

## Response to referee (Xin Song)

### General comments

In this study, Barbeta et al. applied stable isotope techniques to investigate potential water sources of two broad-leaved tree species in a temperate, riparian forest. For this purpose, they made collection of a season-long dataset of  $\delta^{18}\text{O}$  and  $\delta\text{D}$  compositions from tree xylem water, soil water at different depths, and other potential water sources. They show for both of the tree species that different water sources can be appropriately identified with oxygen isotope data. However, the same conclusion cannot be drawn from hydrogen isotopes, as  $\delta\text{D}$  of xylem water on many occasions apparently fell out of the range as encompassed by the potential sources. The authors made a detailed discussion of several possible causes of the observed xylem-soil water  $\delta\text{D}$  mismatch, and concluded that isotopic fractionation in the unsaturated zone and/or within plant tissues could well be the driving mechanism.

This is a well-designed field study that addresses an important topic in stable isotope ecohydrology. The manuscript is well written, and data analysis was carried out in a solid manner. The finding of  $\delta\text{D}$  fractionation in two commonly occurred temperate tree species is a timely reminder that this phenomenon may not be an exception as previously thought, only restricted to a narrow range of species such as mangroves or species from arid regions, but more likely one that is common to a wider range of species. This finding will have important implications for stable isotope ecohydrological and ecophysiological studies.

**We very much thank the referee for his positive assessment of the manuscript. We have amended the manuscript following his comments. Please find below the responses to the referee's comments.**

### Specific comments

1. Line 232: There is a lack of explanation of the rationale behind the modeling exercise of using SW-excess corrected  $\delta\text{D}$ . As far as I understand, given the possibility that fractionation occurs at the soil-root interface or within plant tissues, the purpose of applying such type of correction is to obtain real  $\delta\text{D}$  values of the water that is available for uptake by plant roots (i.e. correcting  $\delta\text{D}$  back to the point before fractionation occurs). If this is the case, then an a priori assumption for doing correction based on a SW-excess line would be that soil water pools are the only sources of water available for tree roots to take up (otherwise an observed "apparent" SW-excess could have been caused by contribution from non-soil sources that do not necessarily follow the SW-line). Was such an assumption met in the present study? How general applicable is this sw-excess based method to other studies? I think it is worthwhile for the authors to discuss further on these important points (this could be done either in M&M or Discussion).

**The referee is right. The SW-excess correction carries out the assumption that tree water uptake occurs exclusively in soil water pools, although stream or groundwater pools are lumped together and kept in the analysis when applying the mixing model. As explained in the text, we ruled out fog and rock water as potential sources because their isotopic signal is much more enriched than xylem water (Fig. 2). The assumption of exclusive root access to belowground (soil and stream/ground) waters seems thus reasonable. We were not able to propose a correction that would also include stream/ground water, because stream/ground water sometimes plotted outside (above) the soil water line (Fig. 2). However in a lot of cases, stream/ground water coincided with the lower part of the soil water line, thus justifying**

their inclusion in the mixing models, even after the SW-excess correction on the xylem waters. Also, based on the observation that the dual isotope approach with the corrected  $\delta^2\text{H}$  yields source contributions that had a stronger relationship with environmental conditions (e.g. the higher the rainfall amount, the higher the top soil water contribution, Table 1, now in the main document), we concluded that our correction and the underlying assumption was justified. We have now better detailed this rationale in the Methods:

*“Correcting xylem  $\delta^2\text{H}$  with SW-excess implies that tree water uptake relies only on soil water pools because the SW-excess is calculated using the slope and intercept of the soil water line. However, the lower part of this line usually overlaps with unenriched stream/ground water. Thus, we expected that  $\delta^2\text{H}$  departures from this line are meaningful in potential cases where trees are accessing not only soil water but also stream water.”*

While adding this remarks in the Discussion:

*“This exercise was made with the purpose of providing a sensitivity analysis. However, its use in other sites and plant species should be made with caution, as it is very likely that the observed  $\delta^2\text{H}$  offset may display different patterns depending on other water sources.”*

2. Line 272: I understand the authors' argument for a lack of sensitivity of d18O to soil water content here, but still the line “changes in the isotopic composition of soil water with rain addition...” seems somewhat contradictory to what is already stated in Line 269 “rainfall amount (...) had a negative effect on top soil water d18O...”. Isn't it like saying that “rain addition has a significant effect on d18O” versus “rain addition may not cause sufficient changes in d18O”?

This is correct. As we already explained in our answer to referee #1, there was a confusion in the interpretation of the results, since it is top soil  $\delta^{18}\text{O}$ , not  $\delta^2\text{H}$ , that is negatively correlated with top soil water content. We have now modified this sentence and the interpretation of the results accordingly:

*“In the top soil, water content was negatively correlated with  $\delta^{18}\text{O}$  ( $P < 0.05$ ), but not with  $\delta^2\text{H}$ . This is surprising because isotopic fractionation occurring during soil water evaporation and water vapour and liquid diffusion should affect both water isotope signals in the same direction. The fact that these water signals respond differently to top soil water content but similarly to rainfall amount (see above) indicates that observed changes in top water isotope signals are primarily governed by precipitation input rather than soil water evaporative enrichment. It may also be that hydrogen isotope of soil water are reflecting extra fractionation processes (e.g. root uptake) compared to their oxygen isotope counterparts.”*

3. Line 164: How often was stream and groundwater collected? This sentence reads as if they were collected every day? Or on every sampling campaign?

Stream and groundwater collected were collected for every sampling date that we sampled the trees and the soil, so fortnightly. We have slightly modified the sentence to clarify this:

*“we collected water from the stream for every sampling date”*

4. Line 276: where is the rock moisture data in Fig. 2?

**Because we did not include rock moisture in the source contribution analysis, we had initially removed it from the plots. Finally, we have decided to include it as it has its importance in illustrating our decision of not considering rock moisture as a potential source (see revised Fig. 2).**

5. Lines 280-284: I'd like to argue here that if xylem water had become progressively enriched due to stem evaporative enrichment over the past winter, we would expect xylem water to deviate not only from the LMWL line but also from SWL. Yet, from fig. 2 soil and xylem water appear to fall into pretty much the same line.

**We have not found much literature reporting branch evaporative enrichment. However, in a recent paper (Bowling et al. 2017, see references in the manuscript), a similar early spring stem isotopic enrichment was attributed to water losses prior to budburst. While we cannot be completely sure that this is the case for the studied trees, there is no apparent reason than branch evaporation would not produce a water evaporation line with a similar slope than soil. We have now changed a "was" by a "could be", which is more appropriate in this case:**

*"This could be indicative of stem evaporative enrichment over winter."*

6. Line 346: change "have also reported isotopic offsets" to "have also been reported to display isotopic offsets"

**Changed.**

7. Lines 360-361: Is there also a possibility that cryogenically extracted soil water does not truly represent bulk soil water? See a recent Ecohydrology paper by Orłowski et al. (2016) Critical issues with cryogenic extraction of soil water for stable isotope analysis

**Yes, this a possibility that we considered. However, internal tests of our cryogenic extraction line showed non-significant discrepancies between spiked and extracted water in sands. In fact, sandy soils are much less affected by methodological issues of cryogenic extraction. Besides, we would expect effects on both water isotopes, not only on  $\delta^2\text{H}$  data.**

8. Line 379: change the second comma to semicolon

**Instead of doing this, we have added a period:**

*"Similarly, we do not think that branch evaporation is responsible for the reported isotopic offset (Martín-Gómez et al., 2017). If it were the case, we would expect the magnitude of the offset to vary over the season with evaporative demand and to affect both hydrogen and oxygen isotopes, i.e., the opposite of what we report here."*

9. Line 381: "fore example" should be "for example"

**Changed.**

10. Line 387: "sympastic" should be "symplastic"

**Changed.**

11. Line 444: this is not a complete sentence

**We have amended this sentence.**

12. Line 445-446: The idea is great, but may not be easy to realize with the current extraction based method that is only capable of extracting bulk water from a plant tissue. Methodological advancement is apparently needed to confer ability of separately analyzing water from different pools (i.e., parenchyma cells versus xylem water) within a given plant sample.

**We agree with the referee. We have thus added the following sentence:**

***“Secondly, to obtain a better understanding of the isotopic dynamics of water pools within plant tissues, notably those associated with plant storage water and its dynamics (Pfautsch et al., 2015) which will require developing new extraction methods for xylem water”.***

13. Line 448: change “fraction” to “fractionation”.

**Changed.**

14. Fig. 5: minus signs are missing in several places of the y-axis.

**We have corrected this.**