

We thank Referee #1 for reviewing our manuscript. We answer the general and specific comments below (in blue) and show how we will revise the paper based on this useful, critical input.

Anonymous Referee #1

This manuscript seeks to determine of tree water source changes as a function of plant water status. Specifically, the study paired point dendrometers, lysimeters, stable isotopes, and root distribution to try and discern if where plants obtained water changed as the water status of the plant and roots changed. They largely conclude that the answer to that question is “yes”. Tree water source changes as a function of plant water status. I consider the question being addressed as an important one that affects our understanding of water sourcing as well as how we model water uptake across time and space. While the question is important, I do have many specific comments that I would like to see addressed.

Specific Comments

Interpretation of mixing models. The major result of this study hinges on the conclusion that, using isotopes and the mixing model, water sources shift. The manuscript presents the results, with confidence intervals during $W=0$ and use the overlap of the confidence intervals to conclude that the values are not different. However, the results of all 3 values from the other two time periods are not reported in the text or Figure 8. Just looking at the one value reported for each of these time periods, I am concerned that there is overlap amongst the values, not allowing the data to conclude that there was a statistical shift in water sourcing across the 3 time periods. If there is no difference in these values, then there is no manuscript.

We thank the Referee for pointing out this issue. We see that our previous choice of sources and the way we reported the uncertainties may have appeared ambiguous. Following comments by Referee #2, we will change our source definition by removing the mobile water end member and only focusing on the shallow and deep bulk soil water. Thus, we will provide new results (and at a higher temporal resolution) for the isotope-based source partitioning in the revised version, methods and results will reflect this change accordingly. Additionally, we will also investigate the effect of weighting the sources by the available water content at each depth.

Small sample size. The data collection is thorough albeit the sample size is low. I understand that this is a tradeoff between replication and detail but it does concern me the level of conclusion being drawn from three stems on two plants. For example, does water source shift change as a function in water status in all types of plants? Plants with different rooting strategies? Plants with different ratios of fine roots? Plants with different hydraulic strategies? I am simply not confident that much can be concluded by 2 plants in 1 lysimeter.

We appreciate the concerns raised by Referee #1 in this comment. We agree that more investigation is necessary to provide more confidence to this result, therefore we will temper the language used in our conclusions and highlight our limitations in the revised manuscript. We do want to highlight that the scientific community has been advocating for such controlled

experiments in order to advance our knowledge regarding plant water source partitioning (Penna et al., 2018). An important advantage of controlled experiments is that we are certain that the plant is not relying on other sources that we were not able to sample as it can occur in field investigations. We will discuss critically in more detail these tradeoffs and place our findings in the context of a field study, where we would not be able to sample all sources available nor capture the spatial distribution of water in the soil as can be accomplished in a lysimeter. We will address the Referee concern in the revised version by improving our sample size regarding the temporal resolution on isotopic composition of sources and xylem, and temper our conclusions to reflect what was found with our limited sample size. Further, we will propose a working hypothesis based on our results and emphasize the need to confirm such observations in field settings and across different species (L-456-459 - we will make sure we make this point clearer and earlier in the revised manuscript). We will propose a common path forward in exploring source water partitioning by incorporating measurements of tree water status, without relying only on isotopic measurements.

Small sample size. Similar to sample size, the isotope sampling is comparing 1 single day across each of the 3 time periods. One single day on 2 plants to conclude that source water partitioning changes as a function of water status is not overly convincing that this phenomenon is consistent or real.

In the original analysis, we used days where end-members were the most distinct within each period to compute xylem source water partitioning. We understand that only one day per period limits the finding of this study. We will improve the temporal resolution of isotopic sampling by including timeseries of isotopic composition of xylem and sources where more days will be shown per period during the experiment as mentioned above. We will also compute the partitioning of sources using other available days within each period. Preliminary time series visualization of isotopic composition of xylem and sources as well as new Bayesian mixing model analysis across other days show that the use of bulk water alone as end-members (see discussion with Referee #2) continues to indicate the larger use of water from deep layers during the period of water deficit. We also observed some variability in the partitioning among the different days within the same period which will be further discussed and analyzed. We will also include figures that will show the isotopic composition throughout the soil profile in relation to xylem water in the different periods to provide more information and transparency to support the analysis. We will address the changes from new analysis on the results and discussions. We will temper the manuscript's conclusion based on the sample size.

Issue of scale. They are comparing 3 broad periods but if water status truly affects water uptake then we would need to see analysis at finer temporal resolution. In other words, water potential (or water status) changes temporally and thus we would need to see a tighter linkages between water status and source partitioning. This would require more days where source water isotopes were sampled to derive this relationship.

We appreciate this fair concern of the Referee in this regard. This shows that clarity is lacking related to our definitions of 'changes in plant water status'. We will provide more detail in

explaining how we defined (and defend) the three distinct periods in the revised manuscript. We wanted to clarify that water status are periods based on continuous measurements of tree water deficit (ΔW). At the daily basis, the deficit of storage in the stem increases as transpiration rates increase and water potentials decrease, resulting in water loss from elastic tissues to offset decrease in water potentials in the xylem as observed in the literature (Zweifel et al., 2001; Steppe et al., 2006; Zweifel et al., 2005; De Swaef et al., 2015). Thus, this stem shrinkage (deficit) pattern is observed daily in trees. However, this offset in stem radius in relation to its fully hydrated state can last longer if the plant is under water stress (as defined by Zweifel et al., 2016, and observed in other field settings). Since plant water stress develops over timescales of days to weeks, understanding plant responses to changes in soil water content and atmospheric demands requires the integration of data from similar timescales. The understanding of changes in water status in our manuscript thus follows similar understanding of plant response to drought in the literature. A single measurement in time cannot provide the same understanding if not integrated over multiple days. Thus, changes in water status ('deficit' or 'no deficit', or simply what could be called 'wet' and 'dry' periods) cannot be defined daily, but whether a change in tree water deficit (ΔW) lasts for multiple days. The use of metrics that are physiological relevant and integrate different aspects of plant hydraulics (i.e. stem capacitance, water potential) in response to water availability are urgently needed to improve our mechanistic understanding in patterns of plant water use and complement stable isotopes observations to go beyond limitations imposed by this technique (i.e. uncertainty regarding fractionation process, extraction techniques, labour intensive). Tree water deficit (ΔW) (relative water content) provides this integrative physiological understanding and allows for inclusion of a temporal element to interpreting plant responses to water availability (Martinez-Vilalta et al., 2019).

We will address the Referee concern by improving the definition of periods in our revised manuscript and better reflect this choice in our discussions, along with more isotopic measurements within each period, as mentioned above.

Role of fine roots in explaining partitioning. The paper states "...that tree water source partitioning is driven by plant water status, and not by patterns of fine root distribution". The analysis never really provides evidence that this response is due to water availability at the expense of fine roots. In other words, the role of roots needs to be more definitively analyzed. Additionally, I am concerned about the conclusions from this that apply to field situations. This was a contained lysimeter that had fine roots throughout as opposed to broader strategies of fine root distributions seen in the field. Thus, there may be a role for fine roots that can't be captured here.

We agree with the Referee and we will remove this statement from our manuscript. We will maintain the findings on fine roots distribution in the lysimeter in the revised manuscript to support the ability of uptake throughout the observed depth, but we will remove statements that go beyond our own observations. We will address this change in the discussion and conclusion of our manuscript.

Technical Corrections

Lines 76-80. Really, 3 of these questions are not effectively addressed in this paper.

We will address this issue in the revised manuscript by narrowing the questions to the true scope of this work. This will result in changes to our overarching research question and the removal of question 3 and 4. This will also result in changes to the discussion in the revised manuscript by narrowing down to the main questions.

Lines 233-236. I do not entirely understand how the study separates out the “water deficit” and “intermittent water deficit”. For example, if you looked just at June 1-3, the values and diurnal cycles look similar to June 23-24. Or June 4-6 look similar to May 17-19. In other words, the classification into 3 broad categories feels coarse and likely biases the results.

As we described above, we provide a physiological definition of tree water status, that is not based on soil measurements but directly inferred by hydrometric measurements in the plant. However, we agree that the definition of periods needs to be further clarified as it is not only a point in time but an integration of multiple days that represents plant response to soil water conditions and atmospheric demands. Therefore, the “water deficit period” (or simply ‘dry period’) only include days where the stem tissues are not fully water saturated and tree water deficit (ΔW) does not cease completely overnight (positive values) over several days. Whereas the intermittent water deficit data (between ‘wet’ and ‘dry’) shows periods where tree water deficit (ΔW) is identified but recovered shortly thereafter. For example, during June 1-3 as questioned by the Referee, we observed two days of tree water deficit (ΔW) (June 1-2), but followed by a recovery day (June 3). Conversely, June 23 and 24 there is no tree water deficit (ΔW), which lasted until the end of the experiment (these two days are also not part of any defined period). On June 4-6 the plant is in between days of tree water of deficit, while this is not the case for May 17-19. In the revised manuscript, we will address this issue by improving the definition of periods.

Lines 342-344, Lines 360- 361. This is a bit overstated based on the data presented.

We will temper the statements in the revised manuscript.

Lines 385-387. This may be true, but I am not convinced that you can conclude that these are anisohydric. There are many isohydric species that have -6 water potentials.

We agree with the Referee. We will remove this statement from our discussion in the revised manuscript. We will adjust the discussion accordingly.

References cited in this response

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