Response to Scott T Allen's short comment

Manuscript: hess-2018-631

Comment on Barbeta et al.

Barbeta et al. argue that fractionation could have occurred upon uptake or within plants because they often observed that xylem water samples were lower in δ2H than any of the potential sources they measured (rock water, stream water, fog, soils from 70-80 cm, and soils from 0-10 cm). They consider a few possible explanations (e.g., "separation between mobile and bound" and "compartmentalization between vessel water and other stem water pools"), but mostly they "argue that an isotopic fractionation in the unsaturated zone and/or within the plant tissues could underlie" their observations.

General comment

We appreciate the interest that Dr. Allen raised on our study, and the time he dedicated to offer his point of view on the conclusion that we have drawn from it (the existence of an 2H/1H separation between plant xylem water and water sources). In short, Dr. Allen questions this conclusion based on three potential explanations (PEs) that, in his opinion, we have not considered: (PE1) non-monotonic variations of soil water isotope composition with depth, (PE2) laser spectral interferences with organics and (PE3) spatial heterogeneity of soil surface water isotope composition. Although these points raised by Dr. Allen were already addressed and discussed in our manuscript, it seems that extra clarifications are required. We added such clarifications in the revised manuscript, notably regarding PE3, and provided also a detailed point-by-point response below.

Of course evaporation causes fractionation in the unsaturated zone, which they show clear evidence of, but they are arguing that there may be an unexplained fractionation that occurs in stems or upon root uptake (similar to that which is sometimes observed in halophytes and xerophytes). Such an argument may be valid if a reasonably comprehensive set of potential explanations have been considered and rejected. However, they did not sample highly likely water sources, and thus there are very probable explanations for their results that were not considered.

In the introduction, the authors state "if H1 is true [i.e., that there is no fractionation upon root uptake], the δ18O and δ2H of xylem water should always lie within the range of values of all water sources." Thus, to test H1, all source waters should be sampled. Although sampling all source waters is an infeasible task, even highly likely sources were not measured (e.g., soils between 10 and 70 cm depth). Thus, the rejection of H1 is not a logical extension of this study's findings, and it is unclear why the authors focus on attributing their findings to fractionation upon uptake or during within-plant transport.

Isotopic fractionation during plant root uptake can be most accurately tested in controlled settings where the "true" value is predictable, not in ambient field conditions where there are many un-controlled complicating factors. Controlled experiments have shown that xylem water accurately reflects soil water isotope values (e.g., Newberry et al 2017); consequently, challenging those findings requires a robust, well-constrained experiment. In the present study, it is not clear that the observed differences between the sampled end members and xylem water samples are due to fractionation during uptake or within the plant, as opposed to numerous other likely explanations. Several of these are listed below.

The rejection of H1 is not a logical extension of our study, as stated by the Dr. Allen. A careful read of our manuscript should not give the reader such impression. Our results are rather embedded in a growing series of papers reporting isotopic offsets in the field (Brooks *et al.*, 2010; Geris *et al.*, 2015; Evaristo *et al.*, 2016, 2017; Wang *et al.*, 2017; De Deurwaerder *et al.*, 2018), but importantly also in controlled experiments (Vargas *et al.*, 2017). Still, further studies are needed to formally reject the mentioned H1.

We agree with Dr. Allen that the rejection of H1 requires a controlled test where the true isotopic value of xylem water is predictable. However, it is noteworthy that the list of controlled experiments actually confirming H1 under controlled experiments is rather short. Dr. Allen cites one single recent paper (Newberry *et al.*, 2017) that did not show isotopic offsets between soil and xylem water. However, two also recent studies showed opposite results (*i.e.* rejecting H1) (explicitly in Vargas *et al.*, 2017; Orlowski *et al.*, 2018). These are not even novel findings as isotopic fractionation occurring during root water uptake had been suggested more than three decades ago (Allison *et al.*, 1983). Importantly, we have since conducted a similar controlled experiment on *F. sylvatica* saplings in which we confirm the occurrence of a 2H/1H fractionation between stem and soil water of the same magnitude as reported here (Barbeta et al. in preparation). We decided to conduct this controlled experiment after a thorough evaluation of the "numerous" likely explanations for the field results reported in the current manuscript. Below, we explain in more details why the explanations proposed by Dr. Allen were found not plausible, and also clarified these explanations in the main text.

1) No soil water samples were collected at depths where roots are often found (10 to 70 cm). Thus, the authors cannot exclude the possibility that the trees' apparent source waters occurred between their shallow (0-10 cm) and deep (>70 cm) samples. If these profiles were only affected by evaporation, then perhaps a profile comprising progressively enriched values towards the top could be expected. However, precipitation infiltrates and mixes heterogeneously with stored waters, creating heterogeneity and obscuring an evaporation profile (for an example that obviously expresses transport effects, see Figure 3 in Sprenger et al 2016). It should not be assumed that soils in intermediate depths (10-70 cm) have isotope values that are in between those of deeper and shallower soils (see Thomas et al., 2013). The un-sampled soil water domain could include winter precipitation that percolated downward into the rooting zone, after undergoing evaporative fractionation near the surface (yielding lower isotope values, due to the water's winter origins, with negative LC-excess, due to evaporation; e.g., see Dudley et al 2018), consistent with the xylem water values shown. My research (including two of the same species) shows that summer use of winter precipitation by plants is a reasonable expectation (Allen et al., 2019). It is reasonable to expect that zones between 10 cm and 70 cm contain roots, and contain winter precipitation with an evaporated signature. Thus, this constitutes a likely source that was entirely overlooked.

Our sampling strategy was designed to capture as much as possible the spatio-temporal variability in soil water isotopes, while keeping the analytical cost within reason. With the aim of optimizing the sampling effort (and sampling processing in the lab) we purposely restricted our sampling of water sources to top soil layers exposed to evaporation (0-10 cm) and deep soil layers (below 60cm) only affected by infiltration and mixing processes, and thus expected to display less variability over the season. Indeed, based on soil texture and climate, we did not expect soil evaporation to affect these deep soil layers at our field site. This was confirmed by a detailed soil isotopic profile collected at the end of the summer in September 2018 (Figure SC1a below).

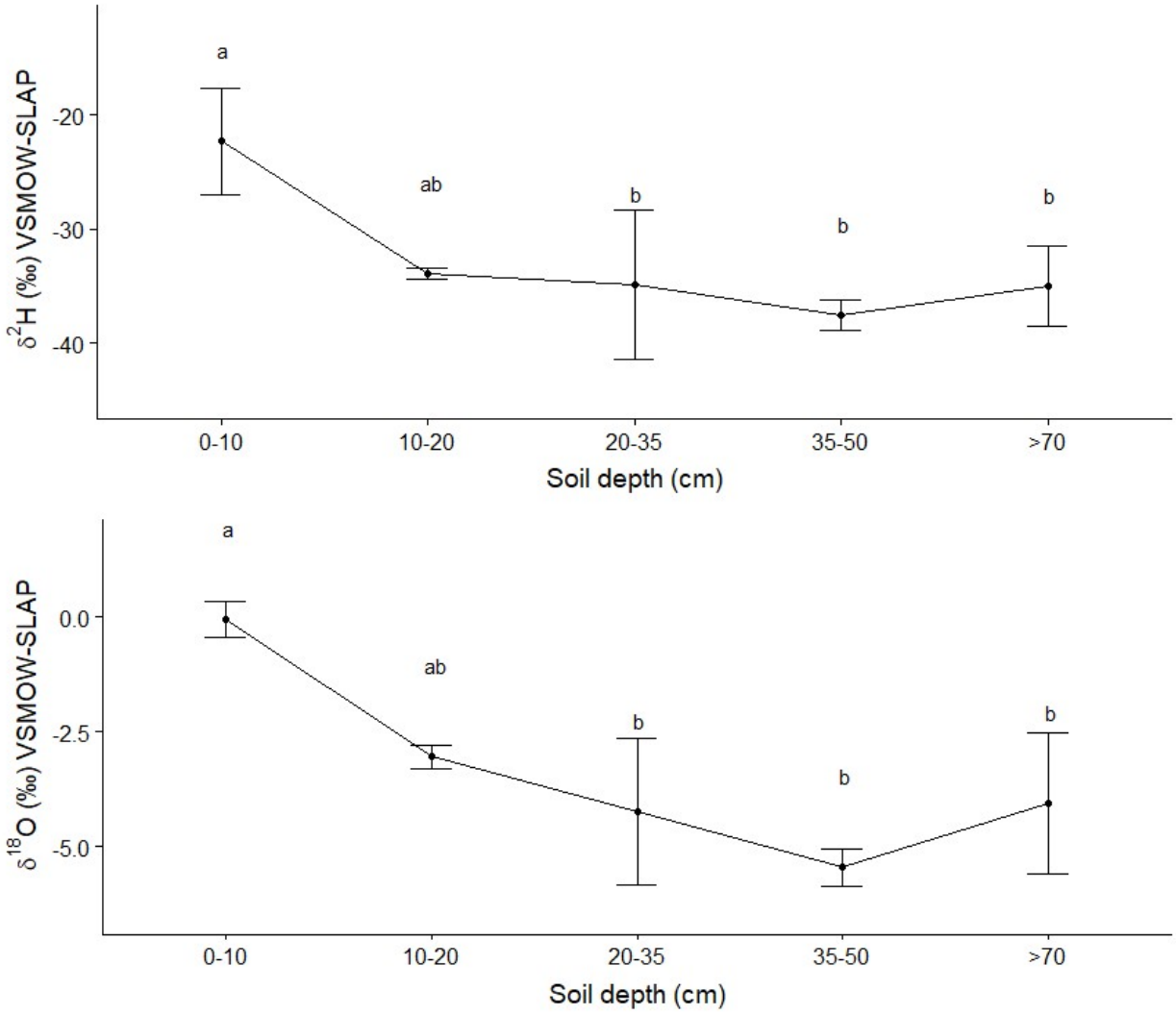

Fig. SC1a. δ1. Mean (±SE, *N* = X locations per depth) soil water isotopic composition ($\delta^{18}O$ and $\delta^{2}H$) at different depths. Different letters indicate significant differences among depths (*P*<0.05).

From this figure we see that there is no significant difference in the $\delta^{18}O$ and $\delta^{2}H$ of soil water among different depths below 20 cm, while the $\delta^{18}O$ and $\delta^{2}H$ of the upper layers are more enriched (not more depleted). We acknowledge that this isotopic profile could change over the course of the season, for instance following a rain event. Summer rain would deplete the topsoil layers but never to values more negative than winter precipitation, and would also add noise to the soil water line regression. In our revised version we have included the statistics of the soil water line regressions for the different sampling campaigns, following the comments of reviewer Juan Pedro Ferrio. In the response to Dr. Ferrio's comments, we included the modifications done in the text to acknowledge that the absence of such an isotopic profile caused by evaporation can lead to uncertainties when applying the SW-excess approach. In cases where the regression of the soil water line was not significant, the calculated isotopic offsets may be less meaningful. However, this only happened in a few cases (Table S3 in the revised manuscript). In addition, the soil water lines were calculated at the plot-scale and for every single date (Table S3), and the fit of these lines did not affect the estimated SW-excess.

Dr. Allen also pointed to an overlooked water source, namely winter precipitation stored in this middle soil layer. A feature of our study site is the varying soil texture in depth. In the allegedly overlooked soil layer (10 to 70 cm depth), the soil texture is coarse sand (Table S1) and the rock fraction is practically zero. On the other hand, the deeper soil layer that we targeted with our sampling strategy is a sandy loam, with higher water retention capacity (Figure SC1b below). Volumetric soil water content data from 2018 suggests that the water storage between 10 and 70 cm layers is minimal during summer, whereas the deeper soil layer holds more moisture all throughout the growing season (Figure SC1b). This is probably caused by higher infiltration rates of the coarse sand horizon, that we did not systematically sample. Although in terms of soil water potential (and thus extractable soil water) these different soil layers should not differ too much, it is very unlikely that the sandy layer would be able to hold winter precipitation until summer. On the other hand, what we sampled as representative for the isotopically unenriched part of the soil (>70cm) is also replenished during winter, which is very rainy in the area. Deep soil only starts drying out in summer, late summer precipitation does not infiltrate into the deeper soil layers, where soil water content only recovers after the first autumn storms (Fig. SC1b). Although we do not have such depth-resolved information for 2017, the GWC data presented in this manuscript illustrates a similar seasonal pattern (Fig. 1 of the manuscript). Importantly, winter rain is depleted in both $\delta^{18}O$ and $\delta^{2}H$ but in our study, xylem water $\delta^{18}O$ was always in the range of soil water $\delta^{18}O$. To sum up, we did not 'entirely overlooked' winter precipitation, as this is very likely the main source of the deep soil layers that we systematically sampled throughout the growing season.

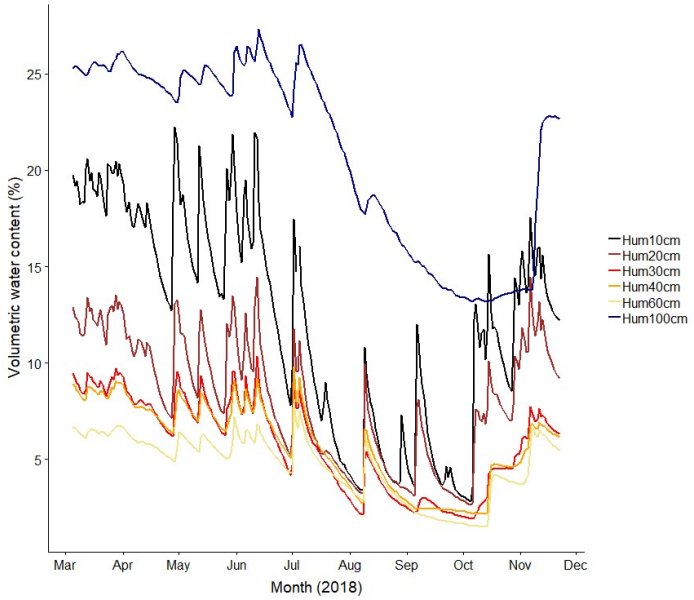

Fig. SC1b. Volumetric water content (Hum) at different depths in one of our study sites during the growing season of 2018. Horizon A (10 and 20cm), horizon B (30, 40 and 60cm), horizon C (100cm).

2) Laser spec analysis issues may compromise inferences. Of course the authors know that using a laser spec can yield uncertain xylem water measurements, and they made attempts to correct those data. However, given that the authors are challenging long-standing knowledge, it is essential to control for the potentially confounding effects of organics (not just "methanol and/or ethanol") in the laser spec

analyses. Although the authors are more attentive to this issue than many, benchmarking a subset of the samples using IRMS would provide a more convincing data set.

Indeed, infrared isotope spectrometers (IRIS) are known to be sensitive to organic volatiles that also absorb light in the mid infrared range explored by these analyzers. IRMS are also sensitive to organic compounds but to an extent that is only proportional to mass contribution of these compounds (Martín-Gómez *et al.*, 2015). As we report in the manuscript, we developed a correction specific for our instrument following previous studies (Schultz *et al.*, 2011; Brian Leen *et al.*, 2012). We found that the corrections applied to the data could not possibly explain the observed SW-excess. In addition, as briefly mentioned in the manuscript, soil-xylem δ^2H offsets of similar magnitude have been reported by other investigators using both IRIS and IRMS, even in controlled settings. Here is a non-exhaustive list of them:

Table SC1a. List of studies showing soil-xylem isotopic offsets comparable to those found in the present study.

Study	Analytical method	Experiment type, study species
Geris *et al.*, (2015)	IRMS	Field, *Pinus sylvestris*
Vargas *et al.*, (2017)	IRMS	Glasshouse, *Persea americana*
Evaristo *et al.*, (2017)	IRMS	Botanical garden, many species
Wang *et al.*, (2017)	IRMS	Field, deciduous shrubs and perennial herb
De Deurwaerder *et al.*, (2018)	IRIS (Picarro with MCM)	Field, rainforest tree species
Brooks *et al.*, (2010)	IRIS (LGR) & IRMS	Field, *Pseudotsuga menziensii*
Evaristo *et al.*, (2016)	IRIS (Picarro) & IRMS	Field, *Swietenia macrophylla*

3) Lateral heterogeneities create challenges for representative sampling. For the 0-10 cm depth, where soil water isotope signatures are most heterogeneous, there were relatively few samples collected. Three cores per plot is minimal. Goldsmith et al (2019; see Figure 7) show that dramatic mischaracterizations of the true variance among surface soil water isotope ratios should be expected when using small sample sizes. The authors cannot retroactively sample the soils, but they should recognize that their sampling probably underestimates the range of lateral variation. It could also be considered that there are fine-scale variations in pore sizes that plants may differentially sample among (Stewart et al., 1999), but an auger cannot.

Given that different pore sizes transport water at different rates, we should expect them to correspond with fine-scale variations in isotope values. Given these limitations in the sampling and analysis (especially a lack of samples from 10-70 cm), it is unjustified to attribute the lack of finding an appropriate source to unexplained fractionation processes in stems or at the root-soil interface. A more defensible conclusion is that the specific sampling regime used here may not have captured the source waters that were actually used by the trees.

We agree with Dr. Allen that the surface spatial heterogeneity in soil water isotopes can be large. Dr. Allen finds that three cores per plot is minimal. Maybe it was not entirely clear in our Methods section but these plots were relatively small (maximum distance between the two most distant trees was 15 m), and all trees within the plot had a soil core below their canopies. Of course, our sampling design

cannot ensure that all the variability in surface soil water isotopes is captured. Horizontal heterogeneity in soil water isotopic composition would certainly generate noise on the SW-excess determination but cannot explain the isotopic offsets between soil and xylem water observed over the entire growing season (over a wide range of environmental conditions) and for all the studied trees (representing differences in size, species, plot, and thus very likely also in rooting depth and lateral root spread).

In the last paragraph of his comment, Dr. Allen points out again to limitations of our sampling design. On the contrary, a strong point of our study is the detailed characterization of the temporal dynamics (bi-weekly sampling campaigns sustained for a whole growing season) of soil water, together with rock moisture, groundwater and stream and fog water. Despite the issues related with cryogenic extraction, which we already consider and discuss in the text, cryogenically extracted water is still a good proxy for soil water isotopes (Newberry *et al.*, 2017). This is especially true when compared with lysimeter-extracted water that subsample soil mobile water, and that is not representative of plant-accessible water that can be held at down to -1500 kPa or more (Slatyer, 1957). A promising avenue for advancing our understanding is the deployment of systems allowing continuous measurements of soil water isotope vapor profiles *in situ* (Oerter *et al.*, 2019), which would provide a different perspective on the spatio-temporal patterns of water isotopes in the soil-root interface.

References (cited by the Dr. Allen)

Allen, S. T., Kirchner, J. W., Braun, S., Siegwolf, R. T., & Goldsmith, G. R. (2019). Seasonal origins of soil water used by trees. Hydrology and Earth System Sciences, 23(2), 1199-1210.

Dudley, B. D., Marttila, H., Graham, S. L., Evison, R., & Srinivasan, M. S. (2018). Water sources for woody shrubs on hillslopes: An investigation using isotopic and sapflow methods. Ecohydrology, 11(2), e1926.

Goldsmith, G. R., Allen, S. T., Braun, S., Engbersen, N., González Quijano, C. R., Kirchner, J. W., & Siegwolf, R. T. (2018). Spatial variation in throughfall, soil, and plant water isotopes in a temperate forest. Ecohydrology, e2059.

Newberry, S. L., Nelson, D. B., & Kahmen, A. (2017). Cryogenic vacuum artifacts do not affect plant waterâAˇ Ruptake studies using stable isotope analysis. Ecohydrology, 10(8), e1892. Sprenger, M., Erhardt, M., Riedel, M., & Weiler, M. (2016). Historical tracking of nitrate in contrasting vineyards using water isotopes and nitrate depth profiles. Agriculture, Ecosystems & Environment, 222, 185-192.

Stewart, J., Moran, C. & Wood, J. Macropore sheath: quantification of plant root and soil macropore association. Plant and Soil (1999) 211: 59. https://doi.org/10.1023/A:1004405422847

Thomas, E. M., H. Lin, C. J. Duffy, P. L. Sullivan, G. H. Holmes, S. L. Brantley, and L. Jin. 2013. Spatiotemporal Patterns of Water Stable Isotope Compositions at the Shale Hills Critical Zone Observatory: Linkages to Subsurface Hydrologic Processes. Vadose Zone J. 12. doi:10.2136/vzj2013.01.0029.

References (authors' response)

Allison GB, Barnes CJ, Hugues MW, Leaney FWJ. **1983**. Effect of Climate and Vegetation on Oxygen-18 and Deuterium Profiles in Soils. *Isotope hydrology, 1983 : proceedings of an International Symposium on Isotope Hydrology in Water Resources Development*: 105–123.

Brian Leen J, Berman ESF, Liebson L, Gupta M. **2012**. Spectral contaminant identifier for off-axis integrated cavity output spectroscopy measurements of liquid water isotopes. *Review of Scientific Instruments* **83**(4).

Brooks JR, Barnard HR, Coulombe R, McDonnell JJ, Renée Brooks J, Barnard HR, Coulombe R, McDonnell JJ. **2010**. Ecohydrologic separation of water between trees and streams in a Mediterranean climate. *Nature Geoscience* **3**: 100–104.

De Deurwaerder H, Hervé-Fernández P, Stahl C, Burban B, Petronelli P, Hoffman B, Bonal D, Boeckx P, Verbeeck H. **2018**. Liana and tree below-ground water competition—evidence for water resource partitioning during the dry season. *Tree Physiology* 38, 1071–1083.

Evaristo J, McDonnell JJ, Clemens J. **2017**. Plant source water apportionment using stable isotopes: A comparison of simple linear, two-compartment mixing model approaches. *Hydrological Processes* **31**: 3750–3758.

Evaristo J, McDonnell JJ, Scholl MA, Bruijnzeel LA, Chun KP. **2016**. Insights into plant water uptake from xylem-water isotope measurements in two tropical catchments with contrasting moisture conditions. *Hydrological Processes* **30**: 3210–3227.

Geris J, Tetzlaff D, McDonnell J, Anderson J, Paton G, Soulsby C. **2015**. Ecohydrological separation in wet, low energy northern environments? A preliminary assessment using different soil water extraction techniques. *Hydrological Processes* **29(25)**, 5139-5152.

Martín-Gómez P, Barbeta A, Voltas J, Peñuelas J, Dennis K, Palacio S, Dawson TE, Ferrio JP. **2015**. Isotope-ratio infrared spectroscopy: A reliable tool for the investigation of plant-water sources? *New Phytologist*. **207**(3): 914-27.

Newberry SL, Nelson DB, Kahmen A. **2017**. Cryogenic vacuum artifacts do not affect plant water-uptake studies using stable isotope analysis. *Ecohydrology* **10**(8): e1892.

Oerter EJ, Siebert G, Bowling DR, Bowen G. **2019**. Soil water vapor isotopes identify missing water source for streamside trees. *Ecohydrology*: e2083.

Orlowski N, Winkler A, McDonnell JJ, Breuer L. **2018**. A simple greenhouse experiment to explore the effect of cryogenic water extraction for tracing plant source water. *Journal of Professions and Organization* **11(5)**: e1967.

Schultz NM, Griffis TJ, Lee X, Baker JM. **2011**. Identification and correction of spectral contamination in 2H/ 1H and 18O/ 16O measured in leaf, stem, and soil water. *Rapid Communications in Mass Spectrometry* **25**: 3360–3368.

Slatyer RO. **1957**. The significance of the permanent wilting percentage in studies of plant and soil water relations. *The Botanical Review* **XXIII**: 585–636.

Vargas AI, Schaffer B, Yuhong L, Sternberg L da SL. **2017**. Testing plant use of mobile vs immobile soil water sources using stable isotope experiments. *New Phytologist* **215**: 582–594.

Wang J, Fu B, Lu N, Zhang L. **2017**. Seasonal variation in water uptake patterns of three plant species based on stable isotopes in the semi-arid Loess Plateau. *Science of the Total Environment* **609**: 27–37.