Response to Barbeta et al.

In my previous comment on Barbeta et al, I suggested that the mismatch between xylem waters and their measured potential source waters may be due to root water uptake from soils that were not sampled. Soils between depths of 10 and ~70 cm samples were not sampled, which is a large range from which roots often take up water. However, the authors' response states that such an explanation was "not found plausible" and offer the following explanation:

"Our sampling strategy was designed to capture as much as possible the spatio-temporal variability in soil water isotopes, while keeping the analytical cost within reason. With the aim of optimizing the sampling effort (and sampling processing in the lab) we purposely restricted our sampling of water sources to top soil layers exposed to evaporation (0-10 cm) and deep soil layers (below 60cm) only affected by infiltration and mixing processes, and thus expected to display less variability over the season." Indeed, based on soil texture and climate, we did not expect soil evaporation to affect these deep soil layers at our field site. This was confirmed by a detailed soil isotopic profile collected at the end of the summer in September 2018 (Figure SC1a below).

From this figure we see that there is no significant difference in the $\delta 180$ and $\delta 2H$ of soil water among different depths below 20 cm, while the $\delta 180$ and $\delta 2H$ of the upper layers are more enriched (not more depleted). We acknowledge that this isotopic profile could change over the course of the season, for instance following a rain event. Summer rain would deplete the topsoil layers but never to values more negative than winter precipitation, and would also add noise to the soil water line regression. ... "

I understand that the cost of sampling limits how the sampling can be conducted; however, this also limits the potential inferences. I do not understand the justification for the authors' assumptions about the unsampled depths. While isotopic variations in the shallowest and deep soils may be a product of different processes, this does not imply that they are bounds for the full range of isotope values. I believe that the new figure (SC1a), showing a profile from a single time that they selected, demonstrates the possibility of a non-monotonic profile where intermediate soil depths contain isotope values that are not bounded by shallower and deeper isotope values. The intermediate soil depths (e.g., 20-50 cm) contain δ^2 H and δ^{18} O values that are lower than those of the shallowest or deepest soils. The "no significant difference" may arise because those intermediate depths are highly variable and contain a wide range of isotope ratios (see the larger SE values at 20-35 cm). While they display SEs, which are measures of confidence in means, those SE values are smaller than the full range of values (which may be more relevant for supporting the argument that the missing source value could not exist in intermediate soils depths). Regardless, this one snapshot into a profile suggests that intermediate values can have lower deuterium values than those seen in the shallowest or deepest measurements.

Upper soils are also affected by transport processes. Summer rains do not "deplete the topsoil layers", but instead mix with shallow soil water, yielding a new (probably lower) δ^{18} O or δ^{2} H value, and / or displace that (former) surface water, causing it to percolate downward. Thus downward percolating water may have previously been in the shallowest soils (and underwent strong evaporative fractionation so it had low LC-excess), and may be sourced from previous precipitation events (e.g., potentially by precipitation inputs that had especially low δ^{18} O and δ^{2} H prior to evaporation effects).

See Figure 6 in Barbecot et al 2018, which shows that LC-excess values in ~20-30 cm depth are lower than those in the shallowest soils, and that these "evaporated" signals are associated with low δ^{18} O values. Note that one of the sites used in this study is from a sandy soil in France, and that sandy soil preserves prior seasons' precipitation signals.

See Figure 4 in in Oerter et al 2019, which shows that a) the most negative $\delta^2 H$ values often occurred in intermediate depths, b) lower LC-excess values can vary non-monotonically (suggesting downward

transport of previously evaporated waters). They state, "*LC-excess* values were relatively high (near 0‰, Figure 4c) in the upper 10- to 15-cm soil depth from April through mid-June, which indicates that the higher $\delta^2 H_{liq}$ and $\delta^{18}O_{liq}$ values at the shallow depths during this time were due to spring season precipitation with higher $\delta^2 H$ and $\delta^{18}O$ values, rather than being caused by evaporative enrichment in shallow soil water (Figure 4c). Deep soil *LC-excess* values below 20 cm from April through the end of June were approximately -30%, with relatively little depth variability. These deep soil *LC-excess* values bearing an evaporative signal are likely derived from winter snowmelt that was partially evaporated or sublimated prior to infiltration." Perhaps most importantly, Oerter et al use these data to argue that previously described mismatches between xylem water and soil waters may be due to previous researchers' limited sampling of soil waters.

To be perfectly clear, I am not saying that manuscript in discussion does not show interesting data. Nor am I saying that we should rule out the possibility that fractionation occurs upon uptake. The authors suggest that they have a forthcoming paper that demonstrates fractionation upon uptake, which would be a very useful thing to demonstrate. Nonetheless, I remain uncertain why it is implausible that the missing source (in this study) might be soil water from depths between 10 and 70 cm, where isotope ratios can be heterogeneous and variable.

Oerter, EJ, Siebert, G, Bowling, DR, Bowen, G. Soil water vapour isotopes identify missing water source for streamside trees. *Ecohydrology*. 2019;e2083. <u>https://doi.org/10.1002/eco.2083</u>

Barbecot F, Guillon S, Pili E, Larocque M, Gibert-Brunet E, et al.. Using Water Stable Isotopes in the Unsaturated Zone to Quantify Recharge in Two Contrasted Infiltration Regimes. *Vadose Zone Journal*, 2018, 17 (1), pp.1-13.