

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

**M. S. Studer et al.**

mirjam.studer@geo.uzh.ch

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We would like to thank Referee #1 for the detailed comments on the manuscript. Here we reply to the major concerns and questions raised. We are glad for all other detailed comments, which we will incorporate into the revised version of the manuscript in order to improve it.

i) Making simple things complicated

We agree with the Referee #1 that scientist should aim at expressing results as simple as possible, however, without losing its exactness. We tried to be as concise as possible by describing exactly what happened. Further simplification would most certainly

Full Screen / Esc

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Interactive Discussion

Discussion Paper



lead to a significant loss of exactness (see comments regarding P15919). Further we do not understand the reviewers comment "It would have been wiser to deal with isotope exchange and partitioning of the applied isotopes and proper discussion of their sources and fate" since that is exactly what we do in this manuscript (P15924, P15928).

ii) Oxygen sources, CO<sub>2</sub> as major oxygen source for assimilates

We strongly disagree with the reviewer. It is correct that the CO<sub>2</sub> dissolved in water is the source of fresh assimilates. However, the oxygen of the CO<sub>2</sub> is known to equilibrate with (leaf) water very quickly, as proofed by extensive literature about CO<sub>2</sub> - H<sub>2</sub>O oxygen exchange (see e.g. Gillon and Yakir 2000, Hesterberg and Siegenthaler 1991, Stern et al. 1999, Uchikawa, and Zeebe 2012).

The equilibration of CO<sub>2</sub> and leaf water is accompanied with an isotope fractionation of 41 ‰  $\delta^{18}\text{O}$  and the isotopic signature of carbohydrates (which have also the H<sub>2</sub>O as direct source) has been shown to correlate with the leaf water isotope signature described by the function  $0.8 \cdot \delta\text{H}_2\text{O} + 30$  (Schmidt et al. 2001). However, this exchange/fractionation is not relevant for any of our calculations. On the one hand the isotope mixing model that we applied to estimate the contribution of the different sources to the leaf water is based on water isotope signatures (of the atmosphere and the stem/xylem water). The oxygen exchange CO<sub>2</sub>->H<sub>2</sub>O is negligible, since the ratios of CO<sub>2</sub>:H<sub>2</sub>O are approximately 1: 500-1000. On the other hand the estimation of the maximum label strength in the fresh assimilates is based on the difference (depletion) compared to the unlabelled control, making the application of a constant correction factor for the isotope fractionation during H<sub>2</sub>O/CO<sub>2</sub> exchange redundant.

iii) Concept of isotope ratios and maximum label strength

It is not clear to us why the maximum label strength and the isotope ratios are considered ambiguous by the reviewer. In the manuscript we demonstrate that this organic matter stoichiometry -based approach can be applied to further investigate processes during biosynthesis and biodegradation. As we develop in the discussion, what we pro-

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pose here is a first attempt to use this approach. As we still know little about exchange processes of C, H and O during transport and biosynthesis, we think this isotope ratio approach could be useful to consider in combination with compound specific analysis to better understand these processes

iv) Reply to specific comments

P15913 L13 and L15: done in the revised version

P15913 L18: We understand the reviewers comment that the van Krevelen diagram is only applicable for compound specific analysis, where we disagree, and we do not understand why he brings an example of  $^{15}\text{N}$  (which is not relevant for the van Krevelen diagram). The van Krevelen diagram was used to characterise mixed organic samples (e.g. charcoal see Preston & Schmidt 2006). In this introductory section we describe the general idea/concept, that if we label fresh assimilates with all three isotopes needed for a van Krevelen diagram and trace the label (i.e. the excess atom fraction of the added isotopes compared to the unlabelled control) in the bulk material we could potentially use it as an indicator for the characteristics of the organic matter formed (following the logic of the van Krevelen diagram).

P15913 L22: done in the revised version

P15913 L23: As mentioned above this is the introductory part with the general concept. It is correct that in this particular study we could not trace it to the bulk soil, due to low isotope label strength. Nevertheless, this does not exclude that this method is incapable of tracing the organic matter from the plant to the soil e.g. by the application of a stronger label. Moreover, the method could be used to study for example the decomposition of the multi-labelled plant OM in the soil. Thus our argumentation is not incorrect here in the introduction.

L15913 L29: done in the revised version

P15914 L18: The leaf area is correctly stated in the manuscript as stated  $641 \text{ cm}^2 =$

C8841

BGD

11, C8839–C8846, 2015

Interactive  
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Interactive Discussion

Discussion Paper



0.0641 m<sup>2</sup>

P15914 L24: Hermetic is defined as "airtight", thus it does not include interplant matter fluxes per definition.

P15914 L8: They are hermetically sealed from the laboratory atmosphere and are aerated with outdoor air (natural isotopic composition, no danger of contamination) to prevent anaerobic conditions in the soil. We clarified this in the revised version.

P15915 L18 Transpiration is a net flux and does not directly affect the (bidirectional) diffusion of molecules between the atmosphere and the substomatal cavity in the leaves. The diffusion is related to the stomatal conductance and the concentration gradient between the atmosphere and the leaf substomatal cavity, i.e. at high humidity there is a high back-diffusion (low concentration gradient), independent on the temperature driven evapotranspiration (discussed on P15925 L2-10).

P15915 L27 and P15916 L10: We agree with the referee that the other study does not necessarily need to be stated because their results are not related to each other (only the experimental setup).

P15917 L 12: We indicated all combustion/pyrolysis temperatures in the revised manuscript. We did not repeat the already measured 18O on TC/EA for financial reason and because the 18O exchange with vapour is negligible compared to the 2H exchange (see next comment).

P15917 L21: Ee reformulated this section in the revised version ("The amount of exchangeable hydrogen (25-27%) and oxygen (2-3%) was measured for the leaf, stem and root tissue using depleted water vapour to equilibrate the samples.")

P15917 L24 The large sample amount (210 samples for CHON,  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  analysis) is the reason why we did not measure analytical replicates (due to financial reasons) and why we indicated the measurement precision only by the standard measurements. The soil samples were treated the same way as plant samples, whereby

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11, C8839–C8846, 2015

Interactive  
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Interactive Discussion

Discussion Paper



C8842

we used a higher amount of material to account for the lower carbon content. And acidification pre-treatment was not necessary (soil pH 4.8).

P15918 L19: The ratios (in brackets) are the "relative recovery of the isotopes", we deleted "three".

L15918 L26: All abbreviations are explained in the paragraph above. This Eq. 1 can not be deleted since it is the basis for the further equations (Eq. 3, 4) and the principle of the mixing model (Eq. 2).

P15919 L9 We model the isotopic signature of the water within the plant at the place of evaporation/condensation (leaf substomatal cavity) with the two sources (Eq. 1). We apply the model after the equilibrium in label distribution was reached, thus we do not have to correct for the signature of the water already available in the plant.

P15919 L10 and L19 Because it is not the soil or the atmospheric water (see Figure A1). It is the isotopic signature of the water at the evaporating/condensing site within the leaf including the isotope fractionation. The referees suggestion to neglect the fractionation would simply be not correct, since it represents a large fraction of the label strength in the water vapour, as can be seen on the following example: the  $^{18}\text{O}$  depletion due to the label addition was 94 ‰  $\delta^{18}\text{O}$  in the water vapour, while the equilibrium fractionation was 9 ‰ at the current environmental conditions (see Fig. A1).

L15919 L12: Yes, it is correct that we measure the water in the plant (leaf water). In this section we describe how we calculate the different sources of the measured leaf water (based on the stem water).

L15919 L8 (probably referring to L15921 L8): We mean the maximum label strength of the precursor of plant organic matter compounds, i.e. the maximum label strength of the fresh assimilates (we added this to the revised version of the manuscript).

P15919 L3 (probably referring to P15923 L5): We disagree with the Referee that it is

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wrong to express atom fraction as delta-notation and vice versa (see Eq. 5 based on Coplen, 2011), but we agree that it would be wrong to use the delta notation for mass balance estimates (see Brand and Coplen 2012), but we do not do that. We express it in the delta-notation just for the sake of comparison with the  $\delta^{13}\text{C}$  measured in the hot water extracts (which clarifies that the maximum label strength in the fresh assimilates cannot be approximated by the isotope label added).

P15925 L3: We call it back-diffusion, because it is in the opposite direction of the  $\text{H}_2\text{O}$  net flux / concentration gradient. Diffusion is always bi-directional.

P15925 L27: We think that the referee refers here to the isoflux. The simple isotope exchange would be relevant for a small fraction (evaporating sites) and not for the entire leaf water (incl. laminar water), if there was no flux into the leaves. As stated in the paragraphs on P15926 our results were comparable to modelling results that do respect the isoflux/isostorage (Farquhar and Cernusak 2005). Nevertheless we want to clarify here again that we do not say that there was a net flux into the leaves, but that approximately 60 % of the leaf water originates from the atmosphere (see Figure below).

L15926 L10: see above "CO<sub>2</sub> as a major oxygen source"

L15927 L6ff. We disagree as we stated on in our comments above.

#### v)References

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11, C8839–C8846, 2015

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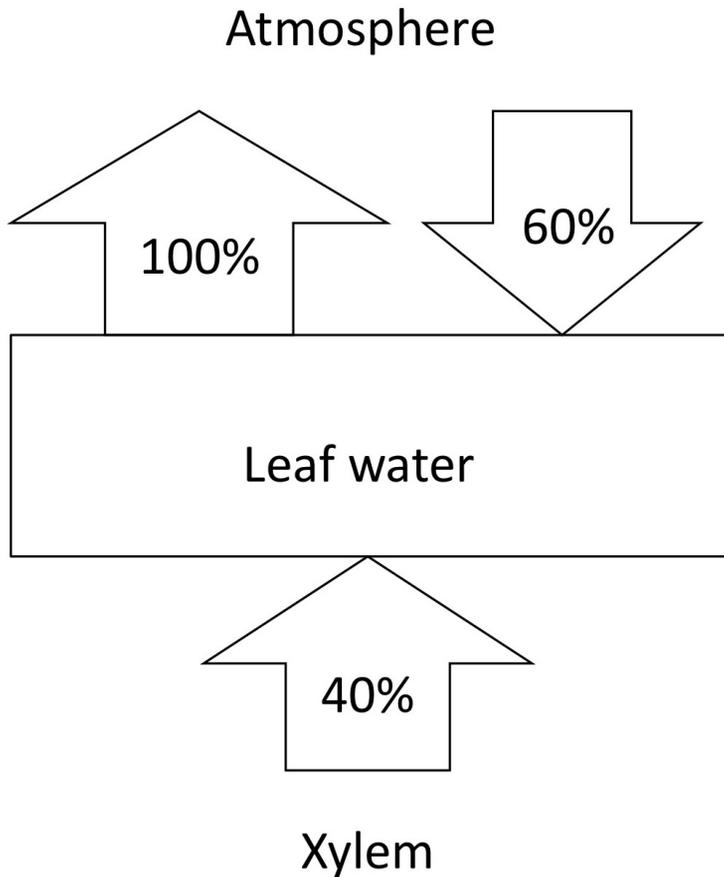
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**Fig. 1.** Schematic diagram of the main fluxes into- and out of the leaf. The net transpiration flux is the sum of the bi-directional diffusion between the atmosphere and the leaf water (-40% in this example).