

Hydrol. Earth Syst. Sci. Discuss., referee comment RC1  
<https://doi.org/10.5194/hess-2020-680-RC1>, 2021  
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## Comment on hess-2020-680

Anonymous Referee #1

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Referee comment on "Insights into the isotopic mismatch between bulk soil water and *Salix matsudana* Koidz trunk water from root water stable isotope measurements" by Ying Zhao and Li Wang, Hydrol. Earth Syst. Sci. Discuss.,  
<https://doi.org/10.5194/hess-2020-680-RC1>, 2021

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The manuscript hess-2020-680: "Insights into isotopic mismatch between soil water and *Salix matsudana* Koidz xylem water from root water isotope measurements" by Zhao and Wang investigates potential reasons for an observed mismatch between soil and xylem water stable isotope values by measuring water stable isotope ratios in different soil water pools (mobile, bulk soil and bound) across soil depths, as well as in roots (across depth) and xylem of three rigorously sampled tree individuals. The authors conclude that the observed isotopic differences between xylem and bulk soil water arise from a combination of ecohydrological separation, i.e. isotopic differences in mobile and bound soil water and plant fractionation during root water uptake.

Root water uptake depth is routinely determined by comparing the isotope composition of xylem water with that of soil water in different depths (and other water sources like stream and groundwater) assuming that extracted water from bulk soil samples represents available water sources. This long-standing principle is lately repeatedly questioned and a lot of uncertainty persists on potential reasons and underlying causes. This work contributes to the discussion and provides new insights. I especially liked, that the authors conducted an experiment under natural conditions. I do not know of any other study that sampled water sources, xylem water and also systematically investigated root xylem within a field experiment to tackle this question and I applaud the authors for conducting this surely very labour-intensive work. The manuscript is well-structured and understandable. Generally, I am in favour of publishing this work. However, I think that a number of critical points (see major points below) should be addressed beforehand in a revised version. I also suggest an English native speaker to proof-read the manuscript and help to further improve some of the expressions.

### Major points:

- You state that plant fractionation, i.e. change of isotope values during root water uptake, is one of two main causes for the observed mismatch. This was observed

before in xerophytic and halophytic plants and for plants in symbiosis with arbuscular mycorrhiza (e.g. Ellsworth & Williams 2007, Poca et al. 2019). However, previous studies on plant fractionation reported depleted (more negative) isotope values in plant xylem as compared to soil water, hence plants discriminated against the heavy isotope (mostly  $^{2}\text{H}$ ). This is not in line with enriched (less negative) xylem values reported here. While it cannot be ruled out completely that water would get enriched in heavy isotopes during rwu, this was not reported before and considering other potential reasons, namely isotopic heterogeneities across soil water pools as well as temporal variability and methodological artefacts, this seems unlikely to me. If you decide to keep it in the manuscript, this discrepancy to other studies should be pointed out and discussed in detail.

- Building on that, some root and all stem xylem samples show an evaporative enrichment in the dual isotope plot (Fig. 2). This is discussed in the manuscript and related to an enriched signal of bound soil water. I think this argument would be strengthened if you provide further description on the sampling procedure. Specifically, I wondered if evaporation during root sample collection could potentially influence obtained results. Did you sample roots right from the soil profile wall or exclude the first few centimetres? How fast was sampling conducted after digging the hole? Also: Was soil thoroughly removed from sampled roots? Regarding xylem sampling, you write "Bark was peeled from the twigs and all leaves were removed to avoid perturbation of xylem water isotopic signatures by fractionation." (L 136-137). So, did you sample twigs that had leaves directly attached to them? Could the enriched signal hence arise from back-diffusion or any other exchange with enriched leaf water before sampling? Were twigs fully suberized or were they green and hence photosynthetically active?
- In my personal opinion, I would not put so much emphasis on the TWW hypothesis. If a reader is not familiar with it, it might be a bit confusing (especially in the abstract). I think it is good that you refer your results to it but I would reduce the importance it has in your manuscript, e.g. the amounts of mentions.  
Also, I think the term "tightly bound water" is misleading. At least in my perception it suggests that plants use an exceptionally tightly bound water pool (as compared simply to bound water). However, in my opinion, they probably just use the water that is available to them and mobile water might infiltrate beyond the root zone too quickly to be available in the long term. I imagine that the sampled mobile water (in lysimeters) mainly originates from percolation during precipitation events, when relative humidity is high, solar radiation is low and plants hence do not transpire a lot. I suggest to use "less mobile water" or simply "bound water". However, I am aware that it is also termed "tightly bound water" in the TWW hypothesis.  
See also the extensive discussion in the commentary by Sprenger & Allen (2020): <https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2020WR027238>  
I leave this decision up to the authors and the editor.
- You took the time to calculate the isotopic composition of tightly bound soil water (from measured bulk and mobile soil water) and you repeatedly argue that plants preferentially use this water source. However, this data is only incorporated into one supplemental figure. I think it would strengthen the story if those values were incorporated into the main figures as well (e.g. Figure 2 and 5).
- SIAR modelling: In my opinion, this does not strengthen the story. The isotopic composition of water sources incorporated are quite similar to each other. Did you check if the modelling results change substantially when running the calculations multiple times? Also, you use bulk soil water in different soil depths as available water sources. Contradictorily, these do not match with observed xylem values and you argue in the text that plants use soil water with differing isotopic composition. Additionally, measurement uncertainty in sources and plant xylem should be considered (see e.g. Kühnhammer et al. 2020). Having that said, I like that you compare rwu fractions derived with bulk soil samples and sampled roots as water sources. If you decide to keep the SIAR modelling, it would be interesting, in my opinion, to also look at tightly

bound water as a potential set of sources and see how this changes the results. You could then also discuss the weaknesses of those purely statistical models and make use of your data to communicate potential issues with the usual approach (e.g. comparing bulk soil water and xylem water) to the scientific community.

- As you sampled roots across soil depths, do you also have information on root length density across the profile or any other measure of root quantity across soil depths? I think this could add some interesting insights into the trees' water uptake strategies

#### Reference suggestions

As you argue a lot with isotopic differences within bulk soil, i.e. in different soil water pools, I think it could be useful to look at and incorporate the findings of the following references as well:

- For a strategic investigation of isotopic differences under different soil water tension see also the new paper by Orłowski & Breuer (2020):  
<https://onlinelibrary.wiley.com/doi/full/10.1002/hyp.13916>
- Sprenger et al. (2018): Measuring and Modeling Stable Isotopes of Mobile and Bulk Soil Water  
<https://access.onlinelibrary.wiley.com/doi/pdf/10.2136/vzj2017.08.0149>
- Berry et al. (2017): The two water worlds hypothesis: Addressing multiple working hypotheses and proposing a way forward  
[https://onlinelibrary.wiley.com/doi/epdf/10.1002/eco.1843?saml\\_referrer](https://onlinelibrary.wiley.com/doi/epdf/10.1002/eco.1843?saml_referrer)

#### Technical comments:

Overall:

- As you are discussing isotopic differences of soil water pools sampled as a reason for the observed mismatch to xylem water, you should pay close attention to specifying which soil water pool you are talking about. I suggest, either you always specify this or you clarify once that when talking about soil water in general you always refer to bulk soil water.
- In the copy I received for review, the figure quality seems too low, axis labels and names seem a bit blurry. It should be verified that all labels and names are easily readable
- Check for consistency of isotope terminology and avoid using (too many) different ones if it does not contribute to the readability of the text. You use all of the following: "isotopic composition", "isotope composition", "isotopes in water", "isotopic signature", "isotope signals", "isotopic signals", "isotope fingerprints", "H and O isotopes", "hydrogen and oxygen isotopes", "water isotopes", "isotopic values", "isotopic patterns" I know that sometimes it makes sense to mix it up a bit to avoid too many repetitions

but I think you can eliminate at least a few of them :-D

Also I would use the term "water stable isotopes" instead of "water isotopes"

- You use xylem to refer to measurements in the trunk. However, water within roots is also transported in the xylem (which you sampled to obtain the root isotopic values). I suggest to specify the use of words here or use trunk instead of xylem

## Specific comments

**Title:** "insights into [an] isotopic mismatch between [bulk] soil water and *Salix matsudana* Koidz xylem water from root water isotope measurements"

**Figure 2:** I think the axis limits should be the same in all subplots (x-axis different for panel c)

**Figure 4 (caption):** remove repetition of lc-excess

**Figure 7:** SD should also be displayed in the opposite direction, are the obtained distributions (of a RWU fraction at a certain depth) normally-distributed? If not, display uncertainty in a different way. Maybe it would make sense to display those distributions as boxplots

**Figure 8:** I like the colour coding depicting the isotopic composition of the different compartments studied. Maybe this would come out even clearer if tree and soil background colours were a little more subtle (maybe grey scale?). Also see my general comment 1 to reevaluate if you should emphasize fractionation as the main cause for the isotopic mismatch

**Figure S2:** Symbols are different sizes in different subplots

**Figure S3:** What is displayed? The figure legends and part of the caption states you display bulk water (BW) and tightly bound water (TW), but the caption also mentions mobile water (MW) – I assume that is a typo. I would also suggest changing the colours in Figure S3 to match with the colours in Figure 3, i.e. bulk soil water should always have the same colour

**Figure S4:** the colours of the boxplots are hard to distinguish

**Line 12:** "at high temporal resolution" it reads as if all beforementioned parameters were sampled over time. However, this is not true for root xylem (only sampled on one occasion). Also, I think the perception of high temporal resolution is quite different depending on who you ask, especially with new in situ methods evolving. Maybe specify that you sampled twice a week/every X days?

**Line 12-13:** "to analyze isotopic dynamics in the soil-root-xylem continuum"  
I don't really see a lot of focus on (temporal) isotope dynamics in your manuscript. You do not really discuss the variations over time (and only sampled roots once), maybe apart from the influence of GWC on soil water isotopes

**Line 17:** I personally would not mention the TWW here again. "and plant fractionation" see my major point 1)

**Line 20:** "isotopic offset occurred at the interface between the soil and *S. matsudana* roots" This statement is a bit misleading I think because if different soil water pools were not isotopically well mixed, the offset does not occur at the interface between soil and roots but because plants only access a certain soil water pool

**Line 28:** "in [the] global hydrological cycle" or "in hydrological cycles", "terrestrial ecosystem[s]"

**Line 42:** exchange "in the movements" with "along the pathway"

**Line 43:** "but also [due] to ecohydrological separation (Brooks et al., 2010) [and] water isotope ..."

**Line 44-45:** add reference to Chen et al. (2020) paper

**Line 57:** put "respectively" at the end of the sentence?

**Line 60:** "incomplete extraction of water during cryogenic distillation could fractionate water isotopes" due to Rayleigh fractionation during the extraction process an incomplete extraction could not only fractionate water stable isotopes in the sample but surely does!

**Line 61-62:** "between [cryogenically extracted] stem water and source water"

**Line 63:** "cryogenic extraction-associated methodological artifact" sounds a bit overly complicated ☹☹☹  
suggestion: "methodological artifacts during cryogenic vacuum extraction"

**Line 65:** "specific process[es]"

**Line 67:** "along [the] soil-root-xylem continuum"

**Line 68:** "leading to the variation in water isotopes" I don't understand the statement of this subordinate clause

**Line 69:** "roots preferentially use tightly bound water according to the TWW hypothesis" In my opinion this statement is misleading as it might be attributed to the long-term availability of water in the soil. Mobile water (transported in big pores) percolates quickly below the plant rooting zone and therefore is only shortly plant available (during precipitation when rh is usually high and solar radiation low). Hence, roots do not prefer the more tightly bound water, they just use the water they have access to. I know however that it is termed like this in the TWW papers and different people might interpret this statement differently.

**Line 78-79:** I would delete "during water transport from root to xylem" as you did not measure isotopes during the transport but at two locations (roots and trunk xylem)

**Line 80-82:** We hypothesize that 1) there is an isotopic deviation between xylem water of *S. matsudana* trees and their potential water sources, and that 2) this deviation might be due to a combination of multiple factors.  
I think the second hypothesis is quite unspecific and that this is the case is already clear from your literature review. Be more precise on what you investigated and how your research contributes to disentangle combination of multiple contributing factors

**Line 98-99:** We selected three sampling sites in the check-dammed channel of the Liudaogou catchment. Designated sites 1, 2 and 3 are located 50, 80 and 100 m upstream

of the dam, respectively.

**Line 100:** "was chose for sampling tree" correct grammar

**Line 101:** "includes" do you mean "consists of"?

**Line 102:** "different soil [depths]" in soil science layers refer to differences in stratification, "in a sampling plot" which sampling plot? As you introduced the numbers before you could just specify

**Line 104:** "from the sampling plot[s]" or "sampling area"? or are you referring to a specific plot?

**Line 109:** We collected root samples of [one] *S. matsudana* tree at each [of the three] sampling site[s]

**Line 112-113:** "was collected and measured its isotopic composition" correct grammar

**Line 113:** "collected disturbed soil samples at 0-160 cm depths" at what interval? Table one suggests 10 cm increments

**Line 114-116:** Did you measure particle size for both disturbed and undisturbed samples? Or was this only done for disturbed samples and samples in cutting rings were used for water retention curves?

**Line 119:** "Our previous results have shown" This reads as it was referring to a previous (published) study. Either you are missing a reference here or you should rephrase the sentence to make clear that this refers to a previous campaign and is up to now unpublished data

**Line 120-121:** I am having difficulty to understand this sentence (referring to TWW)

**Line 126:** delete "For these analyses" otherwise it reads like precipitation samples are connected to beforementioned sample analysis

**Line 124:** "within the period when mobile water was available[, i.e. from August 4 to September 15 2019].

**Line 128:** do you know the depth of the groundwater table at the study site? At which depth did you sample groundwater?

**Line 129-131:** How were soil samples (for isotopic analysis) stored? How was evaporation from samples prevented?

**Line 138:** "placed in 10 mL vials and wrapped in parafilm" I guess you sealed the vials with caps and then secured the caps with parafilm? Parafilm is not 100% gas tight but permeable to water vapor

**Line 141-142:** "Similarly, of 30 mm long pieces of the de-barked twigs were immediately placed in 10 mL vials and wrapped in parafilm." Is this a repetition or did you want to specify the sampling in different tree heights? Maybe streamline to avoid redundancies

**Line 163:** b does not appear in your Ic-excess formula (is essentially the Ic-excess)

**Line 166-167:** In my opinion you do not need the link to the TWW here

**Line 174:** "To compare the isotopic composition of root and soil water at the same depth" Actually you only indirectly compare root and bulk soil water with this. If you aim at comparing those two, I think there is better, different approaches. I would rather say you compare their contribution to xylem water. See also my major comment 5) here.

**Line 177:** "Based on results of the soil water and root water isotope analysis" I do not get what the decision criteria was here. Please specify.

**Line 178:** "soil [and root] water sources were divided into ..."  
Then you can delete the next sentence

**Line 181:** "because plant water use does not generally cause fractionation of hydrogen and oxygen isotopes"



This contradicts with the statement in your paper that fractionation during rwu influences the xylem isotope values. I would rephrase and write: "assuming no fractionation during plant water uptake"

**Line 202:** "overlapped with bulk soil water (Fig 2d)" Actually one needs to look at Figure 2a and d to see the overlap

**Line 208:** unit ‰ is missing

**Line 211:** I would not mention TWW here, as it is part of the discussion

**Line 212-213:** "suggesting that xylem water was isolated from all potential water sources" Actually, it only means that xylem water did not reflect bulk soil water sources and not that it is independent from all potential water sources. Also contradicts with the next sentence and is strictly speaking already discussing the results.

**Line 213-214:** streamline sentence to avoid repetition of tightly bound water, also includes discussion of results already

**Line 232:** "[horizontal] distance"

**Line 233:** exchange "> 80 cm" with "within 80 cm"

**Line 239 & 241:** d2O should be d18O I assume, also stay consistent with the subscripts, here the subscript soil refers to bulk soil water but subscript BW also exists

**Line 243:** "These results show that water isotopes, especially hydrogen isotopes, changed between..."

Water stable isotopes do not change, the ratios of the isotopes change

**Line 244:** "supporting our first hypothesis" belongs into discussion

**Line 246:** Specify heading of subsection, contribution of what?

**Line 248:** "Potential sources of plant xylem water were determined using..."  
you do not determine potential sources but the contribution of these sources to rwu

**Line 256:** "Separation of mobile water and bulk soil water in the soil matrix"  
Your manuscript deals with this separation a lot and it is also a big part of your study design. Why is this not one of your hypothesis/aims?

**Line 257:** exchange "covered" with "experimental"

**Line 261:** "clear [isotopic] separation"

**Line 266-268:** Reference for statement missing

**Line 268-269:** "Due to the seasonal variation in precipitation, winter and summer precipitation have different isotope signals"  
Both half sentences state the same thing. Seasonal variation is not the cause for isotopic differences between winter and summer precipitation

**Line 272:** place "at our study site" at the beginning of the sentence

**Line 274:** "caused by other factors, and not necessarily by seasonal variation in precipitation"  
Observed differences could also stem from isotopic differences of individual precipitation events. Did you check if with high and low intensities are systematically different? If so, high intensity precipitation events might percolate faster into the soil and also contribute bigger water quantities to sampled mobile water.

**Line 277:** "with [increasing] soil depth"

**Line 281-282:** "Although the mixing of mobile and tightly bound water conflicts with the original hypothesis of Brooks et al. (2010)"  
Does it really disagree? Or is it more a question of the degree of mixing or rather that mixing between soil water pools is not complete as previously assumed?

**Line 282-285:** Increased mixing with soil depth was e.g. also observed/mentioned by Sprenger et al. (2016) and Kübert et al. (2020)

<https://agupubs.onlinelibrary.wiley.com/doi/full/10.1002/2015RG000515>  
<https://www.frontiersin.org/articles/10.3389/fpls.2020.00387/full>

**Line 290-291:** "driven by spatiotemporal dynamics of soil water profiles associated with soil evaporation"

What do you mean with that? The temporal (and spatial) differences in infiltration and evaporation and how those influence the sampled soil water sources?

**Line 296-297:** "These results showed that the isotopic offset between plant root water and soil water occurred at the root-soil interface."

I think this statement is misleading. I think this statement is misleading. In my opinion it points towards the explanation that bulk soil water is not representative of available plant water sources (see next paragraph). This heterogeneity does however not only apply to the root-soil interface. Or am I missing something here?

**Line 302:** "that is strongly affected by evaporative effects [in the presented study], isotopically separated from mobile water and groundwater and shows similar enriched isotopic signals [than xylem water]"

**Line 303-304:** "This hypothesis is corroborated by the overlap in isotopic composition between root and bulk soil water at 0-60 cm depths (Fig. 2 and 5)."

Why? As I understand it, this speaks against the statement in the sentence before.

**Line 304-309:** "We considered whether bulk soil water isotopes can represent isotopic values of tightly bound water used by plants. Generally, the water designated 'bulk soil water' includes mobile and tightly bound water due to limitations of water extraction technology when assessing the TWW hypothesis. Thus, the proportion of mobile water in the bulk soil water increases as soil moisture increases, resulting in isotopic deviation between root water and bulk soil water."

I do not understand the argument here. Maybe rephrasing helps :-D

**Line 331-332:** "Under the assumption that plant fractionation does not occur"

Do you mean that the isotopic composition of water does not change during within plant transport? Fractionation is mainly believed to occur during rwu I think. However, as you sampled the roots, you eliminate this influencing factor.

**Line 341:** "also has residence times in branches and roots"

What do you mean with this? How is it different from the time lag due to transport from roots to branches? Do you refer to an influence of xylem water storage?

**Line 346:** "that isotope enrichment may have been present in the unsampled branches"  
Why would you measure a potential enrichment caused by unsampled branches by sampling at different heights? What do you mean with enrichment present in unsampled branches?

**Line 348:** "xylem water was [isotopically] more enriched than..."

**Line 352-353:** "However, we found that the xylem water contained [more of the depleted] isotopic signal of deep roots than [of the] enriched signal from shallow roots. The results show that there was no isotopic fractionation during water transport from root to xylem"

I am not sure if this allows the conclusion that no fractionation during transport took place. However, I agree that it strongly suggests it. I would also specify here that you did not observe an enrichment (fractionation could go both ways) during transport as other authors suggested.

**Line 358:** exchange "covered" with "experimental" or write "period covered"

**Line 359-360:** "isotopic offset [exists] between xylem water and [bulk soil water]"  
As you elaborate in your manuscript, bulk soil water might not reflect all available soil water sources.

**Line 361:** "and water flow paths" what do you mean with that? Infiltration along preferential flow paths?

**Line 369:** "the [estimated] contribution of roots in these depths to xylem water [was] 74%."