Hydrol. Earth Syst. Sci. Discuss.,
https://doi.org/10.5194/hess-2020-446-RC2, 2020

Hydrology and Earth System Sciences
Discussions
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Interactive comment on "A comparative study of plant water extraction methods for isotopic analyses: Scholander-type pressure chamber vs. cryogenic vacuum distillation" *by* Giulia Zuecco et al.

Anonymous Referee #2

Received and published: 18 December 2020

General comments:

The study conducted by Zuecco et al. addresses a very important issue in the ecohydrology community. They investigate two plant water extraction methods and attempt to analyse the differences of the isotopic composition of plant water in relation to the respectively used method. Namely, they compare plant water isotopic compositions extracted with either a Scholander pressure chamber (SPC) or cryogenic vacuum distillation (CVD). They aim at assessing (1) differences in isotopic composition related to the technique used (i.e. SPC or CVD) and additionally (2) want to analyse how differ-

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Discussion paper

ences in the isotopic composition are related to plant species or plant tissue type (from hereon, these two aims will be referred to using the terms aim1 and aim2). While this is a much needed study with a promising topic related to ecohydrology, I have, what I believe, are major concerns regarding the implementation of the method comparison.

While the authors used a fully suberized one- year old shoot to extract water from using the SPC method, they extracted water from several tissues making up a twig/shoot using CVD. These are not the same and therefore cannot be compared. Thus, I feel the authors cannot address aim1. Aim2 addresses differences in relation to tissue type and plant species, but only one sample type is used for SPC. Additionally, the extraction with SPC happened with attached leaves and unpeeled bark. The authors mention in the introduction and the discussion the Geißler et al. 2019 study, but do not take the same precautions when handling the samples (I know that this data set was taken prior to the Geißler manuscript, nevertheless the precautions are valid also for sample handling when not analysing water isotopes (e.g. when analysing xylem sap for nutrients the phloem is peeled off before the shoot is extracted with the SPC)). Is it not likely that when leaves are still attached and intact, to extract xylem sap from the leaves especially when applying high pressures (eg for the Ressi samples) and long extraction times (i.e. "until all the water was collected" L139/140)? Especially on the notice that the samples obtained with SPC in situ were different in colour, the authors could have taken another sample were they peeled the bark and stripped the leaves, thus assessing potential contamination of organics, and of the still living cells in attached leaves.

That said, I want to emphasize, that the topic and the idea for this study is needed, the community is waiting for this assessment and the results that can come from it, so I would encourage the authors to provide either better argumentation of why they can compare samples not from the same tissue, or an additional dataset, in which they compare the same tissues (e.g. complete shoot extracted with CVD or all plant tissues also extracted with SPC) and then address these aims again.

Additionally, I think the manuscript would benefit from a thorough review of a native speaker. Therefore, I will not comment on nor suggest improvements regarding language use or sentence structure.

Specific comments:

INTRODUCTION:

L 41: for completeness sake I would add Marshall et al. (2020) to the vapor equilibration method

L44: I would suggest giving either a comprehensive list of research in which this method is described/applied or one key publication in which the method is extensively described/first published

L45/46: “stored in dead and living cells for months or years” please add a reference

L49-54: Maybe shortening this section would benefit the reader, as they can look it up in the cited literature

L55ff: I would encourage you to shorten this section to the most relevant conclusion, i.e. that the results are ambiguous and different methods result in different outcomes

L69 adding Scholander (1966) to the references would help a comprehensive list (see reference list below)

L73: what about Ellsworth and Williams (2007) and Magh et al. (2020)? Even though Ellsworth and Williams did use SPC in a different way I think it would be worth mentioning with a statement like the one you make here.

STUDY SITE AND SAMPLING:

L88: Reference “Autonomous province of Bozen-Bolzano” is missing URL or in reference list. Please add

L89: what do you mean by “relatively mature”? please specify either the exact age

of the stands (for all three sites) or use appropriate describtion (i.e. either mature or juvenile)

FIG1: there are two red points on each photograph, I assume those are supposed to be the sampling sites? If so, one is really close to the river, the other one not. This probably has an influence on the isotopic composition of the samples and should be accounted for and definitely mentioned in the figure description.

L92+L104 Why after sunset? Especially considering water potentials were expected to be low due to the water deficit? Why not pre-dawn like at the Ressi site?

L105ff soil data are missing here but were added for the other sites. Please add here too.

MATERIALS AND METHODS:

L123 again missing the Scholander reference, please add

L137 please indicate if the samples were taken close to the river or not. The pressures used for extraction seem to indicate that there was no water deficit at the Laas site, but high deficit at the Ressi site, this could be related to the sampling position close to the river?

L140 how long did it take to sample all of the water for the samples? Were the times different for the different sites? Generally, are the samples all the same size and length?

L148ff The proclaimed target was to compare SPC to CVD. In order to do that it would have been good to extract the same tissues with both methods. Please elaborate why this has been done the way it was. Separating the twigs/shoots into smaller units to answer aim2 could still be done only for CVD if the small sample volume with SPC was the problem.

L153 what does this mean? The trees were too small? Too large? Please elaborate.

ISOTOPIC ANALYSIS:

L173-180 please add information about standardization, normalization, and possible corrections

DATA ANALYSIS:

L 190 I think this sentence could benefit from clarification. Maybe rephrase to something like this: “We report d-excess values enabling us to compare samples from different study areas.”

L206ff This compares different plant tissues extracted by CVD with the whole shoot extracted using SPC. The results are not wrong, but misleading when considering the aim was to assess differences between the extraction methods. The tissues compared here are not the same and differences found from this analysis could also be related to differences in plant tissue instead of extraction method.

DISCUSSION:

L271 “in situ” sounds a little misleading because it is often used in relation to vapor equilibration methods. I suggest replacing it with “in the field”.

L277 which advantages are these?

L280 extraction at 3Mpa likely damages living cells in the leaves/shoot, so it could be possible that the samples did not only consist of xylem sap anymore?

L283 how large are these volumes? Would it be possible to calculate a volume weighted isotopic composition of the samples CVD_L and CVD_TWB to get an approximation for the signature of a complete shoot extracted with CVD? Maybe that way, it would be possible to “compare” SPC and CVD with the same plant tissue used. That would only work if the extraction was complete though. Was that calculated? I mean were the CVD samples weighed before and after extraction and then again after drying?

L248ff I understand the volume from the SPC samples was too small for spectral con-

tamination analysis, but an assessment for the CVD samples would have been possible? These data would be interesting especially when comparing the CVD_T and CVD_TWB samples.

L296-300 I feel this is redundant here because it was just discussed in the previous section. I suggest deleting

L310 I don't understand why it was expected for a CVD_WC sample obtained at breast height (?, this is an assumption, please specify the sampling heights and direction for all samples in the material and methods section) to have the same or a similar isotopic composition as a twig sampled from the crown (which likely has a different height). There has been evidence that this cannot be automatically assumed (de Deurwaerder et al. 2020). Please elaborate.

L313ff I don't think this argumentation is valid here. The samples are not comparable because they are not the same.

L319ff Again, this argument bases on the comparison between tissues which are not the same. I agree however, that SPC and CVD are able to extract different water pools/domains and it would be rational to quantify these differences especially when talking about water storage.

L326-329 redundant as the previous sentence says the same, rephrase or delete

L331 missing reference for water storage times. Please add

L339/340 how much older? Maybe a few examples would help here. Also, here you use plant water fractions before you used pools, please be consistent with which term you use throughout the manuscript.

General recommendations to improve the discussion:

I would suggest discussing the possibility of water pool mixture during transportation, because it is highly likely that water pools exchange and cannot be considered com-

pletely separate.

CONCLUDING REMARKS:

This reads like a second abstract/a summary and not like a conclusion. I suggest deleting the summarizing parts and keeping the main message (i.e. lines 363 -371)

References used in this review:

Deurwaerder HPT de, Visser MD, Detto M, Boeckx P, Meunier F, Kuehnhammer K, Magh RK, Marshall JD, Wang L, Zhao L, and Verbeeck H 2020. Causes and consequences of pronounced variation in the isotope composition of plant xylem water. Biogeosciences 17: 4853–4870.

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Magh R-K, Eiferle C, Burzlaff T, Dannenmann M, Rennenberg H, and Dubbert M 2020. Competition for water rather than facilitation in mixed beech-fir forests after drying-wetting cycle. J. Hydrol. 587: 124944.

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Scholander PF 1966. The role of solvent pressure in osmotic systems. P. Natl. Acad. Sci. 55: 1407–1414.

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