In this document we provide a detailed point-by-point response to the comments raised by an anonymous reviewer and by the editor on our manuscript submitted to Hydrology and Earth System Sciences (hess-2021-333). Reviewer and editor comments are in **bold** font. In this document, line (L) numbers refer to the revised version.

<u>COMENTS BY ANONYMOUS REVEWER #3:</u>

This manuscript presents a very interesting meta-analysis in relation to the commonly found (both in field and controlled conditions studies) offsets between precipitation, soil, and plant water stable isotope composition. It explores the potential biotic or abiotic reasons behind these offsets and exhaustively discuss them with current literature. I believe the methodology chosen by the authors (both LC-excess and SW-excess analysis) and statistical approach are correct and that they properly addressed and solved the comments done by previous reviewers. I have a few minor changes that I believe could improve at some points the manuscript:

Thank you very much for these comments and for the time invested in reviewing our manuscript.

Line 29. Rewrite lines 29-30-31. I would put together the sentence "SW-excess was more negative in cold and wet sites…" with the one in line 30 "The climatic effects…" so you can read the results together their implications.

Thank you, we have modified the text accordingly (L31-32).

Line 146. Did you check if there were differences associated to the type of "stem water" was collected? I understood in line 167 that you couldn't address the potential effects of water extraction methodology, but I am not sure you could analyze different type of stem samples.

In our database 92 out of the 112 studies sampled exclusively woody species (shrubs and/or trees). In our database, we only included values of water isotopic composition obtained from samples of lignified tissues (i.e. wood cores or small twigs). For the remaining 20 studies focusing on non-woody species, only water samples from rhizomes, root collars or other similar tissues were considered. Hence, in our study all plant (stem) samples included are assumed to not have undergone any type of isotopic enrichment due to evaporation and we did not consider comparing among sampled organ types. Nonetheless, in our study the effect of "type of stem" would be implicit in the analyses of the effects of growth from (L170-171). Regarding differences in water isotopic composition (and their associated SW-excess and LC-excess) among stem water pools (mainly xylem water and storage water, following Barbeta et al. 2022), the paucity of studies using extraction methodologies alternative to cryogenic water distillation (three out of 112) allowing for the distinction between water pools currently impairs this comparison. Fortunately, in the near future, surely numerous studies applying the recent methodological advances we are witnessing in the field (Barbeta et al. 2022; Chen et al. 2021) will enrich the available data to address this question.

Line 389. Lower/ higher. Always it is useful to add more negative, less negative…

Thank you, we have modified the text accordingly (L390)

Line 521. In this last paragraph I encourage the authors to include some discussion about plant hydraulic strategy and capacitance. Did you explore if there could be any effect associated to the fact that a species is more isohydric or anisohydric? Leaf hydraulic habits are generally associated to stem hydraulic patterns (anatomy, behavior). For example, as isohydric species close stomata quite fast in response to soil water potential changes, one could expect that they would avoid using "storage water", (for example the water in the parenchyma here assessed). We could expect the opposite behavior for anisohydric species and then higher differences between soil and stem water. It is just a hypothesis but, in my opinion, it would be worthy to explore. Indeed, in line 505, the

“hydraulic strategy” it is mentioned but I believe the authors only checked angiosperm vs. gymnosperms.

These are very interesting remarks, also very relevant to our study interpretation. Indeed, the implications of storage water use are discussed in the main text (L436-437). Regarding the discussion on the “hydraulic strategy”, we agree that our approach (comparison of gymnosperms vs. angiosperms, L505-507) constitutes a coarse conceptual simplification and that within these clades there is actually a whole gradient of hydraulic strategies implying the coordination of stomatal behaviour, leaf and wood anatomical traits (e.g. Rosas et al. 2019). On the other hand, the distinction between isohydric and anisohydric species can also be viewed as a continuum and it has been shown that a tight stomatal regulation might not be necessary linked to, for example, vulnerability to drought (Martínez-Vilalta & García-Forner, 2017). Even more, there are species that can depict significant plasticity in their stomatal behaviour (e.g. Guo et al. 2019, Wu et al. 2021), making the distinction between isohydric and anisohydric species even more blurry.

<u>COMENTS BY THE EDITOR (Prof. Dr Markus Weiler):</u>

1) Were the SWLs and LMWLs calculated by orthogonal least squares fitting? They should be because both the X and Y data have uncertainty, and fitting by standard least squares can artificially reduce slopes, which could have consequences for the findings. -> you provided a Figure showing that the slope of a regression line is not statistically different from 1. This is true, but since the correlation is quite low, there is no wonder, that the line is not different from one. However, if you would look at the differences of individually calculated SWL by OLS vs TLS, the can be different up to 5 per mil. The question is, does this matter in the further analysis. So please convince me, that the results in the paper using the SWL is the same if you use OLS or TLS is the same

For the studies included in our meta-analysis, we found that when fitting our soil water lines (SWL’s) for datasets where there was a strong linear correlation between δ^2H and δ^{18}O of soil water, there were no differences in the slope or intercept term of the SWL between the ordinary (or total) least squares and the orthogonal least squares methods (see bottom panels of Figure A in this document for some examples). In contrast, for those studies where there were a few data points where the relationship between δ^2H and δ^{18}O was clearly different from the rest of the data (see for example the graphs corresponding to Goldsmith et al. 2012 or Gómez-Navarro et al. 2019 in Figure A in this document), the orthogonal least squares method underestimated the intercept and overestimated the slope of the SWL, with respect to estimates from the ordinary least squares. In such cases, estimation of the SWL parameters with the orthogonal least squares method would have resulted into an overestimation of the absolute values of SW-excess. Wehr & Saleska (2017) noted that the orthogonal least square fitting is unbiased only when the error variances of the two variables (δ^2H and δ^{18}O in our case) are equal, but the error of δ^{18}O is usually smaller than that of δ^2H. We argue that the ordinary least squares should be a good approximation for fitting our SWL’s, as it should render much less biased estimates of the intercept than the orthogonal method (according to Wehr and Saleska 2017 and also to Zobitz et al. 2006)

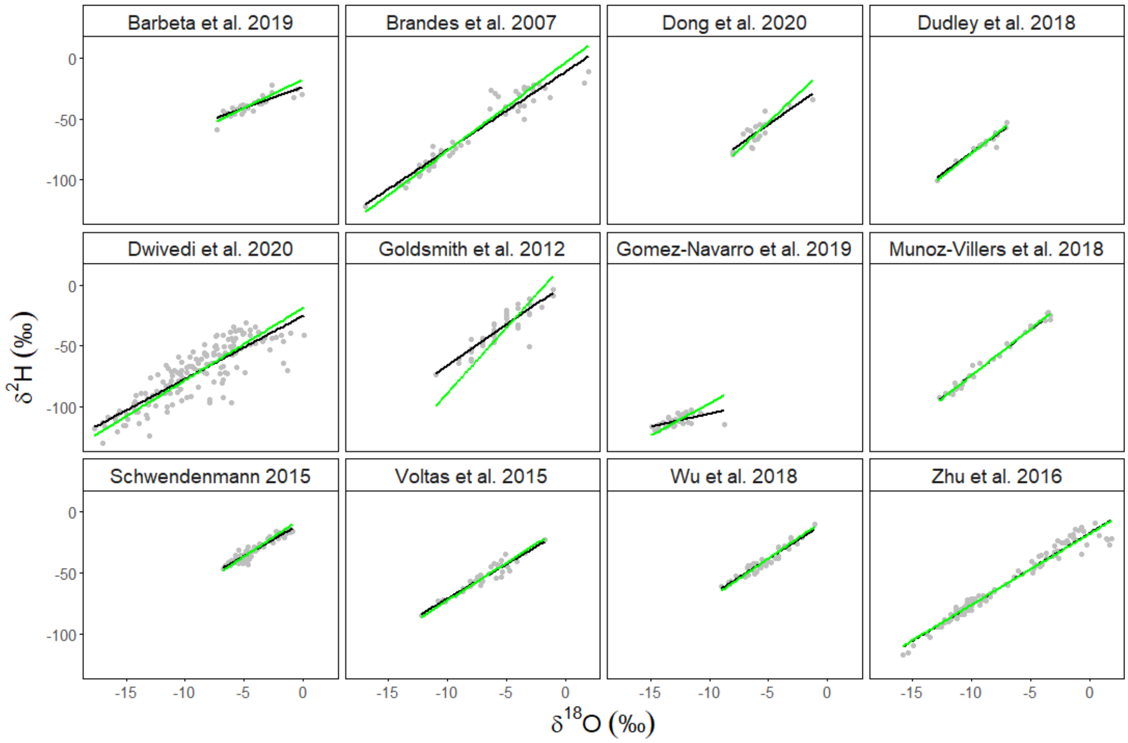

Figure A. Dual plots of soil water isotopic composition (δ^2H and $\delta^{18}O$) from a subset of studies included in the meta-analyses (see Table 1 and list of references in the main text for the corresponding references). The black lines depict the soil water lines (SWL) fitted with the ordinary least squares fitting and the green line depicts the SWL fit with the orthogonal least squares method.

2) I agree with reviewer #2, that "My main concern relates to the approach towards the soil water data. Most studies used in the meta-analyses will have taken soil samples across a range of different depths.". In addition, most studies do not even sample the full range of rooting depth, hence they may miss pools that plants can take up. You argued, that there is no way of dealing with this, as you may not have the data and information to look at shallow or deep separately. But at least, you should provide a clear statement in the abstract and conclusion, that this is a main weakness of this meat-study, that the isotopic composition is sampled at different depths (and with different methods), which may be even more problematic.

In agreement with your remark and those by reviewer #2, we found that there was large variability in sampling methodologies, depths and intervals. Following this suggestion, this is now explicitly stated in the abstract (L23) and in the conclusions (L542). Obviously, we cannot be sure that all potentially relevant water sources and soil layers were sampled in all studies, but this is an inherent limitation to any study using analyses of water isotopic composition to infer plant water sources. During our data collection, we recorded the depth of soil sampling for analyses of water isotopic composition. This information will be provided on the database associated to the publication, but we opted not to calculate SW-excess separately for deep and shallow soils because these were largely indistinguishable (see Figure

B in this document for some examples) and separating soil pools increased the uncertainty of the SWL fit.

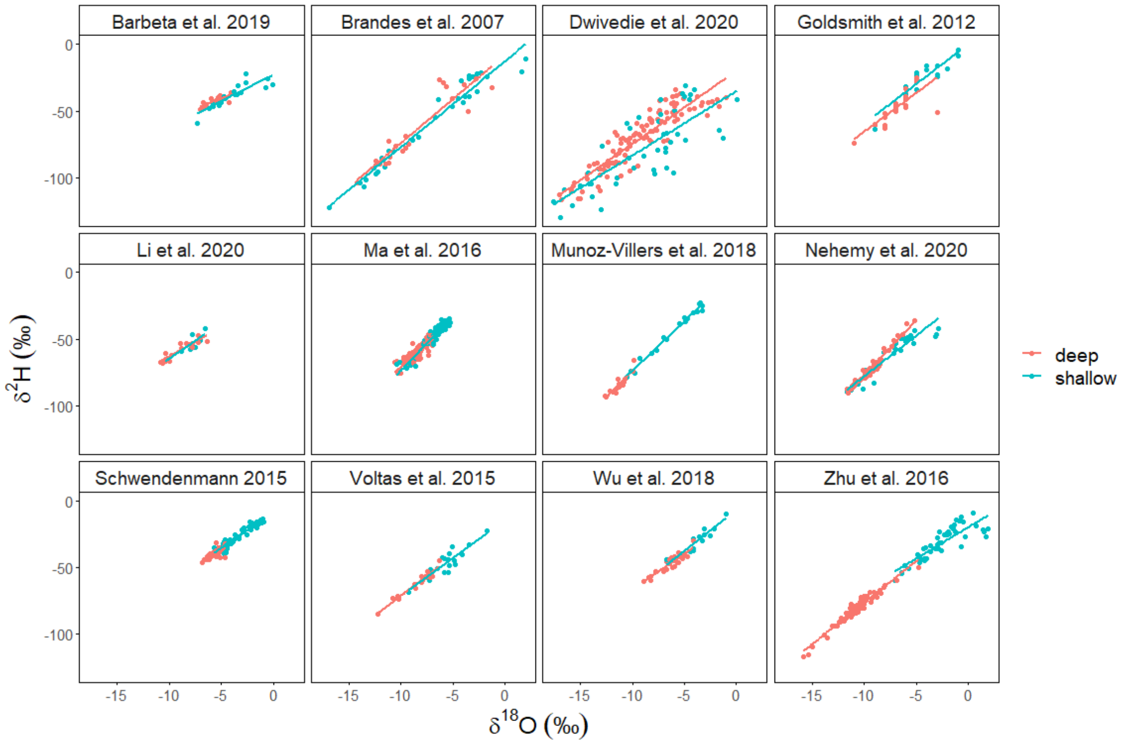

Figure B. Dual plots of soil water isotopic composition (δ^2H and δ^{18}O) for deep (orange lines and symbols, depth ≥ 30 cm) and shallow (cyan lines and symbols, depth < 30 cm) samples from a subset of studies included in the meta-analyses (see Table 1 and list of references in the main text for the corresponding references). From the 112 studies, 92 of them reported sampling depths at shallow and deep layers of the soil profile.

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