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Interactive comment on "Multi-isotope labelling (^{13}C, ^{18}O, ^{2}H) of fresh assimilates to trace organic matter dynamics in the plant-soil system" *by* M. S. Studer et al.

Anonymous Referee #2

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General comments

This is an exciting study, not only due to the methodological challenges associated to the multi-isotope labeling applied, but also as a direct evidence of the isotopic signal that can be transferred from atmospheric water vapor to the leaves. Although some aspects of the methodology could be improved, this is a relevant contribution to our understanding of isotope signature in plant tissues. The conclusions are clearly stated and well supported by the data presented, encouraging further works considering compound-specific analyses, together with a more detailed assessment of soil-plant-atmosphere flows.

Specific comments

The most novel finding of the study is to show a strong "atmospheric water" signal in leaf water, which is transferred to assimilates, under conditions in which a net release of water vapor from the leaf to the atmosphere is expected. Unfortunately, the authors did not provide data on fluxes of water inside the chamber, which would have been a great support to the isotopic data. In particular, I wonder to what extent the transpiration rates were affected by reduced stomatal conductance in response to low light and high CO_2 concentration. Similarly, some estimates of CO_2 assimilation rates would have been helpful to understand the amount of labeling (although they can be indirectly estimated based on biomass growth). Maybe a rough approximation for this could be done if flow rates were measured (and recorded) as part of the control system of the chamber (Fig. A1).

According to the data presented, stem water clearly reflects the water added to the soil. Thus, we can assume net water transfer from the soil to the atmosphere, which would have been an easy explanation for the labeling observed in the leaves. In this regard, the authors should emphasize this point in the abstract and in the methods, otherwise the reader could interpret that the labeling was aimed to produce a net uptake of atmospheric water, parallel to that of CO_2, rather than a back-diffusion of water into a transpiring leaf.

The title and abstract highlight the "multi-isotope labelling" and reflect well the experimental setup. However, the main focus of the discussion (and likely the most relevant result of the paper) seems to be the fate of ^{18}O and ^{2}H from water vapor, as opposed to "source water" (soil water). Indeed, in the way the data is processed, the $^{13}CO_2$ labeling is mainly used as a sort of background value to account for differences in assimilate fixation and translocation. Hence, the paper could be easier to understand if this focus is made clear from the beginning, i.e. in the title, abstract and introduction.

Regarding the methodology, it would be desirable to have some additional details about the "MICE- Multi-isotope labelling in a Controlled Environment" facility, in particular how the chamber conditions were controlled and monitored (i.e. a custom-made control system or a commercial device, type of sensors used, illumination, humidifiers, CO_2 regulation, etc). Alternatively, provide some valid reference where a detailed description of the facility is given.

The calculation of a normalized excess atomic ratio adds complexity to the methods and discussion, and does not provide so much additional information to the pure comparison of atomic ratios together with elemental ratios. It appears that most of the discussion and the conclusions could be fully supported without these values. In addition, as stated by the authors, these calculations raise some concerns due to the mixing of different time-scales.

It would be worth to mention in the discussion the implications of these results for the "dual-isotope approach" proposed by Scheidegger et al (2000), as they could contribute to the observed depletion in the $\delta^{18}O$ of cellulose in response to high humidity (Roden and Farquhar, 2012).

Technical corrections

p. 15912 Line 7: "indicating a considerable diffusion of vapour into the leaves". This sentence is right but could be misinterpreted as a **net** diffusion of vapor from the atmosphere to the leaves. I suggest something like "indicating that despite a net transpiration flow, a significant back-diffusion of vapour took place from the atmosphere to the leaves".

p 15914, lines 14, 17, 24-25, and later on. Mixed use of "cuttings", "stems" and "shoots" is confusing, since the cuttings are also stems and shoots. One possibility is use refer to the new stems as "sprouts", as opposed to the original stem cuttings.

p. 15917. line 15. " with water vapour of *a* known signature..."

p. 15922. lines 5-15. The isotope signature of stem water is almost the same as that of the added water while the roots are more similar to the soil, which is slightly enriched compared to the added water. Since the atmospheric water is depleted, this could mean that the stem still gets some back diffusion of the signal from the leaves, if we consider soil water as the source of water, instead of the added water. Were the stems significantly depleted as compared to roots and soil water?.

p. 15938. Table 3. footnote c. I understand that "normalized excess atom fraction" was calculated rather than measured. Although I still recommend to omit this calculation, it is not clear why it was not done for the cuttings, since the necessary input seems to be available (see Table 2).

References

Roden, J. S. and Farquhar, G. D.: A controlled test of the dual-isotope approach for the interpretation of stable carbon and oxygen isotope ratio variation in tree rings, Tree Physiol., 32, 15 1–14, doi:10.1093/treephys/tps019, 2012.

Scheidegger, Y., Saurer, M., Bahn, M., and Siegwolf, R. T. W.: Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model, Oecologia, 125, 350–357, doi:10.1007/S004420000466, 2000.

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