

# ***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.***

**D. Penna et al.**

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Response to Reviewer #1

“Tracing terrestrial ecosystem water fluxes using hydrogen and oxygen stable isotopes: challenges and opportunities from an interdisciplinary perspective”, by D. Penna et al.

We thank Matthias Sprenger for the time he spent on our manuscript and his detailed comments that have helped us to improve the paper. The reviewer’s comments are reproduced in their entirety and the authors’ responses are given directly afterwards.

[Comment 1] I am happy to read that Daniele Penna and others attending the workshop

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“Isotope based studies of water partitioning and plant-soil interactions in forested and agricultural environments” share the results of their discussions in the submitted commentary (“Ideas and perspectives” as called in Biogeosciences). They provide an adequate overview of the current state regarding recent developments in the application of stable isotopes as a tracer in biogeosciences and discuss also limitations that we are facing at the moment. I like how they frame these limitations as new perspectives and research opportunities to put a positive spin on these challenges. I also welcome their aim to promote interdisciplinary research using stable isotopes. The commentary is in the scope of the journal and I am sure it will be of interest to a broad community using stable isotopes. I suggest publication after minor revision and I provide some critical comments below. Please note that the provided references are of course only meant as suggestions and that reference to my own work are mainly provided to underline my arguments given in the general comments.

I think that the commentary could be stronger if the authors would revisit some parts, as the structure is not very consistent. For example, some parts in Section 2 would fit better to Section 3, as they deal with limitations, while several paragraphs of Section 3 read like a review and would fit better in Section 2. I provide example of these paragraphs in the specific comments.

[Response] We recognize that some improvements to the current manuscript structure are needed and that some sentences do not fit some sections perfectly. We will slightly modify the aim of Section 3 and will modify some lines accordingly in the revised version of the manuscript.

[Comment 2] I would also encourage the authors to acknowledge recent developments that deal with their suggested research agenda, when asking for isotopic variability of groundwater (as done by Scheliga et al., 2017), promoting tests of isotope analysis for vegetation samples (as done by Millar et al., 2018), analysis of spatial variability of soil water stable isotopes (as done by Yang et al., 2016), suggesting dual-labeling studies (as done by Bachmann et al., 2015), incorporating evaporation fractionation in

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catchment models (as done by Knighton et al., 2017, Smith et al., 2018, and Kuppel et al., 2018). While these studies were partly published recently, I still think it would be worth looking into them and considering including them. This would provide examples of developments that might go into the direction that you are suggesting and help the reader to see what is currently being done and tested.

[Response] We thank Matthias Sprenger for suggesting these very recent publications that indeed fit well the topics addressed in our commentary. We will include these references in appropriate sections of the manuscript.

[Comment 3] One comment on the “n water world”: The “Two Water Worlds” were suggested based on the findings by Brooks et al. (2010) that soil waters extracted with suction lysimeters or cryogenically have different isotopic compositions. Thus, this definition of a split into two subsurface pools mainly stems from the limitation to not be able to extract water held at different pressure heads. I obviously agree that the subsurface is a continuum of varying pressure heads (P6 L23). However, we are currently lacking the means to sample the isotopic composition along this continuum (water retention curve), but efforts have been made to compare different methods sampling waters of different mobility with different methods (see Figure 4 by Geris et al., 2015 and the work by Orlowski et al. (2016)). The relevance of different pressure heads for the TWW has been discussed earlier by Berry et al. (2017). The point that I want to raise here: Of course, it would be neat to be able to sample a “n water world”, but is it really practical? Instead, the TWW is relatively loosely defined into “mobile” and “less mobile or tightly bound” waters. However, from a stable isotope perspective (on which the TWW is based), we can only distinguish between “mobile” and “bulk” soil water given our limitations in the sampling procedure with either suction lysimeter and cryogenic extraction/direct equilibration, respectively. I tried to convey this message with my co-authors in Sprenger et al. (2018b). Important with this regard is that i.) the relative contributions of “mobile” and “more tightly bound water” is temporally variable (Figure 5 in Sprenger et al., 2018b) and that ii.) the “mobile” water does not reflect at all the

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total plant available water, but plants can access more tightly bound waters than the suction cup lysimeter (Figure 1 in Berry et al., 2017 and Figure 1 in Sprenger et al., 2018b). There is further a lack of clear definition distinguishing the two water pools.

[Response] We certainly agree with the Reviewer on this relevant point. Particularly, we recognize that there is a definitional problem with the term “water pools” because the definition is not univocal, and this hamper a clear conceptualization of the underlying physical and ecophysiological processes related to this. In the revised version of the manuscript, we will use the Reviewer’s valuable suggestion to reformulate and extend the paragraph, also including the recommended references. Our edits will approximately be the following: “The proposed “two water worlds hypothesis” (McDonnell, 2014) has challenged the assumption of complete subsurface mixing that underlies many catchment models (Pfister and Kirchner, 2017). This hypothesis postulates that more mobile soil water contributes to groundwater recharge and streamflow whereas more tightly bound water tends to be used by plants (McDonnell, 2014; Evaristo et al., 2015). Preliminary evidence from catchment studies based on dual-isotope approach and conducted in different environments showed that bulk soil water was isotopically different from tension lysimeter water collected at the same depth (Brooks et al, 2010), that shallow soil water pool utilized by plants differed in isotopic composition from precipitation, stream baseflow, and soil-lysimeter water pools (Goldsmith et al., 2011), and that xylem water was isotopically similar to soil and rain water, but different from streamflow and groundwater (Penna et al., 2013). This conjecture has stimulated new interpretations of ecohydrological data and new research questions to investigate water flow pathways in catchments (McDonnell, 2014). However, there seems to be a trend to focus on the interpretation of recent data on just confirming or rejecting this one hypothesis. As outlined in Berry et al. (2017) and Sprenger et al. (2016), alternative hypotheses need to be developed and tested to improve our current understanding. Because water held in the soil or moving through the soil and other subsurface layers is a continuum, where water transport is driven by gradients, and not separate “worlds”, we see the necessity to move from the simplistic “two water worlds hypothesis” to an “n

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water worlds” concept, where multiple water reservoirs and flow pathways are invoked and parameterized, doing justice to the properties of the different substrate types and sites. However, in practical terms, we are currently lacking the means to sample the isotopic composition along this continuum, and we are limited to distinguish only between “mobile” and “bulk” soil water sampled by either tension lysimeters or cryogenic extraction and direct equilibration, respectively. Very recent findings highlighted that the relative contribution of mobile and more tightly bound water is temporally variable and that the mobile water does not reflect the total plant available water (Berry et al., 2017; Sprenger et al., 2018b). Although efforts have been made to compare different methods to sample water of different mobility for isotopic analysis (e.g., Geris et al., 2015; Orlowski et al., 2016b), we still lack a clear distinction of these two water pools. Investigating more in detail the relationships between soil water isotopic concentrations and water mobility at high spatial and temporal resolutions (McCutcheon et al., 2017) will help us to move beyond the two water worlds hypothesis.”

Berry, Z. C., White, J. C. and Smith, W. K.: Foliar uptake, carbon fluxes and water status are affected by the timing of daily fog in saplings from a threatened cloud forest, *Tree Physiol.*, 34(5), 459–470, doi:10.1093/treephys/tpu032, 2014.

Brooks, J. R., Barnard, H. R., Coulombe, R. and McDonnell, J. J.: Ecohydrologic separation of water between trees and streams in a Mediterranean climate, *Nat. Geosci.*, 3(2), 100–104, doi:10.1038/ngeo722, 2010.

Evaristo, J., Jasechko, S. and McDonnell, J. J.: Global separation of plant transpiration from groundwater and streamflow, *Nature*, 525(7567), 91–94, doi:10.1038/nature14983, 2015.

Geris, J., Tetzlaff, D., McDonnell, J., Anderson, J., Paton, G. and Soulsby, C.: Ecohydrological separation in wet, low energy northern environments? A preliminary assessment using different soil water extraction techniques, *Hydrol. Process.*, 29(25), 5139–5152, doi:10.1002/hyp.10603, 2015.

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Goldsmith GR, Muñoz-Villers LE, Holwerda F, McDonnell JJ, Asbjornsen H, Dawson TE.: Stable isotopes reveal linkages among ecohydrological processes in a seasonally dry tropical montane cloud forest. *Ecohydrology* 2011, 5:779–790

McCutcheon, R. J., McNamara, J. P., Kohn, M. J. and Evans, S. L.: An evaluation of the ecohydrological separation hypothesis in a semiarid catchment, *Hydrol. Process.*, 31(4), 783–799, doi:10.1002/hyp.11052, 2017.

McDonnell, J. J.: The two water worlds hypothesis: ecohydrological separation of water between streams and trees?, *WIREs Water*, 1, 323–329, doi:10.1002/wat2.1027, 2014.

Penna D, Oliviero O, Assendelft R, Zuecco G, Tromp-Meerveld I, Anfodillo T, Carraro V, Borga M, Dalla Fontana G. Tracing the water sources of trees and streams: isotopic analysis in a small pre-alpine catchment. *Procedia Environ Sci* 2013, 19:106–112

Orlowski, N., Pratt, D. L. and McDonnell, J. J.: Intercomparison of soil pore water extraction methods for stable isotope analysis, *Hydrol. Process.*, 30(19), 3434–3449, doi:10.1002/hyp.10870, 2016b.

Pfister, L. and Kirchner, J. W.: Debates – Hypothesis testing in hydrology: Theory and practice, *Water Resour. Res.*, 53(3), 1792–1798, doi:10.1002/2016WR020116, 2017.

Sprenger, M., Leistert, H., Gimbel, K. and Weiler, M.: Illuminating hydrological processes at the soil-vegetation-atmosphere interface with water stable isotopes, *Rev. Geophys.*, 54(3), 674–704, doi:10.1002/2015RG000515, 2016.

Sprenger, M., Tetzlaff, D., Buttle, J. M., Laudon, H., Leistert, H., Mitchell, C. P. J., Snelgrove, J., Weiler, M., and Soulsby, C.: Measuring and modelling stable isotopes of mobile and bulk soil water, *Vadose Zone J*, 17, 170149, doi:10.2136/VZJ2017.08.0149, 2018b.

[Comment 4] P2 L11: I am not sure if a report (2030 WRG, 2009) sponsored by “The Barilla Group, The Coca-Cola Company, The International Finance Corporation, McK-

insey Company, Nestlé S.A., New Holland Agriculture, SABMiller plc, Standard Chartered Bank, and Syngenta AG” is an appropriate reference.

[Response] Indeed it is not! It was a slip and we will remove it.

[Comment 5] P2 L 29: To my understanding, it is not only that the laser-based instruments are more affordable, but also running it for analysis is cheaper and easier.

[Response] That is true. We will specify this in the revised manuscript.

[Comment 6] P3 L2: I think that the context is missing here. One might ask “which well-mixed conditions”? While I believe to know what you refer to, you aim to reach a wider audience in Biogeosciences and thus, should be clear about the simplifying assumptions.

[Response] We agree with the reviewer. We will modify the sentence as follows: “Consequently, recent studies have revealed problems in the simplifying assumptions that underlie past investigations, especially those related to steady-state and well-mixed conditions, i.e., the assumption that water in the subsurface mixes instantaneously and completely in one common reservoir, so that no differences in isotopic composition would be observed with depth and in space.

[Comment 7] P3 L25-L29: This reads more like “limitations and challenges” and is an example of what I mean that the structure could be improved.

[Response] We agree, and we will move this paragraph to Section 3.2, when explaining sources of heterogeneity in the isotopic composition of soil water.

[Comment 8] P4 L17-L19: Again, when you start a sentence with “One limitation: : :”, this might fit better in section 3 of the manuscript.

[Response] We will reword this sentence to present the characteristics of laser instruments.

[Comment 9] P4 L19: What about carrier gas issues like CO<sub>2</sub>, which potentially cause

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issues for CRDS (Gralher et al., 2018)? Beside consequences for the direct-vapor equilibration method, this would also be relevant for in-situ measurements via the vapor phase.

[Response] Yes, this is a relevant issue, and we will add another statement in this paragraph.

[Comment 10] P4 L23: You mention soils, tree stems and leaf as examples. What about ETpartitioning as done for example by Wang et al. (2010)?

[Response] Even though this is not a recent study, we will add this reference.

[Comment 11] P5 L6: I think that the comparison and review study by Rothfuss and Javaux (2017) is a more appropriate reference here.

[Response] We will add this reference.

[Comment 12] P5 L10: Given the methodological issues with the calculations of source waters by Evaristo et al. (2015), as revealed by Javaux et al. (2016), this might not be a good reference.

[Response] Both Javaux et al. (2016), in their comment, and Evaristo et al. (2016) in their reply recognize that the mistake did not affect the central conclusion of the paper. Therefore, we believe that the reference is still appropriate and we will keep it in the manuscript.

Evaristo, J., Jasechko, S., McDonnell, J.J., 2016. Evaristo et al. reply. Nature 536, E3.

Javaux, M., Rothfuss, Y., Vanderborght, J., Vereecken, H., Brüggemann, N., 2016. Isotopic composition of plant water sources. Nature 536, E1.

[Comment 13] P5 L18: Consider including Zhao et al. (2016) as reference, who compared also different methods for xylem sampling.

[Response] Yes, this reference was already cited elsewhere in the original manuscript,

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and we will add a citation also here.

[Comment 14] P5 L20: Be more specific: What do you suggest to the community? What do you “aim to analyze” (- depends on your research question, or not)? How is this message different to the section 4.3 in the commentary by Berry et al. (2017)?

[Response] We believe that we cannot be more specific because specific suggestions depend on the research question of individual studies. Therefore, we are suggesting to focus on two main issues already mentioned by Berry et al. (2017), somehow reiterating their message. We will include a further citation to the work of Berry et al. (2017).

Berry, Z. C., Evaristo, J., Moore, G., Poca, M., Steppe, K., Verrot, L., Asbjornsen, H., Borma, L. S., Bretfeld, M., Hervé-Fernández, P., Seyfried, M., Schwendenmann, L., Sinacore, K., De Wispelaere, L. and McDonnell, J.: The two water worlds hypothesis: Addressing multiple working hypotheses and proposing a way forward, *Ecohydrology*, 1–10, doi:10.1002/eco.1843, 2017.

[Comment 15] P5 L26: I am not aware that McCutcheon et al. (2016) studied the stable isotopic compositions among different pore spaces. Instead, they specifically stated that “We are not able to determine if pore-scale variability can impact isotopic composition of root-absorbed and draining water”.

[Response] True, that reference is not appropriate there, and we will remove it.

[Comment 16] P5 L31: How about evaporation through the bark as studied by Martín-Gómez et al. (2016)?

[Response] Yes, we will add these findings and the reference.

[Comment 17] P6 L7: Do you think numerical simulations can help assessing such ages, as done by Brinkmann et al. (2018) and Sprenger et al. (2018a)?

[Response] These are relevant studies in this context. We will modify the sentence

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including results from modeling studies.

[Comment 18] P6 L14: Do you think relating tree ring to source waters would be beneficial to study long term dynamics (e.g., Singer et al., 2014)?

[Response] In this paragraph, we discuss the short-term variability of the isotopic composition of water sources. The tree ring timespan goes well beyond this timescale. In addition, the determination of the water source is not straightforward because many processes influence the signature of cellulose, which is only partially related to that of the source water (Gessler et al 2014). Therefore, we think that it is not appropriate to add the suggested reference in this paragraph. However, we will include it in Section 3.2, when discussing wood isotopic composition.

Gessler, A., Ferrio, J. P., Hommel, R., Treydte, K., Werner, R. A. and Monson, R. K.: Stable isotopes in tree rings: towards a mechanistic understanding of isotope fractionation and mixing processes from the leaves to the wood, *Tree Physiol.*, 34(8), 796–818, doi:10.1093/treephys/tpu040, 2014.

[Comment 19] P6 L25: See general comments regarding the “n Water World”.

[Response] Please, see our response to comment 3 above.

[Comment 20] P7 L18- P8 L23: These paragraphs read like a review and do not link to “Limitations and challenges”.

[Response] We will slightly restructure the manuscript focusing the entire Section 3 on summarizing the current knowledge about the source of variability in the isotopic composition of the different ecohydrological compartments, as well as highlighting the limitations and challenges deriving from the heterogeneity of the systems of interest and the uncertainty associated to our current knowledge and measurements efforts. Moreover, we will reword some sentences to highlight the difficulties of studying ecohydrological systems and the related challenges.

[Comment 21] P8 L26: Please note the intensive sampling of groundwater stable iso-

topes by Scheliga et al. (2017).

[Response] Thanks. We will rephrase the sentence and refer to this study.

[Comment 22] P8 L29- P9 L10: To me, you miss pointing out the limitations and challenges in these paragraphs.

[Response] Please, see our response to comment 20.

[Comment 23] P9 L21: Regarding the heterogeneity of soil water stable isotopes, please see the data set by Yang et al. (2016) the intense variability across relatively small area.

[Response] The reference is relevant to highlight the spatial variability of the isotopic composition of soil water, depending on different factors. However, in this sentence, we are referring to the heterogeneity in the isotopic composition of water when moving up in scale in more general terms of ecohydrological processes than soil water processes only. However, we will include the suggested reference in two points of Section 3.2 of the revised manuscript, when mentioning the effect of the isotopic composition of antecedent rainfall on soil water, as well as the effect of local soil properties.

[Comment 24] P10 L23: Consider including the interesting study by Millar et al. (2018)

[Response] Yes, we will add this citation, as it is highly relevant.

[Comment 25] P10 L14: This ratio between water accessible for plants and water extractable by suction lysimeter is time variant (Sprenger et al., 2018b) – this is usually ignored and cannot be accounted for when sampling twice soil and plant isotopes over a year.

[Response] The reviewer likely refers to P11 L14. This is a very relevant study, published online only a few days before we submitted our commentary. We will include the point raised by the reviewer in the revised manuscript.

[Comment 26] P12 L5: I suggest providing examples, where this has been done (e.g.,

Knighton et al., 2017, Smith et al., 2018, and Kuppel et al., 2018)

[Response] Yes, we will do that.

[Comment 27] P12 L1: As done by Bachmann et al. (2015)

[Response] We will add this reference.

[Comment 28] P12 L12: as reviewed by Rothfuss and Javaux (2017)

[Response] We will add the following sentences to the manuscript: “For instance, Rothfuss and Javaux (2017) examined the uncertainty associated to different types of mixing models, stating that graphical and statistical methods have major drawbacks when analyzing root water uptake depths. They found that the latest generation of Bayesian mixing models performs well for that purpose, but only when the number of considered water sources in the soil is high and closely reflects the vertical distribution of the soil water isotopic composition.”

Rothfuss, Y. and Javaux, M.: Reviews and syntheses: Isotopic approaches to quantify root water uptake: a review and comparison of methods, *Biogeosciences*, 14, 2199–2224, doi:10.5194/bg-14-2199-2017, 2017

[Comment 29] P12 L14: Can you provide any studies backing this idea? Is to “introduce further complexity” something positive in this context? This “potential interactions of these tracers with soil, roots and the water itself” would need to be carefully studied and understood for the tracer interpretations, right?

[Response] We agree and we will reformulate as follows: “Additional tracers can be helpful to support and strengthen the observations obtained by using stable isotopes. Trace elements taken up through plants (e.g., gold particles, Lintern et al., 2013) might have particular potential for inferring root water uptake. Also, chemical tracers (e.g., Haase et al., 1996) and tritium (Zhang et al., 2017) have been used to study water uptake depths. However, using other tracers such as fluorobenzoic acids, dissolved ions, isotopic ratios of other elements such as radium or strontium, will introduce fur-

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ther complexity to the system due to potential interactions of these tracers with soil, roots and the water itself. Such effects need to be carefully studied in order to provide meaningful interpretations.”

Haase, P., Pugnaire, F.I., Fernández, E.M., Puigdefábregas, J., Clark, S.C., Incoll, L.D., an investigation of rooting depth of the semiarid shrub *Retama sphaerocarpa* (L.) Boiss. by labelling of ground water with a chemical tracer. *J. Hydrol.* 177, 23–31, 1996. [https://doi.org/10.1016/0022-1694\(95\)02794-7](https://doi.org/10.1016/0022-1694(95)02794-7)

Lintern, M., Anand, R., Ryan, C., Paterson, D., Natural gold particles in Eucalyptus leaves and their relevance to exploration for buried gold deposits, *Nat. Commun.* 4, 2274, 2013. <https://doi.org/10.1038/ncomms3614>

Zhang ZQ, Evaristo J, Li Z, Si BC, McDonnell JJ. Tritium analysis shows apple trees may be transpiring water several decades old. *Hydrological Processes.* 2017;31:1196–1201. <https://doi.org/10.1002/hyp.11108>

[Comment 30] Fig. 1: You’ve added remote sensing to the graph, but do not discuss it in the text.

[Response] We will include more discussion on remote sensing in the text.

[Comment 31] Fig. 1: Plant uptake and transpiration have scales > 10m in your graph, but much of your discussion deals with processes way smaller where water is taken up by roots (scales  $\hat{A}$ n 1m).

[Response] This is a good point. We will modify the figure to highlight the processes of root uptake and leaf transpiration at the scale of individuals (1 m and below) and that of including vegetation processes at larger scales (stands, forests, agroecosystems, etc).

[Comment 32] Fig. 1: How about potential of tree ring samples (in the category “grab sampling”)?

[Response] As we have not discussed this topic in the manuscript, we prefer to leave it

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out from this conceptual sketch.

[Comment 33] Fig. 2A: It seems from Fig. 2B that the green shading represents the range of samples and the black line represents an average value. Are these values then representing average and range from replicates (e.g., five soil samples at 0-5 cm soil depth)? Otherwise, how could you have a range from one sample value (as indicated on the y-axis)? Or does the green shade the range of values out there in nature and the black line represents the individual sample? This is not clear to me.

Fig. 2C: Not sure if I understand how the temporal variability of precipitation and throughfall is higher within events than among events. Is not the variability within the events part of the overall variability among events? Looking at Figure 6 in Freyberg et al. (2017) it seems that variability between events is higher.

Fig. 2C: Why did you not include plant water isotopes?

[Response] The inclusion of this figure in the manuscript stemmed from debates engaged in the discussion groups at the workshop. Introducing this figure aimed at providing a concept of a representative sampling size or scale over which to bulk a sample. It did not focus on averaging multiple samples but on achieving a bulk sample of a typical size that could reflect – upon multiple samplings – the variability (distribution of mean and standard variation) that is representative for the sample type (soil water, plant water etc). Ultimately, these considerations would result into the recommendation of typical sample sizes and frequencies. However, we share the perplexities of all three reviewers on the usefulness of this figure, and we agree with them that the figure is vague and may be confusing. In addition, it is not strictly related to the text and the main focus of this commentary. Therefore, we will remove this figure from the revised version of the manuscript, as well as mention to it in the text.

[Comment 34] Title: As I understand, the manuscript title must start with “Ideas and perspectives:” ([https://www.biogeosciences.net/about/manuscript\\_types.html](https://www.biogeosciences.net/about/manuscript_types.html))

[Response] True, we will implement that in the revised manuscript.

[Comment 35] P2 L24: Repetition of “Stable isotopes of hydrogen and oxygen”

[Response] We will modify the sentence to avoid the repetition.

[Comment 36] P14 L1: Coauthors missing for Bertrand et al. (2014).

[Response] True, we will add the complete reference.

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