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Interactive comment on "Tracing ecosystem water fluxes using hydrogen and oxygen stable isotopes: challenges and opportunities from an interdisciplinary perspective" *by* D. Penna et al.

J. R. Brooks (Referee)

brooks.reneej@epa.gov

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I read with great interest the commentary by Penna et al., as I wished I could have attended the meeting and was very interested in the discussions that ensued. Overall, I think this commentary will be a valuable summary of where we are in using water isotopes to partition stream-soil-plant interactions, and potential directions for the future. However, I feel that the urgent challenges outline here are often very vague in details, and ignore some work that has been published, giving the impression that we know a lot less than we actually do know. I think the impact would be much stronger if the authors gave more examples of the type of research they are suggesting to help

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readers, and I provide some specific comments below on that. The authors also mischaracterize other studies or neglect some key pieces of work. These points can be easily addressed by the authors, and the result will be a valuable reflection on where we are in this field of research. Please see below for specific details.

First, since the audience of Biogeosciences Discussions is much broader than terrestrial ecology and critical zone studies, I think the title needs to read "Tracing terrestrial ecosystem water fluxes…". This commentary does not touch on the broad work of isotopic tracing within aquatic ecosystems. For the same reason, I think the authors should include a phrase that quickly defines critical zone, for those not working in the critical zone. Also, on P2, line 20, specify terrestrial ecosystems.

P2, L25: In mentioning advancements in isotope-based tools and methods, you should mention Sprenger et al. (2015) for an excellent summary.

P3, L13: You should probably include Gat (1996).

P3, L21: You should probably include Allen et al. (2017) when discussing evaporation from plant canopies.

P4, L5-7: When discussing the isotopic enrichment of heavy isotopes with leaf transpiration, the sentence on H and O exchange between CO2 and H2O is really out of place. H does not exchange with CO2, only oxygen, and the amount of oxygen in water is so vast that the oxygen in CO2 does not really impact the water isotopic signature, and the authors don't provide a citation for their statement. Instead, well published processes such as the Péclet effect, and non-steady state processes are not even mentioned. There is a huge literature base on leaf water isotopes, but these sentences make it seem like a neglected area. See for example (Kahmen et al., 2008; Kahmen et al., 2009; Cernusak et al., 2016)

P4 L14-19: if you are going to compare IRMS and the laser techniques for measuring water, you should include the latest IAEA interlaboratory comparison of water isotopic

measures using both techniques (Wassenaar et al., 2018). They have a very interesting figure showing the problem with organics in water isotope analysis. This illustrates that even with this software, the problem is far from solved.

P4 Last line: include “terrestrial” before “ecosystems”.

P5 L2: “How do plants select their water source?”. This phrasing makes it seem like plants are consciously choosing their water sources. This section also seems to discount the vast literature by plant physiologist on plant water uptake through water potential gradients, and soil-plant continuum conceptual model. I don’t believe any of the work with stable isotopes has refuted or made us question this conceptual model. See (Jackson et al., 2000). That said, while we know a lot about plant water uptake, I agree there are nuances we don’t understand that the isotopic work has brought to light.

P5 L11: Please remove Brooks et al. 2010 from the reference list here referring to differences between plant and soil water. Brooks et al. (2010) focused on how soil water, particularly depleted soils at depth could be isotopically different from stream water, and that bulk soil water was different from lysimeter water collected at the same depth. The isotopic depletion found at depth could not be explained by evaporative processes.

P5 L24: I think the most appropriate reference for pore water extraction would be Sprenger et al. 2015. I don’t recall McCutcheon et al. (2017) going into this issue, and I can’t find it with a quick recheck of the paper.

P6 L3: Meinzer et al. (2006) showed it could take months.

P6, L16-19: I would say that other papers prior to this put forward these ideas.

P7, L16: You really need to include the work of Gabe Bowen when discussion spatial variation of precipitation isotopes, and any other spatial variation in water isotopes. See (Bowen & Revenaugh, 2003; Bowen, 2008).

P8 L4: Is this 2019 reference a typo?

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P8 L11: You should include the work of Christine Stumpp here (Stumpp et al., 2007; Stumpp & Maloszewski, 2010).

When addressing heterogeneity within soil water, you gloss over general patterns we do see somewhat consistently. For example, that bound water shows more evaporative effects than lysimeter water collected at the same depth, and that bulk soil water isotopes generally decrease increasing soil depth. I think the section would be stronger if you did talk about these patterns.

P9, L5-6: I think saying “many trees have branches that are plumbed to specific roots” is misleading here. While not with isotopes, xylem transport with dyes and other tracers has been studied for a long time, and mostly mixing does occur, although not completely around the circumference, and it varies with xylem anatomy. For example, see Ellmore et al. (2006). Don’t just highlight the extreme end of segmentation within plants, it’s a continuum. It’s likely only isotopically relevant for lateral vs tap roots.

P9 L9-10: Again, when talking about the spatial and temporal variation in leaf water fractionation processes, you state “this heterogeneity is often neglected..” but this variation has been the subject of many many studies. See my comment above, and many other leaf water papers out there including Helliker & Ehleringer (2002).

P9, L17-21: Please include more examples of work that reflects this across spatial scale work. For example, Sprenger et al. (2018) looked at the isotopic difference in lysimeter (mobile) and bulk water across a range of ecosystems. Brooks et al (2010) looked at 34 sites within one catchment to examine the spatial variation in soil water isotopes, but found depth explained more variation than location within the watershed such as ridge top vs riparian.

P9 L26-P10 L14: Please give examples here as to what you mean. I did not find figure 2 very helpful for these vague paragraphs. What you really mean here is what how good is the isotopic signal to noise ratio for the samples you are measuring. The signal is the variation across the scale of interest such as variance between sources. The

noise is the variance of repeated sampling of what is considered the same pool, such as xylem of multiple trees considered to be part of the same group. The signal should be multiple times greater than the noise. Experimental designs need to determine these variances. Variances generally decrease when samples integrate over larger space or time, but that is true for both the signal and the noise. Figure 2 kind of gets at that, but I felt it was confusing and not well explained.

P10, L23-30: I would also point out what Newberry et al. (2017) found about using oven dried soils, and our general method of testing the extraction protocol. I think it's important to highlight here. Also include Sprenger et al. (2015) review on pore water methodology.

P11, L4-18: These are very good points.

P11, L20-24: I agree this would be an exciting area to see researched in more detail. I think it would help readers if you gave a specific example of a physiological or ecohydrological process you would envision being aided by these techniques. Concrete examples help readers fully understand. Maybe expand on a labeling study, and explain how more high-resolution monitoring would have aided to more insights.

P12, L5-20: Again while a very important point, this paragraph is vague, and would be aided by more concrete examples.

P12, L21-29: I would go further here and say that studies need to do a better job of quantifying the variance within and between pools by duplicating every 10th or 20th sample. If your 10th sample is soil at 10 cm, collect two in the field, relatively near each other depending on study objectives.

P13 L14: Change to "natural and anthropogenic terrestrial enviornments. . .".

Overall, I look forward to seeing the authors develop this commentary further.

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