Interactive comment on "Diurnal variation in the isotope composition of plant xylem water biases the depth of root-water uptake estimates" *by* Hannes P. T. De Deurwaerder et al.

Anonymous Referee #1

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De Deurwaerder and colleagues present a composite work where they (i) run a model simulating diurnal variations and vertical heterogeneity in xylem water isotopic composition (δxyl) and perform a multi-variate sensitivity analysis. They also (ii) present results of sampling campaigns where δxyl temporal and spatial variations were observed in twelve tree and liana species. The authors explain these variations and thus the departure of the generally accepted hypothesis of homogeneous δxyl on account of their model output. Finally they warn the isotopic community against the "danger" in using water stable isotopes as tracers for RWU analysis.

The manuscript is well written, figures and tables are of good quality and appropriate

referencing supports the text. Finally the manuscript content falls within the scope of BG.

My general comments are listed below:

1- I note that the authors do not confront their model results to collected data, nor thoroughly test their model hypotheses on independent data. I do not see a particular problem, but it should be mentioned clearly that aforementioned items (i) and (ii) are only "softly" coupled in the study;

2- highlighting the temporal as well as spatial (longitudinal) dynamics of δxyl is of evident interest. However the prevalence of such dynamics may not put in "danger" – as the authors say – the determination of fractional root water uptake for other non-wooden species. The abstract should be rewritten accordingly. The isotopic community should be on the "safe" side if researchers extract water from a plant tissue for which it has been proven that its stable isotopic composition reflects that of RWU. Of course, this should be investigated for each investigated plant species, preferably under controlled conditions (see for example: Barnard et al., 2006);

3- The authors provide no information about the soil compartment; what about the soil water isotopic composition profile temporal and spatial variabilities? Are the isotopic differences in xylem water reflected by the span of isotopic composition values in soil water? This would offer the possibility to rule out possible evaporation effects mostly during sampling and transport (which is not listed as other reasons for the observed diurnal variations of δxyl). If soil water isotopic information is not available, it should be stated as limitation of the study;

4- I found on several occasions that the authors did not fully understand basic principles driving isotopic fractionation (see my specific comments);

5- In general, I do not think that such field experiments, where a significant number of environmental driving factors are unknown, should be used to question the entire

isotopic research methodology. I urge the authors to discuss this point as well and measure their words.

The authors will also find a list of specific questions/remarks/corrections/issues:

L24ff. What does "i-H2O-xyl" refer to? To "plant xylem water uniform isotope composition" or "plant xylem water isotope composition"? In either case, "δ" is to be preferred over "i-H2O-"

L32-33. "field data show excessive i-H2O-xyl variation during the day or along stem length ranging up to 25.2‰ in δ2H and 6.8‰ in δ18O" does not read well. I propose something like: "the hydrogen (oxygen) isotope composition of plant xylem water showed strong temporal (i.e., daily) and spatial (i.e., along the stem) variation ranging up to 25.2 (6.8) ‰"

L36. Please rephrase: "danger" is not the proper word.

L46-47. There is no such thing as the depth of root water uptake in the case of several soil water sources. Only in the context of direct inference is this true. But the authors do not refer to the later (and outdated) technique.

L49. This is not true: the isotopic technique is of course destructive (you have to take a soil core), very labor intensive (e.g. extraction of soil and plant xylem water).

L50 (also L47). You should mention that it is fractional RWU and not absolute RWU you are talking about. You cannot solve for water mass balance with the isotope technique, which constitutes its greatest limitation when compared to other techniques.

L52. How would you determine fractional root water uptake at the ecosystem level?

L56. This should be "δxyl". Why the "i" instead of "δ" here? Also, why the "H2O"? (is there another molecule investigated here?)

L58-60. Peclet effect is measureable in the xylem vessels upstream of the evaporative sites. This assumption is not systematically made. Instead, authors investigate the

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prevalence of isotopic fractionation depending on the plant tissues they sample, e.g. in Barnard et al. (2006). Please revise.

L67-69. There can only be kinetic fractionation playing a role during the transport of water through the root membranes, since there is no liquid-vapor phase change that would involve equilibrium fractionation. Please revise.

L69-72. Not only kinetic fractionation is a result of the difference in mass of the water isotopologues, but fractionation in general (e.g., equilibrium and kinetic fractionations).

L94-95. Why would you make the assumption that δxyl is constant over time (over which period of time anyway)? At this point of the MS, it is not clear. Actually, no one makes this assumption in the field, rather they sample from e.g. the base stem among individuals at e.g. a sub-hourly temporal resolution and sub-daily temporal extent.

L100-101. What do you mean by "diurnal changes in the soil-plant-atmosphere continuum"? Which changes?

L113. What exact "water potential gradients" do you refer to?

L114-116. Why would you need to use a mixing model, especially since you did not sample soil water and determine its isotopic composition? You may as well simulate a sinusoidal pattern for the δxyl. Please elaborate/explain.

L117-119. You should write the isotopic equations with "δ" instead of "δ2H" as the model does not focus on 1H2HO, to the contrary of what the authors say. For the model to focus on 1H2HO, it would mean that 1H2HO and 1H218O would follow different physical processes, which is not the case (both isotopologues undergo mass-dependent fractionations, i.e. εeq(2H)/ εeq(18O)≈8 and εk(2H)/ εk(18O)≈0.88). Also write 2H instead of Deuterium and do it consistently throughout the MS. The latter is just an element's isotope and does not deserve (anymore) its own letter (see IAEA tech reports guidelines).

L130-131. This assumption is only reasonable when soil water redistribution no longer

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occurs, e.g., this does not stand shortly after a rain event.

L125ff. Report the dimension of each variable and parameter throughout the MS.

L142. From Eq. (3), I understand that the water "potentials" are in fact "hydraulic heads". This should be clarified.

L143-144. Add here that k_i and $\delta I \dot{Z} \acute{z} \delta I \acute{S} E_{,,} \delta I \acute{S} a$ are also specific to the ith soil layer.

L193-196. I am missing background information to understand what the "30 days sequence" of the "model runs of Huang et al. (2017)" refers to. Please elaborate.

L201-203. Why would you need external data (Meissner et al. 2012) and not simply do your model exploration on basis of a synthetic experiment?

L208-209. I did not hear of such standard practice and I doubt there is. Could you add a reference for this?

L223-225. Split the sentence and add detail. It is hard to understand. Also following the Rayleigh distillation model, the error should always be negative in the case of incomplete water recovery, which does not match to your normal distribution of error in the null model.

L228-229. How so? And why would it be relevant to take into account the analyzer systematic error at this point of your model testing?

L235-244. Are you talking about RWU depth of rooting depths here? How do you define the latter term? Why would you use the direct inference model (which is a very simplistic view on RWU, i.e., one single root sampling from on single layer at a time) if you use a multi-source mixing model (Phillips and Gregg, 2003), which allows the plant to sample simultaneously from different layers? Please explain this apparent contradiction. Overall this section is quite difficult to read and I ask that the authors simplify it.

L258. Is there a specific reason why you did not use Van Genuchten's soil retention

curve?

L309. Delete "kinetic". It is not even sure that you would have fractionation at all, considering that you may boil (==fractionation free process) the water here rather than evaporate it.

L321. Fresh weight does not take into account possible loss of water during transport/storage. You should have weighted the samples prior extraction again.

L331-336. Since you are measuring with a Picarro, which does not give ratio (but performs already the delta conversion), you need to say that you "corrected the Picarro raw delta readings into calibrated delta values thanks to the values of the aforementioned 'internal laboratory References' expressed on the international V-SMOW scale". No need to display the equation (12) but you may detail these "internal laboratory References" (e.g., value).

L336-334. Still at this point, I do not know what the difference is between i-H2O-xyl and δxyl...If there is none, please use the latter term. In addition, use another letter than ε for the normalized "i-H2O-xyl": it usually stands for isotopic fractionation, defined as the deviation of the fractionation factor to unity. It seems even odd that you would consider such a letter...

L369. I still do not understand what is the concept of RWU depth if you consider the multi-source mixing model approach.

Fig. 2. Panel (a): how do you come up with a night δxyl at 1.3 m above −60‰ Also, I don't see why panels (a) and (d) look so different for day 1, since if I understand correctly, the cumulative SF is a function of time (if sap flow remains constant).

L371. "isotopic composition of soil water is dominated by depleted deuterium". Please correct phrasing: soil water can be depleted in 2H in comparison to another water volume, but there is no such thing as "depleted 2H".

L373. An isotopic composition, which is a number, cannot be "enriched". Please cor-

rect.

L375. "depleted deep soil water"

L384. "...RWU originating from deeper, more depleted soil layers". Please correct: water from a given soil layer might be depleted, not the soil layer in itself.

L399-400. This belongs to the discussion section.

L407-418. Nowadays no study is published where RWU depth is investigated with the direct inference method. Analyses are performed with Bayesian mixing models. So I wonder if this section, although interesting theoretically, would benefit practically to the community.

L446 and Fig. 4. See my previous comment on the use of "ε". The caption of a figure should not point to another figure or table. Write here the name of the species (no need to write them in the figures though).

L452. Add in the text that growth forms refer to lianas and trees.

L455-457. This belongs to the discussion section. Also the link between "easily accessible and abundant groundwater reservoir" and the fact that the diurnal intra-individual variance is minimized is not clear. I suggest moving to the discussion and elaborating on this.

L471-472 and Table 2. How many individuals (which you could consider as replicates) of each species were sampled during the experiment? Discuss the implication of having n=1 with respect to δxyl variance.

L486-492. The authors say that the intrisinc problem of the "isotopic tracing method" is that there is a soil water isotopic gradient in case there is evaporation and under heterogeneous soil water potential gradient? I don't understand this at all (!) The isotopic methodology for studying plant RWU relies on heterogeneous isotopic gradients in soil water. This is a solution, not a problem here...

L493-506. I disagree. There is a clear problem in determining fractional RWU profiles on basis of measurements of the transpiration isotopic composition, which is highly temporally dynamic and spatially heterogeneously distributed; many observation of leaf water confirm the non-reaching of isotopic steady state. In addition, how would a "change of cloud cover degree" have an "instantaneous" influence on δxyl? This contradicts the results of your synthetic experiments, where depending on sap flow rate, there is a marked isotopic memory effect of the antecedent water moving upward it the xylem vessel.

L516-523. The model provides an explanation, sure, but does not validate your hypotheses from the confrontation with experimental data. This is missing from your study and should be mentioned.

L534-546. My understanding from the literature is that hydraulic redistribution is intermittent and localized, thus does not affect that much the bulk soil water isotopic composition, rather it affects the direct environment of the roots.

L578-587. Not to forget we need to monitor soil water isotopic composition to verify if δxyl spreads within the range of isotopic values observed in the soil profile.

References: âĂČ Barnard, R. L., de Bello, F., Gilgen, A. K., and Buchmann, N.: The δ18O of root crown water best reflects source water δ18O in different types of herbaceous species, Rapid Commun. Mass Spectrom., 20, 3799-3802, doi:10.1002/Rcm.2778, 2006.

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