected by evaporation, then perhaps a profile comprising progressively enriched values towards the top could be expected. However, precipi-

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tation infiltrates and mixes heterogeneously with stored waters, creating heterogeneity and obscuring an evaporation profile (for an example that obviously expresses transport effects, see Figure 3 in Sprenger et al 2016). It should not be assumed that soils in intermediate depths (10-70 cm) have isotope values that are in between those of deeper and shallower soils (see Thomas et al., 2013). The un-sampled soil water domain could include winter precipitation that percolated downward into the rooting zone, after undergoing evaporative fractionation near the surface (yielding lower isotope values, due to the water's winter origins, with negative LC-excess, due to evaporation; e.g., see Dudley et al 2018), consistent with the xylem water values shown. My research (including two of the same species) shows that summer use of winter precipitation by plants is a reasonable expectation (Allen et al., 2019). It is reasonable to expect that zones between 10 cm and 70 cm contain roots, and contain winter precipitation with an evaporated signature. Thus, this constitutes a likely source that was entirely overlooked.

2) Laser spec analysis issues may compromise inferences. Of course the authors know that using a laser spec can yield uncertain xylem water measurements, and they made attempts to correct those data. However, given that the authors are challenging long-standing knowledge, it is essential to control for the potentially confounding effects of organics (not just "methanol and/or ethanol") in the laser spec analyses. Although the authors are more attentive to this issue than many, benchmarking a subset of the samples using IRMS would provide a more convincing data set.

3) Lateral heterogeneities create challenges for representative sampling. For the 0-10 cm depth, where soil water isotope signatures are most heterogeneous, there were relatively few samples collected. Three cores per plot is minimal. Goldsmith et al (2019; see Figure 7) show that dramatic mischaracterizations of the true variance among surface soil water isotope ratios should be expected when using small sample sizes. The authors cannot retroactively sample the soils, but they should recognize that their sampling probably underestimates the range of lateral variation. It could also be considered

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that there are fine-scale variations in pore sizes that plants may differentially sample among (Stewart et al., 1999), but an auger cannot. Given that different pore sizes transport water at different rates, we should expect them to correspond with fine-scale variations in isotope values.

Given these limitations in the sampling and analysis (especially a lack of samples from 10-70 cm), it is unjustified to attribute the lack of finding an appropriate source to unexplained fractionation processes in stems or at the root-soil interface. A more defensible conclusion is that the specific sampling regime used here may not have captured the source waters that were actually used by the trees.

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