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

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We would like to thank Referee #2 for his encouraging and helpful comments on the manuscript. We will incorporate the referee's suggestions and reply here to questions raised and topics that we do not address in the revised version.

i) Further data on gas exchange rates

We agree with the referee that it would be interesting to have further data on the transpiration and respiration fluxes or the stomatal conductance. Unfortunately this data was not assessed. To determine fluxes based on the monitored data is theoretically possible, but in this experiments we did not record regularly the plant activity without

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the interfering effects of the humidifiers/dryer. A previous study looked in more detail on the effect of different stomatal conductances and transpiration rates on the label incorporation into the leaf water (Reynolds 2007). In the presented study the focus was on tracing the label within the organic matter.

ii) Highlight the net water flux out of the leaves and back-diffusion of ^{18}O and ^{2}H

We agree with the referee that more emphasis should be given to these issues. We changed the title in the revised manuscript to "Multi-isotope labelling of organic matter by diffusion of $^{2}\text{H}/^{18}\text{O}$ -vapour and ^{13}C -CO₂ into the leaves and its distribution within the plant" and added clarifying statement in the abstract and introduction.

iii) More details on the MICE-facility

We added following paragraph in the method section: "The environmental conditions in the MICE facility are automatically controlled and monitored by a software (programmed with LabVIEW, National Instruments Switzerland Corp.) switching on/off the light sources (Xenon, HELLA KGaA Hueck & Co) and valves to in- or exclude instruments to regulate the CO₂ and H₂O concentration, which is measured by an infrared gas analyzer (LI-840, LI-COR Inc.). The chamber air is fed by a vacuum pump (N 815, KNF Neuberger AG) through perforated glass tubes within a water reservoir to humidify the air or through a Peltier cooled water condenser to dry the air (Appendix Fig. A1). Further the chamber air can be fed through a Plexiglas tube filled with Soda lime to absorb the CO₂ or CO₂ is injected from a gas cylinder."

iv) Indication of the normalized excess data

We would like to present the normalized excess data because it is easier to relate to the general van Krevelen diagram. The normalized isotopic ratios are in the same magnitude as the elemental ratios, this result proofs the concept that the isotope ratios have the potential to indicate changes in the characteristics of organic matter after correction for maximum label strength and (future) corrections for the elemental exchange. This

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is not so obvious from the Fig. 3b.

iv) Relation to other publications

We added following paragraph at the end of the discussion section 4.1: "Furthermore, these results demonstrate that the leaf water isotopic composition is strongly affected by the atmospheric signature at humid conditions and that thus the applicability of the dual-isotope approach (Scheidegger et al., 2000), e.g. to reconstruct past climate conditions by tree ring analysis, is only valid if the source water and atmospheric vapour $\delta^{18}\text{O}$ are similar. The back-diffusion of atmospheric vapour at high humidity could be another factor next to the evaporative enrichment (as demonstrated by Roden and Farquhar, 2012) to overshadow the effects of stomatal conductance on the leaf $\delta^{18}\text{O}$ signature."

v) Technical corrections

P15912 L7: We agree and changed the sentences in the following way: "The leaf water isotopic composition was between the atmospheric and stem water, indicating a considerable back-diffusion of vapour into the leaves (58 - 69 %) in opposite direction to the net transpiration flow that itself is reflected by the stem water resembling soil water values."

P15914 L14, 17, 24-25 We clarified now in the method part our definition of (new) stems vs. (original) cuttings vs. shoot (=leaves, petioles and stems). We think that replacing stems with "sprouts" would also be misleading, because sprouts would include petioles and leaves.

P15917 L15: included in the revised version

P15922 L5-15 Over all sampling dates (including the unlabelled samples), the stem water ($-9.9 \pm 0.5 \delta^{18}\text{O}$ and $-73.9 \pm 4.1 \delta^{2\text{H}}$) was significantly depleted compared to the root water ($-6.3 \pm 1.0 \delta^{18}\text{O}$ and $-57.8 \pm 3.7 \delta^{2\text{H}}$) or the soil water ($-5.6 \pm 1.1 \delta^{18}\text{O}$ and $-62.5 \pm 2.7 \delta^{2\text{H}}$). However, we do not think that the depletion in $\delta^{18}\text{O}$ of the

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stem water detected during the labelling experiment reflects a depletion induced by the labelling, since there was no consistent depletion in $\delta^{2\text{H}}$ of the stem water compared to the unlabelled control (Table below) and the depletion in $\delta^{18\text{O}}$ in the stem water does not follow the dynamics of the depletion detected in the leaf water. While there was a gradual decrease in the leaf water during the first days (Fig. 1), the depletion in the stem water was "strong" already on the first sampling date, but not significant in the two following sampling dates.

P15938 Table 3: It is correct that we measured all input data also for the cuttings (and that thus the footnote is not accurate). However, we could not calculate the isotope ratios for the cuttings, since they were not always (and never significantly) depleted in $^{18\text{O}}$ and $^{2\text{H}}$ or even enriched compared to the unlabelled control (Table 2). For other tissues that did not show a significant depletion in their average values (e.g. petioles $\delta^{2\text{H}}$) the calculation was possible, since the signature of the single tissues were always depleted compared to the control. We will adjust the footnote to clarify this.

vi) References

Reynolds Henne, C. E.: A study of leaf water $\delta^{18\text{O}}$ composition using isotopically-depleted $\text{H}^{18\text{O}}$ -vapour, in Climate-isotope relationships in trees under non-limiting climatic conditions from seasonal to century scales (PhD thesis), pp. 77–92, University of Bern., 2007.

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Sampling date [days]	$\delta^{18}\text{O}$ [‰]	$\delta^2\text{H}$ [‰]
0	-8.3 ± 0.4	-72.5 ± 2.6
1	-10.1 ± 0.3	-72.8 ± 3.2
2	-9.5 ± 0.6	-68.8 ± 2.1
8	-9.5 ± 0.7	-78.1 ± 3.9
14	-10.5 ± 0.4	-75.9 ± 5.1

Fig. 1. Isotopic composition of the stem water. Data represent the average ± one standard deviation of three plant replicates.

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