Reply on RC2  
Javier de la Casa et al.

Author comment on "Isotopic offsets between bulk plant water and its sources are larger in cool and wet environments" by Javier de la Casa et al., Hydrol. Earth Syst. Sci. Discuss., https://doi.org/10.5194/hess-2021-333-AC3, 2021

AC: We thank this reviewer for their positive evaluation of our manuscript and more importantly for the time invested and the constructive comments.

RC: This is an interesting study that adds to several other recent meta-analyses on the topic, by exploring the potential role of various biotic and abiotic factors in determining the offset between plant and source (in this case: soil) water stable isotope composition.

AC: We thank this reviewer for these positive comments about our work.

RC: I recommend that the authors reconsider their title (main contribution). As it stands, revealing the offset is not the main novelty that this study brings. Indeed, the second line of the abstract already states that many studies have reported this, and this has also been demonstrated by recent other meta-analysis studies. This study does include several really interesting novel aspects, that I would recommend the authors put the focus more on, e.g. the outcomes of the specific hypotheses that were tested, the relationships with air T and VWC, combined use of LC-excess and SW-excess (but see comment below). The abstract and conclusion could be slightly edited to highlight the novelty a bit more as well.

AC: We appreciate this comment and we propose the following alternative title:

Large isotopic offsets between bulk plant water and its sources in cool and wet environments

RC: 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. It is known that at many sites, the water stable isotope composition of soil water changes with depth (and the slope of the soil water lines, which is the basis of the analyses here, does as well); the variation with depth is also often used to determine proportional plant water uptake patterns of different soil depths, and Amin et al. 2020 have shown that uptake occurs mainly from the upper part of the soil profile. The authors do not provide information on their approach to dealing with data from different depths, but it appears that they have not taken this into account and have used all soil water data available to derive soil water lines. The extend of the offset between the plant and actual source water could therefore be significantly over- or underestimated. It would be good if the authors provide a clear rationale for their approach and discuss the implications of this, and/or investigate how different the results might be e.g. for SW-excess of shallow and deep soil water as the source. Similarly, it is questionable that the authors currently consider VWC at a range of depths, but not the isotope composition.

AC: In order to fit a soil water line (SWL) for a given field campaign there is no alternative but to use soil water isotopic composition from different depths. The theory leaves little room for interpretation in this matter: in the absence of any water inputs (precipitation), for any period (days to weeks) during which soil water is subject to evaporation, then soil water isotopic compositions along the entire soil profile (at all depths) will align along an evaporation line in the dual isotopic space. A poor linear fit usually results from incomplete mixing of different water inputs (precipitation events), at different depths (Bennetin et al. 2018). That is why we only calculated values of SW-excess for those campaigns where the linear fit was statistically significant. In any case, the soil water line is always based on soil data from different depths. Therefore, it is not possible to derive an "SW-excess of shallow and deep soil water as the source" as this reviewer suggests, since using either deep or shallow water would not result in a linearly fitted soil water line. In addition, SW-excess is not an estimation of the soil water pool contributing to plant water but rather an estimation of the separation in the dual isotope space between the soil and xylem water, irrespective of the processes underlying this separation.

RC: Although there is only a small proportion of the studies that does not (only) report soil water from vacuum distillation, there is a lot of literature that has demonstrated that there are strong differences in the pore spaces that are sampled via vacuum distillation versus lysimeters. Those results also show that the LC-excess of soil water obtained via vacuum distillation can be very different from soil water obtained via suction lysimeters. I agree with the authors that it is beyond the scope of this study to address this here as well, but I would argue that the data of the suction lysimeters should not be included in the analyses here. Alternatively the authors could indicate which studies have used which type of data and explore whether these are outliers/affect the results.

AC: The reviewer raises an important issue that we did take into account during our data collection, by carefully listing each time the extraction technique was used to obtain soil water. Among all the studies that we retained for the calculation of SW-excess, only four used suction lysimeters (Jespersen et al. 2018, Lovelock et al. 2017, Yin et al. 2015, Zhang et al. 2011), and an extra ten studies combined cryogenic extraction and suction lysimeters (Dwivedi et al. 2020, Geris et al. 2015, Geris et al. 2017, Grossiord et al. 2017, Hervé-Fernández et al. 2016, Li et al. 2020, Marttila et al. 2018 and Muñoz-Villers et al. 2018, Nehemy et al. 2020, Snelgrove et al. 2020). We added these references to the edited manuscript (L60). Unfortunately, in most of these studies, no distinction was made between extraction techniques when presenting the data (unlike in Brooks et al. 2010, for
example). Furthermore, we found that excluding the isotopic composition of soil water obtained from suction lysimeters did not have any impact on our overall results and interpretation, as there were no statistical differences between the SW-excess estimated for studies that used exclusively cryogenic vacuum distillation to extract soil water, and those that included water sampled with lysimeters $P=0.64$)

This is now clearly stated in the revised manuscript, by adding the following sentence in the Results section:

“Finally, for those studies that included soil water samples obtained by lysimeters, the estimated SW-excess was not significantly different from that estimated from studies extracting all soil water with cryogenic vacuum distillation ($P = 0.64$).”

**RC:** Following on from the comment above, I suggest the authors do not refer to precipitation, stream water and groundwater as the ‘mobile’ water, as this is often used to describe the freely draining or large pore space (i.e. not tightly-bound) soil water as typically sampled by lysimeters.

AC: Thank you for this comment, we have modified the text according to this suggestion and a similar one by reviewer 1 (L27, L147, L278 & L494).

**RC:** Finally, I wonder why the LC-excess for the soil water itself was not also included in the analyses to understand offsets. This could help with the interpretation of the relationship between plant LC-excess and SW-excess, and the role that climate or soil properties may have.

AC: Here we used the joint analysis of plant LC- and SW-excess to test whether significantly negative values of SW-excess were likely to depict genuine offsets between plant water and its most likely source (the soil), or if instead these significantly negative SW-excess values would be caused by a plant accessing water pools of meteoric origin, instead of soil water. We believe that the discussion of the soil LC-excess and how it could be affected by climatic drivers would be beyond the scope of this study.

**RC:** Regarding the abstract:

L 21: make it clear here that you are looking at offsets in plant water.

AC: We now specify that these are “plant-source” $^2$H offsets.

**RC:** L 21: make it clear here that LC-excess is calculated based on the LMWL (not the GMWL)

AC: Our text in the abstract already reads “a line conditioned excess (LC-excess) that describes the $^2$H deviation from the local meteoric water line”. No changes have been performed.
RC: L 30: I did not see direct evidence for the statement that offsets are caused by isotopic heterogeneity within plant stems. I’d leave this and the last sentence out of the abstract and keep it in the discussion as a possible explanation/implication.

AC: Thank you for the appreciation, we have removed this sentence from the abstract as suggested by this reviewer and also by reviewer 1.

RC: Introduction:

L56-59: would be easier to follow if this sentence was split. Also in L 58 change ’that showed’ to ‘showed that’

AC: This sentence has been split into two. Now it reads: “More recently, an isotopic offset between plant stem water and pot soil water has been identified in various glasshouse experiments with non-halophytic and non-xerophytic plant species (Vargas et al., 2017; Barbeta et al. 2020). In addition, another recent glasshouse study showed that this isotopic offset was larger in plants forming symbiotic associations with mycorrhizal fungi (Poca et al., 2019).”

RC: L62: seems a bit strange to refer to Poca et al. 2019 as an ‘early study’?

AC: We have modified the text in line with this suggestion which now reads “Another recent glasshouse study”.

RC: L116: it would be good to provide more information on the approach to combine LC-excess with SW-excess.

AC: We have added a sentence to this paragraph to clarify this issue: “When we found that estimated values of both SW-excess and LC-excess for a given plant were significantly different from zero, this would indicate that there could be a genuine mismatch in isotopic composition between plant water and its most likely sources.”

RC: L146: here is a list of source waters. If I understand correctly though only soil water was directly tested. The other data were not even extracted, so this would be good to clarify here. I understand precipitation was analysed indirectly via LC-excess, but again the data were not used directly in this study.

AC: We have removed from the text “precipitation, groundwater and streamflow” since these were not used in subsequent analyses.

RC: Methodology:

It is not immediately clear what a ‘sampling campaign’ corresponds to. Is this a ‘sampling occasion’ at a specific site and within each plot? The word ‘grouped’ in line 168 might be why I’m confused, because if you group all dates and plots
from a study site, it is unclear why you would have more campaigns than studies. Or does each study report on data from on average 5 study sites? It would be helpful to see the word ‘sampling campaign’ reappear as a heading in the right place in Table 1.

AC: We have clarified the definition of campaign as follows: “For our analyses, we considered that the experimental unit was the sampling ‘campaign’. We defined a campaign as a data collection event that occurred within a study site (or plot) within a limited time interval, thus each study consisted of one or more campaigns, depending on the number of sampling events.” (L168). We have added a column to Table 1 indicating the number of ‘campaigns’ included per study and clarifying that this number is the number of plots times the number of sampling events, per study. Also, we included a new column for total data regarding the combination of species sampled in each campaign.

RC: Soil water content is considered as an effect of climate, however, this is also a strong function of the soil properties. Climate might explain the main variations in VSM overtime at a specific site, but different soil types under the same climate can have vastly different absolute values at one moment in time. In this study, VSM is simply a variable across all campaigns and time locally is not considered (e.g. in the way it is plotted in Figure 4b). I’d therefore suggest rewording the role VSM represents (not simply to look at climate effects only).

AC: We agree that soil volumetric water content (VWC) is not solely a function of climate, but also of soil properties. In section 2.3. of the Methodology we detail the collection of these and other data, the title of this section is “Climatic, environmental and biological data”. We believe that the term “environmental data” is sufficiently broad enough to include soil VWC. We have carefully revised our text to make sure that soil VWC is not referred to as merely “climatic data”.

RC: Results:

Figure 2: the caption seems to be incorrect/unclear. I don’t see n reported in the figure. The small numbers on the left-hand side appear to refer to the ID in table 1. This is helpful, but several study sites appear more than once. Therefore, the statement in the first few sentences referring to ‘for each study site/sampling site’ might be incorrect. I also find the think bars in the figure misleading. They suggest a range rather than a mean value, and a range that either starts or stops at 0. It would be better to have the mean as a coloured marker, also so that the SE is clearly equal distance either side of the mean.

AC: We have changed this figure and clarified its caption according to this suggestion. The Y-axis depicts the study sites and since some studies included more than one study site, that is why some numbers are repeated. The figure no longer depicts bars, but only the symbols with their corresponding error bars.

RC: L307-312: this is very difficult to follow. Maybe split up or report the values in a table?

AC: We have modified the text according to this suggestion: “The results of the LMMs including climatic and environmental variables in the fixed part of the model indicated that
the slope of the SWL slope was sensitive to various climatic drivers. The slope of the SLW decreased with warmer temperatures (Table 2). In contrast, the slope of the SWL increased with soil VWC of the upper soil layers (Table 2) and with integrated soil water content (Table 2). Finally, the slope of the SWL also increased with annual and monthly precipitation (Table 2).

RC: L335-339 and Figure 4: I wonder why only Tair and Soil VWC for the upper 1 m are plotted in the figure? It would be good to see the data for PET as well and equally it would be interesting to see if the slope of the relationship for VWC of different soil depths changes and how?

AC: In this revised version of our manuscript, we present the suggested graphs in the supplementary material (Figure S1). Yet, please note that our results indicated that upper soil VWC measured at 0-7, 7-28 and 28-100 cm depth had a significant negative effect on SW-excess and that the estimated effects for all these variables are presented in Table 2, together with the estimated effect of potential evapotranspiration (PET). We opted to only present in the main manuscript graphically the effects of monthly air temperature and integrated soil water content because we believe that these were the most informative ones.

RC: Table 2: is it correct that ‘estimate’ refers to the ‘slope estimate’? If yes, I suggest to edit the heading as such.

AC: We have edited the caption of Table 2 which now reads: "Results (t and P), sample size (n) estimated slope and standard error (except for the null models where ‘Estimate’ is the intercept) according to the linear mixed models.”.

RC: Discussion:

L405-411: this appears to be misplaced as the authors report additional analysis here (would fit better in the methods and results section)

AC: We agree that this fragment reports the result of a particular analysis. This analysis is already reported in Results (L341):

"Importantly, a more detailed analysis of the residuals of the relationship between SW-excess and the SWL parameters (slope and intercept) revealed that only the temperature effects had a direct effect on SW-excess (Table S3). On the other hand, the observed effects of soil VWC on SW-excess appeared to be a consequence of the direct effect of soil VWC on the SWL slope and intercept (Table S3)."

However, we considered it important to repeat what the results in the analysis were? to improve the readability, i.e., to avoid that the readers have to go back and forth to find the results in another section. Still, we have slightly modified the sentence so it is more focused on the discussion of the results. The sentence reads as follows:

"Therefore, we ran the subsequent analysis of the residuals to that teased apart as direct and indirect environmental effects on SW-excess (Table S3). This analysis revealed that the negative effect of soil VWC on the SW-excess was mediated by the variability in the parameters of the SWL".
RC: L 407: change ‘run’ to ‘ran’

AC: Typo edited accordingly, thank you.

AC: Again, thank you for the time invested and the constructive comments.

Literature cited


Zhang, C., Zhang, J., Zhao, B., Zhu, A., Zhang, H., Huang, P. and Li, X.: Coupling a two-tip linear mixing model with a δD-
δ18O plot to determine water sources consumed by maize during different growth stages,
F. Crop. Res., 123(3), 196–205,