Response to the interactive comment of Juan Pedro Ferrio (Referee)

Authors' responses in bold.

General comments

The work by Barbeta et al. highlights one critical issue in the application of stable isotopes as hydrological tracers for the study of plant water uptake patterns. Methods are described properly, but some clarifications are needed. In general, the data is clearly presented and the discussion is well written and focused. Some improvements could be made in the figures in order to make them more self-explanatory. Overall, the manuscript is timely, shows good quality data and makes a significant contribution to ecohydrology.

We appreciate the positive assessment of the reviewer. Following his comments, we have amended the manuscript to improve its clarity and added additional analyses that were not shown previously. We agree with the reviewer that those analyses are helpful for the understanding of the data presented (notably, for the SW-excess).

Specific comments

The calculation of the SW-excess, instead of the deviation from the LMWL, is properly justified in the methods section, and appears as a reasonable alternative for the context of this study. However, I am concerned about the fact that soil data did not always show a single evaporative line (e.g. 5/5/17; 23/5/17; 4/7/17). It would be useful to show the fitting statistics for these regressions (e.g. r2, intercept, slope, p-value). One way to consider this uncertainty is to take into account the confidence intervals of this slope in order to recalculate the errors associated to the SW-excess, and eventually include this as a kind of "analytical error" term in the models.

We agree with the reviewer that the fitting of the soil water line may influence the calculation of the SW-excess. Importantly, non-significant correlations of the different soil water samples from the same sampling date and plot can reduce the relevance of the SW-excess. In the revised version of the manuscript, we now present a new table with the r-squared, intercept, slope and *p*-value of the soil water lines for each plot and sampling date (Table S3). In addition, we re-ran the analyses without those values corresponding to cases in which the soil water line regression was not significant. However, those cases did not have a significantly different SW-excess compared to cases with significant soil water line regressions. Accordingly, with have now added the following text:

In Results:

"The linear regression of the soil water line was significant for most of the sampling dates and plots, as shown in Table S3. Consequently, we removed from the multivariate analysis of the SW-excess those data corresponding to sampling dates and plots that did not present a significant soil water line regressions. Still, the SW-excess did not significantly differ between cases with significant soil water lines and cases with non-significant soil water lines (P = 0.45)."

In Discussion:

"In addition, in our study, the correlation of soil water $\delta^{18}O$ and $\delta^{2}H$ was not always significant (Table S3), although this did not seem to affect the SW-excess quantitatively. However, the concept of the SW-

excess becomes less meaningful when soil water isotopes are not significantly correlated, since the error associated to the regression coefficients could be of the same magnitude than that of the calculated SW-excess"

Regarding the degree of mixing between precipitation and different soil water pools (e.g. line 59, lines 70-75, lines 380-381), and the effect of recent rainfall on soil water d18O and d2H (lines 287-290), it is particularly suitable the discussion about rewetting drying cycles presented in (Tang and Feng 2001). Indeed, (Tang and Feng 2001) also found little effect of recent precipitation below 50 cm depth, with the exception of particularly strong rain events.

Unfortunately, we did not know this study from Tang and Feng, that reports seasonal dynamics of the isotopic mixing of rain and soil water, depending on rain amount and soil water status. We have now included citation of this study in the fragments mentioned by the reviewer.

Figure 4. The point that coarse roots show larger fractionation than twigs does not support the "fractionation during water uptake" hypothesis, but favours the option of some kind of isotopic-exchange undergoing in stored water. During a previous field study (Martín-Gómez et al. 2017), we did some preliminary tests comparing twig water with water extracted from trunk cores. Interestingly, and in line with the present study, we found a depletion in d2H of trunk water of about 10‰ as compared to soil and twig samples, although this was not consistent across tree species and sampling times (Martín-Gómez et al., unpublished). The apparent "bypass" of the root fractionation along the path from soil to twigs described by Barbeta et al., and the differences between xylem sap and distilled xylem water shown by Zhao et al. (2016), suggest that fractionation processes associated with water storage could be the key for the observed changes, and certainly deserve further studies.

This preliminary data on the relative depletion in δ^2H of water in trunk cores mentioned by the reviewer does indeed coincide in sign and magnitude with our results and those of Zhao et al. (2016). We thus agree with reviewer that the hypothesis of fractionation between plant-internal water pools has more support by our findings than fractionation occurring during root water uptake. We think that we clearly state this in the Discussion of this manuscript. Nevertheless, further experiments targeted to this mechanism are required to test this hypothesis.

Technical comments

Figure 1. Although the measurements were taken at different time intervals, it would be desirable to adjust all the panels to a single scale in the X axis. In Figures 2, 6, 7, 8a, to facilitate interpretation, I would combine fill colours with different symbol shape, and keep them unified throughout the manuscript. For example, circles could represent xylem water, squares soil water, diamonds for fog and rainfall, upward triangles for stream water, ground water and rock water.

We appreciate the constructive comments of the reviewer. This figure has already been modified during a previous round of review. In fact, the time axis in Figure 1 was originally presented as it is now suggested by the reviewer but was changed to its present form following the advice of a previous reviewer. We think that the current version (with separate time axes) is more correct and keeps a good readability. Regarding the dual isotope plots, given that nowadays a large portion of paper reads are online, the color palette used for the figures is clear enough to distinguish between the different groups depicted and adding different shapes would not increase substantially the clarity of the figures.

Line 165. If I understood well, 3 of the beech trees and 1 of the oaks were sampled from the roots, whereas in the rest of the trees the sampling was based on twigs. According to Figure 4, the observed d2H-depletion was much stronger in coarse roots than in twigs. However, I wonder whether the significant test shown simply indicates that twigs and roots have different SW-excess, or it shows the significance of the SW-excess (i.e. divergence from SW-excess=0). In this regard, the text citing the figure does not clarify this point: "we found differences in SW-excess when the xylem water was collected from coarse roots rather than from twigs (Fig. 4)." In any case, since SW-excess in roots is about twice that found in the twigs, it is worth to indicate them separately in the rest of the graphs.

We have now clarified this point in both the figure legend and the text. We have modified the sentence in Discussion mentioned by the reviewer (see below). We also specified in the legend of Figure 4 that the significance highlighted in the plot with an asterisk refers to the difference in the isotopic composition and SW-excess between the water extracted from coarse roots and twigs. We found more appropriated pooling the data of coarse roots and twigs in the other figures. Figure 4 already highlights this difference, and the messages provided by the other figures would be more difficult to interpret if we add this additional factor. However, we included the effect of this factor in the analysis of the SW-excess (Table S2).

In Discussion:

"The SW-excess of xylem water collected from coarse roots was significantly more depleted than when xylem water collected from twigs"

References

Martín-Gómez P, Aguilera M, Pemán J, et al (2017) Contrasting ecophysiological strategies related to drought: the case of a mixed stand of Scots pine (Pinus sylvestris) and a submediterranean oak (Quercus subpyrenaica). Tree Physiol 37:1478–1492. doi: 10.1093/treephys/tpx101

Tang KL, Feng XH (2001) The effect of soil hydrology on the oxygen and hydrogen isotopic compositions of plants' source water. Earth Planet Sci Lett 185:355–367. doi: 10.1016/S0012-821X(00)00385-X

Zhao L, Wang L, Cernusak LA, et al (2016) Significant Difference in Hydrogen Isotope Composition Between Xylem and Tissue Water in Populus Euphratica. Plant Cell Environ 39:1848–1857. doi: 10.1111/pce.12753