Response to SC2 by Dr Scott Allen

In this second comment, Dr. Allen further developed his arguments on the possibility of a missing water source that could explain our observation of a persistent hydrogen isotope composition (δ^2H) offset between plant xylem water and all considered water sources. Although the mechanisms brought about by Dr. Allen are sound, they do not give a quantitative explanation of why the offset would occur for δ^2H only, and not $\delta^{18}O$. Dr. Allen quoted only a fragment of our response, but we had already explained why a non-monotonic soil isotopic profile should impact both δ^2H and $\delta^{18}O$ (although not necessarily in exactly the same way). Nonetheless, to fully address Dr. Allen's comments, we have reinforced the idea in our manuscript that a more detailed sampling design (i.e. with more soil water samples from intermediate depths) would have helped capture better the SW-excess and raised any doubt on the existence and persistence of these "unexplained hydrogen isotope offsets" (to quote our own title).

Dr. Allen also brought up a study that explained isotopic mismatches by isotopic differences between the soil water pools that are accessed by plants, using a recently developed technique (Oerter *et al.*, 2019). This response is not the place to comment on this other study. Instead, we would like to mention that the same authors have also published another study, using the same technique, where they conclude to have "found some support for H isotope discrimination effects during water uptake by *Quercus gambelii*" (Oerter & Bowen, 2019). This is in full agreement with the results and conclusions of our own study. The reason why studies, even from the same author, have contrasting results is because the mechanisms leading to the "unexplained hydrogen isotope offsets" have not been identified yet and may vary between soil types, plant species and climate conditions. However, we feel it is rather dishonest to systematically ignore studies that claim that H isotope discrimination effects during plant water uptake may occur. Instead we offer the reader an array of explanations for our results, and remain rather cautious when it comes to identifying the exact mechanisms.

A point-by-point response is provided below.

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 $\delta^{18}O$ and δ^2H of soil water among different depths below 20 cm, while the $\delta^{18}O$ and δ^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^2H and $\delta^{18}O$ 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.

The reason why we presented a detailed soil water isotopic profile in our previous response was not to refute the idea that non-monotonic soil water isotopic profiles could have occurred under certain circumstances. We agree that this isotopic profile does show some soil water isotope depletion in intermediate depths (20-35cm), although not significant. However, as we already mentioned in our response to the first comment, such a non-monotonic profile would imply that we should see also a depleted xylem water $\delta^{18}O$, which was not the case. In addition, such non-monotonic isotopic profiles would need to be sustained over time and space, because the isotopic offset was observed in all campaigns and sampled trees. Xylem δ^2H was persistently more depleted than soil water δ^2H, that is, following rain events and subsequent mixing processes, under relatively dry conditions, both at the beginning of the season and right before autumn leaf shedding, representing a wide range of soil moisture (and isotopic) conditions as well as probably different prevalent depths of water uptake.

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) $\delta^{18}O$ and δ^2H 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 $\delta^{18}O$ and δ^2H prior to evaporation effects).

If summer rain mixes with shallow soil water, it should "deplete the top soil layers" (via mixing) because it is expected to be depleted compared to an initial surface soil water that has undergone soil evaporation. Dr. Allen argues that alternatively winter rain may have been displaced to intermediate depths by subsequent spring rain events, without mixing, and stayed there throughout the growing season. As explained in our answer, the intermediate soil layers are very sandy and cannot hold much water. It is thus much more likely that winter precipitation would remain in the deep soil layers that we sampled.

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 $\delta^{18}O$ 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.

It is not clear to us what is the link between the data shown by Barbecot et al. and our results. First, the data is from a single sampling date, which does not imply a persistent pattern over the growing season, as we observed. Second, Dr. Allen highlights that the LC-excess was more negative in the 20-30 cm that in shallower layers (for that particular date), but as he correctly mentions as well, those negative LC-excess are associated with low $\delta^{18}O$ values. In Fig. 3 of the same paper, it is clear that the modeled $\delta^{18}O$ and $\delta^{2}H$ of infiltrating water are proportional, i.e., they follow the same temporal pattern. Again, we can assume that an intermediate soil layer is isotopically depleted compared to shallower and deeper layers, but as shown by Barbecot et al (2018), this depletion should be occur for both $\delta^{18}O$ and $\delta^{2}H$.

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.

We appreciate the review of previous literature brought up by Dr. Allen. We have already read Oerter et al. (2019), they present an interesting dataset of vapor and liquid water isotopes. In their Fig. 4, the clearest pattern we can see is a rather gradual depletion of soil water $\delta^{18}O$ and $\delta^{2}H$ with depth, i.e. similar to what we found at our field site. It is true that the most depleted layer is not always the deepest, but the one ranging from 60 to 80 cm. We agree that this could suggest percolation of evaporated water, but this could also suggest different infiltration depths of rainwater from events with distinct isotopic composition. We leave these interpretations for those who know well that site and analyzed the data.

As we interpreted it, Oerter et al. (2019) report that soil water vapor probes were able to solve the isotopic mismatches of previous studies in that same site, not other ones. All along the paper, we did not find the statement mentioned by Dr. Allen that "described mismatches between xylem water and soil waters may be due to previous researchers' limited sampling of soil waters". Rather, they state that "Part of the problem in finding the missing water source supplying plant transpiration in some ecosystems, may be an inability to adequately quantify the isotopic composition of potential soil water pools". Specifically, they are referring to different soil water pools that could be accessed or not by roots and that may differ in their isotopic composition. This is already discussed in our manuscript (2nd paragraph of the Discussion).

Importantly, a study published during the discussion of our manuscript, also led by Erik Oerter (Oerter & Bowen, 2019) and using the very same technique (soil and root vapor probes) found very similar isotopic mismatches between soil and plant water isotopes to the ones reported by us. They conclude that while further investigations are needed (we agree), there is some evidence for isotopic fractionation during root water uptake.

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.

We appreciate the interest of Dr. Allen in our work. We hope that we have argued satisfactorily our points of view, so the potential reader may create her/his own. We have now amended our manuscript to acknowledge the possibility of an effect of our sampling strategy on our results. We have done so by including all the arguments that we consider relevant, either provided by Dr. Allen, or by our own responses. Please see the revised version of the manuscript in the next step of the peer-review process. Finally, it is worth saying that our goal is not to demonstrate the existence of isotopic discrimination during root water uptake, but to test specific hypothesis formulated based on previous research, and to do so with data obtained through the most rigorous possible experimental design.

References cited by Dr. Allen

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References

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