Part A: Letter to the Editor

Dear Mister Wang

The two reviews we've got on our manuscript were helpful and we included most of the comments in the revised version as stated below. The Referees opinions on the quality of the manuscript diverged significantly. In our opinion the quality of the review from Referee #1 was low. The Referees comments made the impression that he is not familiar with basic physical and chemical processes such as the equilibration and exchange of the oxygen isotopes between CO_2 and water, the bi-directional diffusion of molecules in the air (between leaves and the atmosphere) and the fractionation processes relevant for the leaf water isotopic enrichment. Thus his conclusions with regard of the oxygen isotopic signature in organic matter were wrong. Furthermore we had the impression that the review of Referee #1 was done in a hurry, not doing justice to a complex topic as it is for the presented manuscript. On the contrary Referee #2 seemed to be well familiar with this topic. Still we used both reviews to ensure that the addressed points are now very clear, precise and correct to provide high quality science.

We are confident that this revised version of the manuscript meets the quality standards of Biogeosciences. We hope that we addressed satisfactorily all the issues raised by the referees and we thank you for handling this review process.

Yours sincerely,
Mirjam Studer, Markus Leuenberger, Rolf Siegwolf, Samuel Abiven

Part B: Incorporation of the comments by Referee #1

Referee main statement 1

"It would have been wiser to deal with isotope exchange and partitioning of the applied isotopes and proper discussion of their sources and fate rather than complicating the issue with not straight-forward models."

Authors response:

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 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).

Referee main statement 2

"With respect to oxygen and hydrogen (water), the three water sources you have is the label (atmosphere), the soil and xylem. However, with respect to oxygen, a further source must be considered: From uptaken CO2 during photosynthesis. Thus, most of the oxygen of primary photosynthetates comes from CO2 and not from uptaken water. Therefore, the concept of the three labels is questionable as these sources are not linked to each other. While most of the CO2 goes into the photosynthetates, most of the water vapor goes into the plant water with subsequent or simultaneous isotope exchange without uptake."

Authors response:

We strongly disagree with the reviewer. It is correct that the CO_2 dissolved in water is the source of fresh assimilates. However, the oxygen of the CO_2 is known to equilibrate with (leaf) water very quickly, as proofed by extensive literature about CO_2 - H_2O 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_2 and leaf water is accompanied with an isotope fractionation of 41 ‰ $\delta^{18}O$ and the isotopic signature of carbohydrates (which have also the H_2O as direct source) has been shown to correlate with the leaf water isotope signature described by the function $0.8*\delta_{H2O}+30$ (Schmidt et al. 2001). However, this exchang/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_2->H_2O is negligible, since the ratios of CO_2:H_2O 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_2O/CO_2 exchange redundant.

Referee main statement 3

"The concepts of "maximum label strength“ and labeled isotope ratios (18O/13C, 2H/13C) are ambigous and make simple things (isotope labeling with 13C, 18O and 2H) more complicated than it is. There are many more specific comments, which are given below."

Authors response:

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 propose 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.

Specific comments by the Referee

P15913 L 12: Replace "molecular formula“ by "molecular composition“.

Authors response: done in the revised version

P15913 L15: You should include and refer here Fig. 3a.

Authors response: done in the revised version

P15913 L18: This "logic“ is ambiguous as such can be done only using dual-labeled compounds e.g. 13C and 15N-labeled amino acids and detection of both isotopes in amino acids.

Authors response: 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}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: Delete "of high speciïnˇA˛city“ as different organic molecules might be labeled with different isotopes in different plant organs.

Authors response: done in the revised version

P15913 L23: I disagree as your approach does not help for the detection of labled plant material in (bulk) soil. Therefore, the whole argumentation has to be deleted.

Authors response: 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.

P15913 L29: Write "plant compartments... and soil (organic matter?)“.

Authors response: done in the revised version

P15914 L18: How can a leaf area of 6.5 m2 correspond to only 3 g leaf (and stem) biomass?

Authors response: The leaf area is correctly stated in the manuscript as stated 641 cm^2 = 0.0641 m^2

P15914 L24: What do you mean by "hermetical“? Is it only separated from the gases or also from the intrplant matter fluxes? The whole procedure is unclear. A schematic of the labeling chamber would probably clarify a lot.

Authors response: Hermetic is defined as "airtight", thus it does not include interplant matter fluxes per definition. We extended the description of the facility according to the Referee#2's comments.

P15915 L8: If pots are "hermetically“ sealed, how can they be aerated?

Authors response: 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: If air humidity and temperature is high, why water vapor should be taken up? Instead, transpiration of plants should be high.

Authors response: 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: Unclear what that study has to do with this one. Either delete this sentence or explain the added values of the new study in comparison to the previous one.

Authors response: 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).

P15916 L10: This is already discussion and should be moved there.

Authors response: Was removed, see comment above

P15917 L12: Please give exact conditions of oxidation and pyrolysis (temperature, catalysts etc.). Please explain why 18O analysis was not undertaken by TC/EA.

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

P15917 L21: Unclear what you did and why.

Authors response: We 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: Precision of working standards is not of interest. Instead, please give accuracy (how did you do calibration ?) and precision of real (your) samples. How soil samples were treated and analysed?

Authors response: The large sample amount (210 samples for CHON, $\delta^{13}C$, $\delta^{18}O$ and $\delta^{2}H$ 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 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: Three isotopes (13C, 18O, and 2H)!

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

P15918 L26: Please explain each single abbreviation in eqn. 1. Alternatively, delete eqn. 1 as you do not need it (see eqn 2).

Authors response: 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: What about water already in the plant?

Authors response: 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: Why don't you call it simply "soil water“? Why couldn't you simply use the isotope signature of your soil water for this variable? AND P15919 L19: Why don't you simply call it "atmospheric water“? Why can you not simply take the isotope signature of your labelling atmosphere for this variable?

Authors response: 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}O depletion due to the label addition was 94 ‰ $\delta^{18}O$ in the water vapour, while the equilibrium fractionation was 9 ‰ at the current environmental conditions (see Fig. A1).

P15919 L12: What you measure is in the plant!

Authors response: 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).

P15919 L8: Not clear what you mean by "maximum label strenght". Therefore, I suggest deleting this section or referencing it to the maximum isotope labels.

Authors response: 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: It is useless and wrong to give a d13C value for 10at% CO2. Please delete.

Authors response: (The Referee probably refers to P15923 L5) We disagree with the Referee that it is 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 $\delta^{13}C$ 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: Why back diffusion? Delete "back". The same appies for the rest of the paragraph.

Authors response: We call it back-diffusion, because it is in the opposite direction of the H_2O net flux / concentration gradient. Diffusion is always bi-directional.

P15925 L27: Can you exclude simple isotope exchange without water uptake by this approach?

Authors response: 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).

P15926 L10: The three water sources you have is from the label (atmosphere), from the soil and from xylem. However, with respect to oxygen, a further source must be considered: From uptaken CO2 during photosynthesis. Thus, most of the oxygen of primary photosynthetates comes from CO2 and not from uptaken water. Therefore, the concept of the three labels is questionable as these sources are not linked to each other. While most of the CO2 goes into the photosynthetates, most of the water vapor goes into the plant water with subsequent isotope exchange.

Authors response: see above reply to referees main statement 2

P15927 L6ff.: Tracing organic matter with this approach is not possible given the facts mentioned in the previous comment.

Authors response: We disagree as we stated on in our comments above.

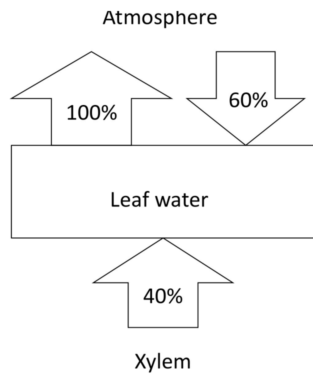

Figure 1: Fluxes into- and out of the leaf. The net transpiration flux is the sum of the bi-directional diffusion between the atmosphere and the leaf water (-40% in this example).

References used for the authors responses

Brand, W. A. and Coplen, T. B.: Stable isotope deltas: tiny, yet robust signatures in nature, Isotopes Environ. Health Stud., 48, 393–409, doi:10.1080/10256016. 2012.666977, 2012.

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Farquhar, G. D. and Cernusak, L. A.: On the isotopic composition of leaf water in the non-steady state, Funct. Plant Biol., 32, 293–303, doi:10.1071/FP04232, 2005.

Gillon, J. S. and Yakir, D.: Internal conductance to CO_2 diffusion and $C^{18}OO$ discrimination in C3 Leaves, Plant Physiol., 123, 201–213, 2000.

Hesterberg, R. and Siegenthaler, U.: Production and stable isotopic composition of CO_2 in a soil near Bern, Switzerland, Tellus B, 43, 197–20, 1991.

Preston, C. M. and Schmidt, M. W. I.: Black (pyrogenic) carbon: a synthesis of current knowledge and uncertainties with special consideration of boreal regions, Biogeosciences, 3, 397–420, doi:10.5194/bg-3-397-2006, 2006.

Schmidt, H.-L., Werner, R. A. and Rossmann, A.: O-18 pattern and biosynthesis of natural plant products, Phytochemistry, 58, 9–32, doi:10.1016/S0031-9422(01) 00017-6, 2001.

Stern, L., Baisden, W. T. and Amundson, R.: Processes controlling the oxygen isotope ratio of soil CO_2: Analytic and numerical modeling, Geochim. Cosmochim. Acta, 63, 799–814, 1999.

Uchikawa, J. and Zeebe, R. E.: The effect of carbonic anhydrase on the kinetics and equilibrium of the oxygen isotope exchange in the CO_2–H_2O system: Implications for $\delta^{18}O$ vital effects in biogenic carbonates, Geochim. Cosmochim. Acta, 95, 15–34, doi:10.1016/j.gca.2012.07.022, 2012.

Part C: Incorporation of the comments by Referee #2

Referee main statement 1

"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 CO2 concentration. Similarly, some estimates of CO2 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)."

Authors response:

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 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.

Referee main statement 2

"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 CO2, 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 18O and 2H from water vapor, as opposed to "source water" (soil water). Indeed, in the way the data is processed, the 13CO2 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.

Authors response:

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}H/^{18}O-vapour and ^{13}C-CO_2 into the leaves and its distribution within the plant" and added clarifying statement in the abstract and introduction.

Referee main statement 3

"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, CO2 regulation, etc). Alternatively, provide some valid reference where a detailed description of the facility is given."

Authors response:

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 CO_2 and H_2O 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 CO_2 or CO_2 is injected from a gas cylinder."

Further replies related to the stem water signature see detailed comments.

Referee main statement 4

"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."

Authors response:

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 is not so obvious from the Fig. 3b.

Referee main statement 5

"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 _18O of cellulose in response to high humidity (Roden and Farquhar, 2012)."

Authors response:

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 $\delta^{18}O$ 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 $\delta^{18}O$ signature."

Specific comments by the Referee

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".

Authors response: 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."

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.

Authors response: 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.

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

Authors response: included in the revised version

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?

Authors response: Over all sampling dates (including the unlabelled samples), the stem water (-9.9 ± 0.5 $\delta^{18}O$ and -73.9 ± 4.1 $\delta^{2}H$) was significantly depleted compared to the root water (-6.3 ± 1.0 $\delta^{18}O$ and -57.8 ± 3.7 $\delta^{2}H$) or the soil water (-5.6 ± 1.1 $\delta^{18}O$ and -62.5 ± 2.7 $\delta^{2}H$). However, we do not think that the depletion in $\delta^{18}O$ of the stem water detected during the labelling experiment reflects a depletion induced by the labelling, since there was no consistent depletion in $\delta^{2}H$ of the stem water compared to the unlabelled control (Table below) and the depletion in $\delta^{18}O$ 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.

Table1: Isotopic composition of the stem water. Data represent the average ± one standard deviation of three plant replicates.

Sampling date [days]	**$\delta^{18}O$ [‰]**	**$\delta^{2}H$ [‰]**
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

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).

Authors response: 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}O and ^{2}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 $\delta^{2}H$) 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.

References used for the Authors responses

Reynolds Henne, C. E.: A study of leaf water $\delta^{18}O$ composition using isotopically-depleted $H_2{}^{18}O$-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.

Part D: List of most relevant changes made

Changes related to Referees comments

- Change of title
- Clarification of the fact that there is back-diffusion of H2O in the opposite direction to the net H_2O flux out of the leaves in the Abstract (and in the main text)
- Addition of details on the MICE facility used for the experiment in the methods
- Removal of notes related to the study conducted in parallel to this study in the methods
- More details on the isotopic analysis performed in the methods
- Addition of a paragraph in the discussion to highlight the importance of our findings for the interpretation of ^{18}O signatures measured in cellulose.
- Correction of the Table 3

Additional changes not related to Referees comments

We adjusted the abbreviation of the Equations according to the style of Biogeosciences discussions and corrected the caption of Figure 1.

Part E: Detailed changes in the Manuscript

Mirjam Studer 2/20/15 2:55 PM
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Multi-isotope labelling of organic matter by diffusion of $^{2}H/^{18}O$-vapour and ^{13}C-CO_2 into the leaves and its distribution within the plant

Mirjam Studer 2/4/15 4:14 PM
Deleted: (^{13}C, ^{18}O, ^{2}H)

Mirjam Studer 2/4/15 4:16 PM
Deleted: fresh assimilates

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Deleted: to trace organic matter dynamics in the plant-soil system

M. S. Studer[1,2], R. T. W. Siegwolf[2], M. Leuenberger[3], S. Abiven[1]

[1] Department of Geography, University of Zurich, Winterthurerstr. 190, 8057 Zurich, Switzerland

[2] Laboratory of Atmospheric Chemistry, Paul Scherrer Institute, 5232 Villigen PSI, Switzerland

[3] Climate and Environmental Physics, Physics Institute and Oeschger Centre for Climate Change Research, University of Bern, Sidlerstr. 5, 3012 Bern

Correspondence to: Dr. S. Abiven (samuel.abiven@geo.uzh.ch)

Abstract

Isotope labelling is a powerful tool to study elemental cycling within terrestrial ecosystems. Here we describe a new multi-isotope technique to label organic matter (OM).

We exposed poplars (*Populus deltoides x nigra*) for 14 days to an atmosphere enriched in $^{13}CO_2$ and depleted in $^{2}H_2{}^{18}O$. After one week, the water-soluble leaf OM ($\delta^{13}C$ = 1346 ± 162 ‰) and the leaf water were strongly labelled ($\delta^{18}O$ = - 63 ± 8 ‰, $\delta^{2}H$= - 156 ± 15 ‰). 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. The atomic ratios of the labels recovered ($^{18}O/^{13}C$, $^{2}H/^{13}C$) were 2 - 4 times higher in leaves than in the stems and roots. This either indicates the synthesis of more condensed compounds (lignin vs. cellulose) in roots and stems, or be the result of O and H exchange and fractionation processes during transport and biosynthesis.

30 We demonstrate that the three major OM elements (C, O, H) can be labelled and
31 traced simultaneously within the plant. This approach could be of interdisciplinary
32 interest for the fields of plant physiology, paleoclimatic reconstruction or soil science.
33

34 ## 1 Introduction

35 Artificial labelling with stable isotopes facilitates the observation of bio(geo)chemical
36 cycling of elements or compounds with minor disturbance to the plant-soil systems. It
37 has provided many insights into plant carbon allocation patterns (e.g. Simard et al.
38 1997; Keel et al. 2006; Högberg et al. 2008), water dynamics (e.g. in Plamboeck et al.
39 2007; Kulmatiski et al. 2010) and soil organic matter processes (e.g. in Bird and Torn
40 2006; Girardin et al. 2009) in terrestrial ecosystems. Only a few studies used labelling
41 approaches with more than one stable isotope, for example to study the interactions
42 between the carbon and nitrogen cycle (e.g. in Bird and Torn 2006; Schenck zu
43 Schweinsberg-Mickan et al. 2010). However, to our knowledge isotopic labelling of
44 organic matter (OM) with its three major elements, carbon (C), oxygen (O) and
45 hydrogen (H), has never been done in ecosystem studies before, even though
46 combined $\delta^{13}C$, $\delta^{18}O$ and $\delta^{2}H$ analyses have been widely used to study plant
47 physiological processes and to reconstruct past climatic conditions (Hangartner et al.,
48 2012; Roden and Farquhar, 2012; Scheidegger et al., 2000; Werner et al., 2012).
49 Similarly, an artificial labelling with those isotopes would be useful to clarify basic
50 mechanisms related to the plant water-use efficiency or the oxygen and hydrogen
51 signals in tree rings, but also to study other OM dynamics in the plant-soil system
52 such as OM decomposition in the soil.
53 The C, O and H contents of organic matter have been applied to distinguish major
54 groups of compounds, by plotting the atomic ratios O/C and H/C in a van Krevelen
55 diagram (Kim et al., 2003; Ohno et al., 2010; Sleighter and Hatcher, 2007). This
56 approach is based on the distinct molecular composition of organic compounds. For
57 example the glucose molecule ($C_6 H_{12} O_6$) is characterized by high O/C (= 1) and H/C
58 (= 2) ratios and is the precursor of other compounds, such as cellulose (($C_6 H_{10} O_5)_{[n]}$
59 O/C = 0.8, H/C = 1.7, Fig. 3a). Condensation or reduction reactions during
60 biosynthesis lead to other compound groups with lower atomic ratios (e.g. lignin) or
61 similar H/C, but lower O/C ratios (e.g. lipids, proteins) compared to glucose.
62 Following the logic of the van Krevelen diagram, we wanted to test, if we can use the
63 isotopic ratios $^{18}O/^{13}C$ and $^{2}H/^{13}C$ of the labels recovered in plant-soil bulk materials

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after labelling the fresh assimilates with those stable isotopes, to detect the utilization of the labelled assimilates for the synthesis of different OM compounds. With this multi-labelling approach we would gain information about the characteristics of the OM formed by simple isotopic analysis of bulk material. This has several advantages compared to compound specific analysis, such as being much less laborious and less expensive and yield integrated information on the bulk organic matter sampled.

In this study we added the ^{13}C, ^{18}O and ^{2}H labels via the gaseous phase in the plants' atmosphere (CO_2, water vapour). Pre-grown plants were exposed to the labelled atmosphere continuously for fourteen days under laboratory conditions and the labels added were traced in different plant compartments (leaves, petioles, new stems, stem cuttings, roots) and soil organic matter at different points in time. We applied a simple isotope mixing model to estimate the fraction of ^{18}O and ^{2}H that entered the leaf by diffusion from the atmosphere into the leaf intercellular cavities and plotted the atomic and isotopic ratios of the OM formed in van Krevelen diagrams to test if the multi-isotope labelling approach can be used to detect changes in the OM characteristics.

2 Material and Methods

2.1 Plants and soil

The soil (cambisol) was sampled from the upper 15 cm in a beech forest (8° 33' E, 47° 23' N, 500 m elevation), coarse sieved (2.5 x 3.5 cm) and large pieces of hardly decomposed organic material were removed. The soil had a clay loam texture, a pH of 4.8, an organic C content of 2.8 % and a C/N ratio of 11. The plant pots (volume = 8.2 dm^3) were filled with 3018 ± 177 g soil (dry weight equivalent). 15 Poplar seedlings (*Populus deltoides x nigra*, Dorskamp clone) were grown indoors from 20 cm long stem cuttings for five weeks before they were transferred into labelling chambers (described below). They were kept in the chamber for acclimatization for one week prior to labelling. At the beginning of the labelling experiments, the average dry weight of fresh plant biomass (without the original stem cutting) was 3.3 ± 0.1 g and the average total leaf area was 641 ± 6 cm^2 per plant. At the end of the experiment (last sampling) the dry weight was 5.4 ± 1.1 g and the total leaf area was 1354 ± 161 cm^2. The leaf area was measured with a handheld area meter (CID-203 Laser leaf area meter, CID Inc.).

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96 ## 2.2 Labelling chamber, procedure and environmental conditions

97 The labelling chambers (MICE - Multi-Isotope labelling in a Controlled Environment
98 - facility) provide a hermetical separation of the shoots (leaves, petioles and new
99 stems) from the roots, rhizosphere and the soil. The plant shoots are enclosed by one
100 large polycarbonate cuboid (volume 1.2 m^3) with a removable front plate and five 2
101 cm wide gaps in the bottom plate to slide in three plants in each row. Small
102 polycarbonate pieces, Kapton tape and a malleable sealant (Terostat IX, Henkel AG &
103 Co.) wrapped around the stem cuttings were used to seal off the upper from the lower
104 chamber. The belowground compartments (soil and roots) are in fifteen individual
105 pots, which are hermetically sealed from the laboratory and aerated with outdoor air.
106 This setup ensures that all plants receive the same labelling treatment and prevents the
107 diffusion of labelled atmospheric gases into the soil.
108 The environmental conditions in the MICE facility are automatically controlled and
109 monitored by a software (programmed with LabVIEW, National Instruments
110 Switzerland Corp.) switching on/off the light sources (Xenon, HELLA KGaA Hueck &
111 Co) and valves to in- or exclude instruments to regulate the CO_2 and H_2O
112 concentration, which is measured by an infrared gas analyzer (LI-840, LI-COR Inc.).
113 The chamber air is fed by a vacuum pump (N 815, KNF Neuberger AG) through
114 perforated glass tubes within a water reservoir to humidify the air or through a Peltier
115 cooled water condenser to dry the air (Appendix Fig. A1). Further the chamber air can
116 be fed through a Plexiglas tube filled with Soda lime to absorb the CO_2 or CO_2 is
117 injected from a gas cylinder.
118 The isotope labels (^{13}C, ^{18}O and ^{2}H) were added continuously for 14 days via gaseous
119 phase to the plant shoots. We used CO_2 enriched in ^{13}C (10 atom% ^{13}C-CO_2,
120 Cambridge Isotope Laboratories, Inc.), and water vapour depleted in ^{18}O and ^{2}H ($\delta^{18}O$
121 = - 370 ‰ and $\delta^{2}H$ = - 813 ‰, waste product from enrichment columns at the Paul
122 Scherrer Institute). Thus the labelled gases added were enriched by 8.90 atom% ^{13}C
123 and depleted by 0.07 atom% ^{18}O and 0.01 atom% ^{2}H relative to the ambient air.
124 The soil moisture was maintained at 100 % field capacity and the relative air humidity
125 was 74 %, in order to promote the back-diffusion of water into the leaves. The light
126 intensity was low (80 ± 25 µmol m^{-2} s^{-1} photosynthetic active radiation), and the CO_2
127 concentration was kept at 508 ± 22 ppm in order to maintain a high atmospheric

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128 carbon supply. The day-night cycles were twelve hours and the temperature within the
129 labelling chamber was 31 ± 3 °C throughout the experiments.

130 2.3 Sample collection

131 The plant-soil systems were destructively harvested at five sampling dates (three
132 replicates each) to detect the dynamics of the labelling over time. The first sampling
133 was done one day before the labelling experiment started (unlabelled control, referred
134 to as t = 0). Subsequently plant-soil systems were sampled after 1, 2, 8 and 14 days of
135 continuous labelling.

136 At each sampling date the plant-soil systems were separated into leaves, petioles,
137 stems, cuttings, roots (washed with deionised water and carefully dabbed with tissue)
138 and bulk soil (visible roots were removed with tweezers). The leaves (sub-sample of
139 six leaves) were sampled all along the stem (homogeneously distributed). The
140 uppermost leaves, newly formed during the experiment (completely labelled), were
141 excluded, since we wanted to study the tracer uptake and translocation dynamics in
142 already existing leaves prior to the treatment. In one out of the three plant replicates
143 we took two leaf sub-samples from distinct positions along the shoot. We sampled six
144 leaves from the upper and six leaves from the lower half of the shoot (thereafter
145 referred to as "top" and "bottom", respectively). Leaves, stems, roots and bulk soil
146 were collected in airtight glass vials and frozen immediately at - 20 °C for later
147 cryogenic vacuum extraction of the tissue water. Cuttings and petioles were dried for
148 24 hours at 60 °C.

149 The tissue water was extracted with cryogenic vacuum extraction by heating the
150 frozen samples within the sampling vials in a water bath at 80 °C under a vacuum
151 (10^{-3} mbar) for two hours. The evaporating water was collected in U-vials submersed
152 in a liquid nitrogen cold trap. After thawing (within the closed U-vials), the water
153 samples were transferred into vials and stored frozen at - 20 °C for later $\delta^{18}O$ and $\delta^{2}H$
154 analysis. To study the water dynamics, additional water vapour samples from the
155 chamber air were collected by peltier-cooled water condensers (in an external air
156 circuit connected to the plant labelling chamber) and analysed for $\delta^{18}O$ and $\delta^{2}H$.

157 The dried plant residues of the cryogenic vacuum extraction were used for isotopic
158 bulk analyses (described below). The leaf water-soluble organic matter was extracted
159 by hot water extraction. 60 mg milled leaf material was dissolved in 1.5 ml of
160 deionised water and heated in a water bath (85 °C) for 30 min. After cooling and

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161 centrifugation (10'000 g, 2 min), the supernatant was freeze-dried and analysed for
162 $\delta^{13}C$. $\delta^{2}H$ analyses were not possible on the hot water extracts (mainly sugars), due to
163 incomplete equilibration with ambient water vapour (Filot, 2010).

164 2.4 Isotopic and elemental analyses

165 All samples were milled to a fine powder with a steel ball mill and weighed into tin
166 ($\delta^{13}C$ analyses) or silver ($\delta^{18}O$ and $\delta^{2}H$ analyses) capsules and measured by isotope-
167 ratio mass spectrometry (IRMS). The $\delta^{13}C$ samples were combusted in an elemental
168 analyser (EA 1110, Carlo Erba) and the resulting CO_2 was transferred in a helium
169 stream via a variable open-split interface (ConFlo II, Finnigan MAT) to the IRMS
170 (Delta S, Thermo Finnigan; see Werner et al. 1999). The samples for $\delta^{18}O$ analyses
171 were pyrolysed in an elemental analyser (EA 1108, Carlo Erba) and transferred via
172 ConFlo III interface (Thermo Finnigan) to the IRMS (Delta plus XL, Thermo
173 Finnigan). The samples for $\delta^{2}H$ analyses were equilibrated with water vapour of a
174 known signature prior to the IRMS measurements, to determine the isotopic signature
175 of the non-exchangeable hydrogen (as described in Filot et al. 2006; Hangartner et al.
176 2012). After equilibration the samples were pyrolysed in a thermochemical elemental
177 analyser (TC/EA, Thermo-Finnigan) at a temperature of 1425 °C and the gaseous
178 products were carried by a helium stream via a ConFlow II open split interface
179 (Thermo Finnnigan) into the IRMS (Isoprime, Cheadle). The amount of exchangeable
180 hydrogen (25-27%) and oxygen (2-3%) was measured for the leaf, stem and root
181 tissue using depleted water vapour to equilibrate the samples. The measurement
182 precisions of the solid sample analyses were 0.12 ‰ $\delta^{13}C$, 0.54 ‰ $\delta^{18}O$ and 1 ‰ $\delta^{2}H$
183 and were assessed by working standards measured frequently along with the
184 experimental samples. The precisions were lower than reported for measurements of
185 natural abundance, since highly labelled sample material was analysed.
186 Elemental C, H and N content of solid samples was analysed in an elemental analyzer
187 (CHN-900, Leco Corp.) and the elemental O content by RO-478 (Leco Corp.).
188 The liquid samples from the cryogenic vacuum extraction (tissue water) were
189 pyrolysed in an elemental analyser (TC/EA, Thermo Finnigan) and the evolving CO
190 and H_2 gases were transferred via the ConFlo III interface (Thermo Finnigan) to a
191 IRMS (Delta plus XL, Thermo Finnigan) for oxygen and hydrogen isotope ratio
192 analysis (Gehre et al., 2004). The precision of the liquid sample measurement was ±
193 0.75 ‰ $\delta^{18}O$ and ± 1.59 ‰ $\delta^{2}H$.

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194 ## 2.5 Calculations

195 Isotopic ratios were expressed in delta (δ) notation as the deviation (in ‰) from the
196 international standards Vienna Pee Dee Belemnite (V-PDB, $^{13}C/^{12}C = 1.11802 \times 10^{-2}$)
197 and Vienna Standard Mean Ocean Water (V-SMOW, $^{18}O/^{16}O = 2.0052 \times 10^{-3}$ and
198 $^{2}H/^{1}H = 1.5575 \times 10^{-4}$). The significance of changes in isotopic signature between the
199 sampling dates and the unlabelled control (t = 0) were statistically tested by t-tests
200 performed by R software (R Core Team 2014).

201 In the following paragraphs we describe first the calculations for the leaf water source
202 partitioning (Eqs. 1 - 4). These equations are given for the oxygen isotope (^{18}O), but
203 they apply also for hydrogen (^{2}H). Then we describe the calculations for the relative
204 recovery of the isotopes ($^{18}O/^{13}C$ and $^{2}H/^{13}C$) in the bulk organic matter (Eqs. 5 - 7).

205 The leaf water isotopic signature (at steady state) can be described by a model of
206 Dongmann et al. (1974) to calculate leaf water $H_2{}^{18}O$ enrichment, a derivative of
207 Craig & Gordon (1965) (Eq. 1). According to this model, the isotopic signature of the
208 leaf water (L) is the result of kinetic (ε^k) and equilibrium (ε^*) fractionation processes
209 during evaporation of the source water (S) within the leaves and the back-diffusion of
210 atmospheric water vapour (V) into the leaves as affected by relative air humidity (h).

211 $$\delta^{18}O_L = \delta^{18}O_S + \varepsilon^k + \varepsilon^* + \left(\delta^{18}O_V - \delta^{18}O_S - \varepsilon^k\right) \cdot h \qquad (1)$$

212 We used a two-source isotope mixing model (Eq. 2, principles described in Dawson et
213 al. 2002) to assess the contribution of the two main water pools (soil and atmospheric
214 water) to the leaf water based on its isotopic signatures. An overview on the input data
215 for the mixing model is given as in Appendix A (Fig. A1).

216 $$f_{source,2} = \frac{\delta^{18}O_{leaf,water} - \delta^{18}O_{source,1}}{\delta^{18}O_{source,2} - \delta^{18}O_{source,1}} \qquad (2)$$

217 , where $\delta^{18}O_{leaf,water}$ is the isotopic signature (in ‰) of water extracted from the leaves
218 at a specific sampling date and $\delta^{18}O_{source,1}$ and $\delta^{18}O_{source,2}$ are the theoretical isotopic
219 signatures of the leaf water if all water would originate either from the soil (source 1)
220 or the atmospheric (source 2) water pool.

221 The first source, thereafter referred to as "evaporating source", represents the water
222 taken up from the soil by the roots, which is transported via the xylem to the leaf,
223 where it evaporates. The isotopic signature of the evaporating source (Eq. 3) is

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224 estimated by the maximum leaf water enrichment that would occur at 0 % relative air
225 humidity i.e. by the first part of the Dongmann approach (solving Eq. 1 with h = 0).

226 $$\delta^{18}O_{source,1} = \delta^{18}O_{stem,water} + \varepsilon^{k} + \varepsilon^{*}_{atm} \quad (3)$$

227 , where $\delta^{18}O_{stem,water}$ is the isotopic signature (in ‰) of the water extracted from the
228 stem tissue (approximating the xylem water) and ε^{k} and ε^{*}_{atm} are the kinetic and
229 equilibrium fractionation terms, respectively, at the specific sampling date.
230 The second source, thereafter called "condensation source", refers to the water vapour
231 that diffuses from the atmosphere into the leaves and condensates at the cell walls.
232 The contribution of this source would be maximal at 100 % relative humidity, which
233 results in Eq. 4 when solving Eq. 1 with h = 1.

234 $$\delta^{18}O_{source,2} = \delta^{18}O_{atm,vap} + \varepsilon^{*}_{atm} = \delta^{18}O_{atm,cond} - \varepsilon^{*}_{pelt} + \varepsilon^{*}_{atm} \quad (4)$$

235 , where $\delta^{18}O_{atm,vap}$ is the isotopic signature of the water vapour of the chamber
236 atmosphere and ε^{*}_{atm} is the equilibrium fractionation inside the chamber at the specific
237 sampling date. The signature of the atmospheric water vapour was measured on its
238 condensate ($\delta^{18}O_{atm,cond}$) collected in the peltier water trap, which was therefore
239 corrected with the equilibrium fractionation during condensation inside the peltier-
240 cooled water condenser (ε^{*}_{pelt}).
241 The kinetic fractionation due to the difference in molecular diffusivity of the water
242 molecule species (ε^{k} = 20.7 ‰ $\delta^{18}O$ and 10.8 ‰ $\delta^{2}H$) was estimated according to
243 Cappa et al. (2003) for a laminar boundary layer (Schmidt-number q = 2/3,
244 Dongmann et al. 1974). The equilibrium fractionation due to the phase change during
245 evaporation and condensation at different temperatures was calculated as in Majoube
246 (1971) with the conditions present at the specific day. The condensation (dew point)
247 temperature inside the peltier-cooled water condenser ($T_{pelt,DP}$) was determined based
248 on the remaining humidity and the air pressure of the air leaving the condenser
249 (details on the calculation are given in Appendix B). The equilibrium fractionation
250 factors during the labelling experiment were on average ε^{*}_{atm} = 8.9 ± 0.2 ‰ for $\delta^{18}O$
251 and 72.7 ± 2.7 ‰ for $\delta^{2}H$ at T = 31.3 ± 2.7 °C inside the labelling chamber and ε^{*}_{pelt} =
252 11.1 ± 0.2 ‰ for $\delta^{18}O$ and 103.3 ± 3.3 ‰ for $\delta^{2}H$ at $T_{pelt,DP}$ = 6.0 ± 2.5 °C inside the
253 water condenser.

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254 We compared the distribution of the assimilated labels (^{13}C, ^{18}O, ^{2}H) in the leaf, stem
255 and root tissue by its isotopic ratios. Therefore we converted the δ-notation to atom
256 fraction (Eq. 5) according to Coplen (2011).

257 $$x(^{13}C)_{t=x} = \frac{1}{1 + \frac{1}{(\delta^{13}C_{t=x}/1000 + 1) \cdot R_{V-PDB}}} \quad (5)$$

258 , where $\delta^{13}C_{t=x}$ is the isotopic signature (in ‰) of the bulk tissue at sampling date x
259 and R is the ratio of the heavier to the lighter isotope ($^{13}C/^{12}C$) of the international
260 standard V-PDB. The atom fraction of ^{18}O and ^{2}H was calculated accordingly, but
261 using $R_{V\text{-}SMOW}$ as reference and neglecting the ^{17}O isotope amount.
262 For the Van Krevelen approach we calculated the elemental ratios. The relative
263 label distribution ($^{18}O/^{13}C$ and $^{2}H/^{13}C$) within the plant organic matter (OM) was
264 calculated based on the excess atom fraction measured in each tissue (Eq. 6).

265 $$\frac{x^E(^{18}O_{tissue,OM})_{t=x/t=0}}{x^E(^{13}C_{tissue,OM})_{t=x/t=0}} = \frac{x(^{18}O_{tissue,OM})_{t=x} - x(^{18}O_{tissue,OM})_{t=0}}{x(^{13}C_{tissue,OM})_{t=x} - x(^{13}C_{tissue,OM})_{t=0}} \quad (6)$$

266 , where $x^E(^{18}O)_{t=x/t=0}$ and $x^E(^{13}C)_{t=x/t=0}$ is the excess atom fraction of the labels detected
267 at a specific sampling date (t = x), relative to the unlabelled control (t = 0). Eq. 6 and
268 7 was analogously calculated for the $^{2}H/^{13}C$ ratio.
269 In a second step we corrected the isotopic ratios ($^{18}O/^{13}C$ and $^{2}H/^{13}C$) with the
270 maximum label strength of the precursor, i.e. of the fresh assimilates (Eq. 7), which
271 was assumed to be the excess atom fraction of ^{13}C in the leaf water-soluble organic
272 matter (wsOM) and the excess atom fraction of ^{18}O and ^{2}H in the leaf water (relative
273 to the unlabelled control).

274 $$\frac{x^E_{norm}(^{18}O_{tissue,OM})_{t=x/t=0}}{x^E_{norm}(^{13}C_{tissue,OM})_{t=x/t=0}} = \frac{x^E(^{18}O_{tissue,OM})_{t=x/t=0}}{x^E(^{13}C_{tissue,OM})_{t=x/t=0}} \cdot \frac{x^E(^{13}C_{leaf,wsOM})_{t=x/t=0}}{x^E(^{18}O_{leaf,water})_{t=x/t=0}} \quad (7)$$

275 # 3 Results

276 ## 3.1 Labelling of the leaf water and water-soluble OM

277 The ^{18}O and ^{2}H label added as water vapour to the chamber atmosphere ($\delta^{18}O$ = - 370
278 ‰, $\delta^{2}H$ = - 813 ‰), was mixed with transpired water, which was isotopically
279 enriched compared to the added label (Fig. 1). The isotopic signature of the water

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280 vapour within the chamber air stabilized after four days at a level of - 112 ± 4 ‰ $\delta^{18}O$
281 and - 355 ± 7 ‰ $\delta^{2}H$. Thus the atmospheric water vapour signature was depleted in
282 ^{18}O by 94 ± 4 ‰ and in ^{2}H by 183 ± 7 ‰ compared to the unlabelled atmosphere.
283 The leaf water was strongly depleted and its isotopic signature was stable at a level of
284 - 64 ± 7 ‰ for $\delta^{18}O$ and - 158 ± 13 ‰ for $\delta^{2}H$ already after two days of labelling with
285 the depleted water vapour (Fig. 1). The leaf water was thus on average depleted by 63
286 ± 7 ‰ for $\delta^{18}O$ and 126 ± 14 ‰ for $\delta^{2}H$ compared to the unlabelled leaf water
287 signature and it was between the signature of the atmospheric water vapour and the
288 water added to the soil ($\delta^{18}O$ = - 9 ± 0 ‰, $\delta^{2}H$ = - 74 ± 2 ‰). This indicates that a
289 substantial amount of the leaf water originated from the atmospheric water pool,
290 suggesting that it entered the leaf via diffusion through the stomata. The depletion of
291 the water within a leaf was dependent on its position on the shoot (Fig. 2c,e). The leaf
292 water of the leaves sampled in the upper half of the shoot was 7 ± 2 ‰ and 18 ± 8 ‰
293 less depleted in $\delta^{18}O$ and $\delta^{2}H$ than the leaves sampled at the lower half. The isotopic
294 signature of the stem water ($\delta^{18}O$ = - 10 ± 0 ‰ and $\delta^{2}H$ = - 74 ± 4 ‰), as well as the
295 root ($\delta^{18}O$ = - 6 ± 1 ‰ and $\delta^{2}H$ = - 58 ± 4 ‰) and the soil water ($\delta^{18}O$ = - 6 ± 1 ‰
296 and $\delta^{2}H$ = - 63 ± 3 ‰), was not significantly depleted and reflected the signature of
297 the water added to the soil (Fig. 1).
298 At the second sampling date, the leaf water seemed to be more depleted than the water
299 vapour within the chamber air (Fig. 1). This is the result of different sampling
300 procedures. The leaf sampling was performed at one point in time (three hours after
301 the light switched on), while the atmospheric water vapour collected by condensation
302 represents an average on the previous 24 hours. Therefore the depletion of the water
303 vapour is underestimated before the equilibrium of the isotopic signature in the
304 atmosphere was reached. In the following the average values of signatures detected
305 after the equilibrium was reached are given (t = 8 and t = 14). We tried to estimate the
306 contribution of the isotopic signature of the atmospheric water vapour that enters the
307 leaf by diffusion with a two-source mixing model (Tab. 1). The results were obtained
308 by the two water isotopes ^{18}O and ^{2}H separately. Both indicated a substantial
309 contribution of the atmospheric water vapour to the leaf water isotopic signature,
310 whereby the estimates based on the oxygen isotope yielded a higher contribution (69
311 ± 7 %) than the hydrogen estimates (58 ± 4 %). The estimates for the leaves sampled
312 at different position on the shoot varied by 5 %, whereas the contribution of
313 atmospheric water to the leaf water was higher in the leaves sampled at the bottom

314 (71 ± 4 % based on ^{18}O and 60 ± 2 % based on ^{2}H) than in the leaves at the top (66 ±
315 2 % and 55 ± 0 %, respectively) of the shoots.
316 The ^{13}C-CO_2 added (8938 ‰ $\delta^{13}C$) was assumingly also strongly diluted by respired
317 ^{12}C-CO_2, but we did not measure the isotopic signature of the CO_2 within the chamber
318 air. The leaf water-soluble OM was significantly enriched already after one day of
319 labelling and levelled off towards the end of the experiment. At the last two sampling
320 dates its isotopic signature was on average 1346 ± 162 ‰ $\delta^{13}C$.

321 **3.2 Labelling of the bulk organic matter**

322 All three applied labels could be detected in the plant bulk material (Tab. 2). We
323 measured the isotopic signature of the non-exchangeable hydrogen, which was
324 estimated to be 74 ± 1 % of the total OM. After fourteen days of continuous labelling,
325 the leaves, petioles, stems and roots were enriched by 650 - 1150 ‰ in $\delta^{13}C$, depleted
326 by 4 - 17 ‰ in $\delta^{18}O$ and 6 - 31 ‰ in $\delta^{2}H$. Thus the plant biomass was significantly
327 labelled even under the extreme environmental conditions (high temperature and low
328 light availability) that were critical for net C assimilation (increasing tissue respiration
329 and reducing photosynthesis, respectively). However, the labelling was not strong
330 enough to trace the OM within the large OM pools of the cuttings and soil organic
331 matter, in which the change in isotopic signature was close to the detection limit or
332 could not be detected. The measured depletion in ^{18}O of the bulk soil can be
333 accounted for natural variability, since the same effect has been observed in non-
334 treated soil (data not shown here).
335 The labelling of the leaf bulk OM occurred in parallel to the labelling of the leaf water
336 and water-soluble OM (Fig. 2). The leaf OM was enriched in ^{13}C after one day (Fig.
337 2b) and depleted in ^{18}O and ^{2}H after two days (Fig. 2d,f). The incorporation of the
338 label into the leaf OM was, as the labelling of the leaf water, dependent on the
339 position on the shoot. The biomass of the leaves at the top was more enriched in ^{13}C
340 (by up to 673 ‰) than the biomass of the leaves at the bottom of the shoots, and in
341 contrast to the leaf water, more depleted in ^{18}O and ^{2}H (by up to 9 and 21 ‰,
342 respectively) at the top than at the bottom. This indicates a higher overall assimilation
343 in the leaves at the top of the shoot.

344 ### 3.3 Atomic and isotopic ratios to characterize organic matter

345 The atomic ratios of the plant bulk OM were in the range of 13.7 - 115.4 C/N, 0.70 -
346 0.83 O/C and 1.56 - 1.72 H/C (Tab. 3). The leaf OM was characterized by the lowest
347 C/N and O/C ratios and concurrently by highest H/C ratios (Fig. 3a). The other plant
348 tissues indicated a linear trend in decreasing O/C and H/C and increasing C/N ratios
349 in the order of stems, petioles, roots and cuttings.
350 The recovery of the three isotopes varied between the leaf, stem and root tissue, while
351 they were similar between the sampling dates (Fig. 3b). The isotopic ratios of the
352 excess atom fractions were $3.5 \pm 0.4 \times 10^{-3}$ $^{18}O/^{13}C$ and $5.3 \pm 0.5 \times 10^{-4}$ $^{2}H/^{13}C$ in the
353 leaves, $1.4 \pm 0.1 \times 10^{-3}$ $^{18}O/^{13}C$ and $2.9 \pm 0.6 \times 10^{-4}$ $^{2}H/^{13}C$ in the stems and $1.0 \pm 0.2 \times$
354 10^{-3} $^{18}O/^{13}C$ and $1.0 \pm 1.4 \times 10^{-4}$ $^{2}H/^{13}C$ in the roots after the equilibrium in the leaf
355 water and water-soluble OM labelling was reached. Thus the $^{18}O/^{13}C$ ratios were on
356 average 2.6 (± 0.2) times lower in the stems and 3.8 (± 0.7) times lower in the roots
357 than in the leaves (Tab. 3) and the $^{2}H/^{13}C$ ratios 1.9 (± 0.2) and 3.1 (± 0.6) times lower
358 in the stems and roots, respectively, than in the leaves.
359 After correction for the maximum label strength (^{18}O, ^{2}H and ^{13}C excess atom fraction
360 within the leaf water and the water-soluble OM, respectively), the isotopic ratios were
361 in the range of 0.17 - 0.43 $^{18}O/^{13}C$ and 0.14 - 0.23 $^{2}H/^{13}C$. The normalized isotopic
362 ratios were thus in the magnitude order of the atomic ratios reported for OM
363 compounds (Tab. 3, Fig. 3c), however lower than expected for fresh organic matter
364 (in the range characteristic for condensed hydrocarbons).

365 ## 4 Discussion

366 ### 4.1 Diffusion of atmospheric water vapour into the leaf

367 The strong depletion in $\delta^{18}O$ and $\delta^{2}H$ observed in the leaf water indicates a high back-
368 diffusion of labelled water vapour from the atmosphere into the leaf. The diffusion is
369 dependent on the gradient between atmospheric and leaf water vapour pressure and
370 the stomatal conductance (Parkhurst, 1994). The higher the atmospheric water vapour
371 pressure (the smaller the gradient), the more water molecules diffuse back into the
372 leaf. The latter is further enhanced the larger the stomatal conductance is (Reynolds
373 Henne, 2007). Here we maintained the atmospheric vapour pressure constant at a high
374 level, ensuring a high back-diffusion at a given stomatal conductance. In our
375 experiment the leaf water $\delta^{18}O$ and $\delta^{2}H$ signature is determined by i) the signature and

376 the amount of labelled (depleted) water vapour diffusing into the leaf intercellular
377 cavities, ii) by the enrichment due to transpiration (kinetic and equilibrium
378 fractionation) and iii) by the influx of xylem water, which is isotopically enriched
379 relative to the labelled water vapour. The latter is proportionally enhanced by
380 increasing transpiration rates as a result of the diffusion convection process of H_2O
381 (Péclet effect, Farquhar and Lloyd 1993).
382 The distinct label signal in the water sampled in leaves at different positions on the
383 shoot indicates differences in the transpiration rate. Meinzer et al. (1997)
384 demonstrated in large poplar trees that shading or lower irradiance leads to lower
385 stomatal conductance and transpiration rates. Thus the back-diffusion in the leaves on
386 the bottom might have been reduced due to lower stomatal conductance. However, the
387 increased transpiration in the leaves at the top, lead to an even stronger dilution of the
388 isotopic signal in the leaf water due to i) increased evaporative leaf water enrichment
389 and ii) the Péclet effect (enhanced influx of xylem water, which was enriched
390 compared to the labelled atmospheric water vapour).
391 The amount of leaf water that entered the leaf by back-diffusion was estimated to be
392 58-69 %. This result is in contradiction to the common perception that most of the leaf
393 water is taken up from the soil via roots. However it is in line with the observations
394 made by Farquhar & Cernusak (2005), who modelled the leaf water isotopic
395 composition in the non-steady state and estimated the contribution of atmospheric
396 water to the leaf water to be approximately two-thirds of the total water supply.
397 Albeit, our estimates are based on a modelling approach that does not take into
398 account the Péclet effect or daily fluctuations in the isotopic signatures as described
399 below, our estimates correspond very well the findings of Farquhar & Cernusak
400 (2005).
401 The model used to estimate the quantitative contribution of the two water sources is
402 based on the measured signature of the leaf water ($\delta^{18}O_{leaf,water}$) and the estimated
403 signatures of the water at the evaporating and condensation site ($\delta^{18}O_{source,1}$ and
404 $\delta^{18}O_{source,2}$, respectively). The "dilution" of the (laminar) leaf water with the relatively
405 enriched xylem water through the Péclet effect is included in the $\delta^{18}O_{leaf,water}$. This
406 explains the lower contribution of atmospheric water (- 5 %) estimated in the leaves
407 sampled at the top (due to the Péclet effect resulting from higher transpiration rates)
408 compared to the leaves sampled at the bottom of the shoot.

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409 Some inaccuracy in the two-source mixing model estimates might have been
410 introduced by daily fluctuations in the environmental and labelling conditions. The
411 mixture ($\delta^{18}O_{leaf,water}$) was sampled after three hours of light, whereas the estimation
412 of the two sources ($\delta^{18}O_{source,1}$ and $\delta^{18}O_{source,2}$) is based on daily average values of
413 environmental parameters and the atmospheric water vapour ($\delta^{18}O_{atm,vap}$) label
414 strength. In our experiment, fluctuations in $\delta^{18}O_{atm,vap}$ were caused by adding the
415 labelled vapour mainly during night-time, when transpiration was low. Thus the
416 atmospheric label strength was assumingly highest before the lights were switched on
417 and gradually diluted during the day by transpired water vapour. Hence the actual
418 $\delta^{18}O_{atm,vap}$ at the time of plant sampling was probably more depleted than the
419 measured average signature. Therefore $\delta^{18}O_{source,2}$ and its contribution to the leaf
420 water was slightly overestimated. The effect of the temperature fluctuations (± 3 °C)
421 via changes in the equilibrium fractionation was minor for the outcome of the mixing
422 model < 1 %.

423 Nonetheless, the strong depletion of the leaf water in ^{2}H and ^{18}O proofs, that back-
424 diffusion of atmospheric water vapour into the leaf is an important mechanisms for
425 leaf water uptake. This supports the hypothesis that atmospheric water vapour
426 diffusion might be as important as the flux of water from the xylem into the leaf (at
427 least under humid conditions) and be an important mechanisms for the reversed water
428 flow observed in the tropics (Goldsmith, 2013). Furthermore, these results
429 demonstrate that the leaf water isotopic composition is strongly affected by the
430 atmospheric signature at humid conditions and that thus the applicability of the dual-
431 isotope approach (Scheidegger et al., 2000), e.g. to reconstruct past climate conditions
432 by tree ring analysis, is only valid if the source water and atmospheric vapour $\delta^{18}O$
433 are similar. The back-diffusion of atmospheric vapour at high humidity could be
434 another factor next to the evaporative enrichment (as demonstrated by Roden and
435 Farquhar, 2012) to overshadow the effects of stomatal conductance on the leaf $\delta^{18}O$
436 signature.

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437 **4.2 Tracing organic matter?**

438 The O/C and H/C ratio of the plant bulk material was close to the signature of
439 cellulose (Fig. 3a). The leaves had a lower O/C ratio with a constant high H/C ratio
440 indicating that its OM contains more reduced compounds such as amino-sugars or
441 proteins, which is also supported by its low C/N ratio. The trend of decreasing O/C

442 and H/C ratios observed in the other tissues is in the direction of condensation
443 reactions. This trend most likely indicates the increasing lignification of OM from
444 shoots, to roots, to cuttings.
445 The same trend has been observed in the ratios of the labels added from the leaf, to
446 the stem, to the root OM (Fig. 3b,c). The lower isotopic O/C and H/C ratios in the
447 root and stem tissue compared to the leaf tissue could indicate the utilization of the
448 labelled assimilates for the synthesis of more condensed compounds (e.g. lignin) in
449 those tissues. However, other factors affecting the isotopic ratios of the OM are the
450 maximum label strength, the exchange of hydrogen and oxygen with xylem water
451 during transport and biosynthesis and the isotopic fractionation during metabolism.
452 The isotopic ratios (Fig. 3b) were around three magnitudes smaller than the expected
453 atomic ratios of OM (Sleighter and Hatcher, 2007). This is mainly due to the different
454 maximum label strength, which was highest for the ^{13}C and lowest for the ^{2}H. After
455 correction for this factor, the isotopic ratios were in the range of the atomic ratios
456 characteristic for condensed hydrocarbons (Fig. 3c). The isotopic ratios might be
457 lower than expected due to inaccurate approximation of the maximum label strength
458 of fresh assimilates (by the leaf water and water-soluble OM), or be the result of ^{18}O
459 and ^{2}H label losses during transport and biosynthesis.
460 One reason for the label loss might be the use of other (more enriched) sources during
461 biosynthesis. For example O_2 (enriched by 23 ‰ $\delta^{18}O$) has been identified as a further
462 source for aromatic compounds, such as phenols and sterols (Schmidt et al., 2001).
463 However, for hydrogen, water is the only known source (Schmidt et al., 2003) and
464 therefore the use of other O or H sources during biosynthesis can not explain the
465 (major) loss of the ^{18}O and ^{2}H label.
466 Another potential reason would be the kinetic fractionation during biosynthesis that
467 leads to distinct isotopic signatures of different OM compounds (described in Schmidt
468 et al. 2001, 2003; Badeck et al. 2005; Bowling et al. 2008). However, assuming
469 constant isotopic fractionation during the experimental period (constant
470 environmental conditions), the isotopic ratios would not be affected, since they are
471 based on the excess atom fraction relative to the unlabelled OM.
472 A third reason for the loss of the ^{18}O and ^{2}H label could be the exchange of hydrogen
473 and oxygen atoms with water. O and H exchanges with tissue water during transport
474 and the synthesis of new compounds (as recently discussed for oxygen in phloem
475 sugars and cellulose in Offermann et al. 2011 and Gessler et al. 2013). O of carbonyl

476 groups (Barbour, 2007; Sternberg et al., 1986) and H in nucleophilic OH and NH
477 groups or H adjacent to carbonyl groups (Augusti et al., 2006; Garcia-Martin et al.,
478 2001) exchange with water. Thus biochemical reactions lead to different isotopomers
479 of organic compounds (Augusti and Schleucher, 2007). The proportion of O and H
480 exchanged can be considerable, e.g. during cellulose synthesis around 40 % of O and
481 H are exchanged with the tissue water (Roden and Ehleringer, 1999; Yakir and
482 DeNiro, 1990). The exchange with water explains to some extend the stronger relative
483 ^{18}O and ^{2}H signal in the leaf OM compared to the stem and root OM, since the leaf
484 water was labelled, while the stem and root water was not. Especially the $^{18}O/^{13}C$
485 isotopic ratios were increased in the leaf OM compared to the relations observed in
486 the atomic ratios (Fig. 3a). The leaf OM has the lowest O/C atomic ratios while it has
487 the highest $^{18}O/^{13}C$ isotopic ratios of all plant compartments (Tab. 3). This effect is
488 less expressed for the $^{2}H/^{13}C$ ratios, since only the fraction of hydrogen that does not
489 exchange with ambient water vapour is measured. The non-exchangeable fraction (74
490 %) is hydrogen bound to carbon (Filot et al., 2006), which is hardly exchanged with
491 xylem water.

492 5 Conclusions

493 We present a new technique to label organic matter at its place of formation by the
494 application of labels through the gaseous phase ($^{13}CO_2$ and $^{2}H_2{}^{18}O$). In this study we
495 could show that in a humid atmosphere, the atmospheric water vapour isotopic
496 signature dominates the leaf water signature, due to a strong back-diffusion of water
497 vapour into the leaf. Further we detected differences in the relative distribution of ^{13}C,
498 ^{18}O and ^{2}H in the leaves, stems and roots. This could indicate the synthesis of
499 different compounds in the particular tissues (change in OM characteristics), but it
500 could also be the result of exchange and fractionation processes during transport and
501 biosynthesis. To further test these two possibilities a better estimation of the
502 maximum label strength by compound specific sugar analysis would be needed,
503 which has been further developed for $\delta^{13}C$ (Rinne et al., 2012) and for $\delta^{18}O$ (Zech et
504 al., 2013) recently, but does not yet exist for $\delta^{2}H$ analysis.
505 The multi-isotope labelling technique can be used to assess the amount of vapour
506 diffusing into the leaves and to trace the dynamics of the labelled organic matter. It
507 could be applied in soil sciences, e.g. to track the decomposition pathways of soil OM
508 inputs, or in the field of plant physiology and paleoclimatic reconstruction, e.g. to

509 further investigate the O and H exchange and fractionation processes during transport
510 and metabolic processes or the importance of the ambient air humidity besides its
511 isotopic composition for the climate signal stored in tree-ring cellulose. Furthermore
512 the multi-isotope labelling technique has the potential to make changes of OM
513 characteristics visible (e.g. C allocation into the non-structural vs. structural pool), for
514 example after a change in climatic conditions, and to trace the labelled OM during its
515 decomposition within the soil.

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676 **Tables**

677 **Table 1.** Diffusion of atmospheric water vapour into the leaf water. $\delta^{18}O$ and $\delta^{2}H$
678 signatures of leaf water and its two sources: i) the evaporating source (Eq. 3),
679 estimated by the stem water signature plus kinetic and equilibrium leaf water
680 enrichment (assuming full evaporation without back-diffusion), and ii) the
681 condensation source (Eq. 4), assessed by the atmospheric water vapour signature plus
682 equilibrium fractionation to account for the gas-liquid phase change. The contribution
683 of the second source (diffusion and condensation of atmospheric water vapour) to the
684 leaf water ($f_{source,2/leaf,water}$) was estimated by a two-source isotope mixing model for
685 ^{18}O and ^{2}H separately (Eq. 2). Presented are the average values of three plant
686 replicates for each sampling date ± one standard deviation

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Sampling date (days)	Leaf water[(1)]		Source 1: Evaporating source[(2)]		Source 2: Condensation source[(2)]		$f_{source,2/leaf,water}$[(2)]	
	$\delta^{18}O$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)	$\delta^{2}H$ (‰)	^{18}O (%)	^{2}H (%)
0	-1.0 (±0.5)	-32.0 (±1.8)	21.3 (±0.4)	10.9 (±2.6)	-8.8	-99.7	74.2 (±1.2)	38.8 (±0.3)
1	-11.7 (±1.8)	-53.0 (±5.9)	19.5 (±0.3)	10.3 (±3.2)	-27.3	-143.3	66.6 (±3.9)	41.2 (±3.2)
2	-65.6 (±6.5)	-162.3 (±8.6)	20.0 (±0.6)	14.4 (±2.1)	-47.6	-196.0	126.6 (±9.8)	84.0 (±4.1)
8	-65.2 (±2.0)	-159.9 (±3.8)	20.0 (±0.7)	5.3 (±3.9)	-98.6	-274.8	71.8 (±1.5)	59.0 (±0.8)
14	-60.4 (±10.7)	-152.3 (±21.2)	19.3 (±0.4)	9.5 (±5.1)	-101.8	-275.8	65.8 (±8.7)	56.8 (±6.8)

[(1)] directly measured
[(2)] calculated

687

687 **Table 2.** Multi-isotope labelling of bulk organic matter. $\delta^{13}C$, $\delta^{18}O$ and $\delta^{2}H$ signatures
688 (in ‰) of the plant-soil compartments (three replicates ± one standard deviation)
689 measured before and after 1, 2, 8 and 14 days of continuous labelling. A significant
690 enrichment ($\delta^{13}C$) and depletion ($\delta^{18}O$, $\delta^{2}H$) compared to the unlabelled control (t =
691 0) is highlighted with * (t-test, P < 0.05). The degree of labelling is indicated by the
692 change in the isotopic signature of the last sampling date (t = 14) compared to the
693 control

	Sampling date (days)					
$\delta^{13}C$ (‰)	0	1	2	8	14	14 - 0 [(1)]
Leaves	-30.8 (±0.4)	161.5* (±37.4)	189.7 (±128.7)	570.7* (±81.0)	812.5* (±235.0)	843.3 ±235.0)
Petioles	-32.8 (±0.2)	163.9* (±56.2)	212.8* (±75.2)	908.5* (±277.3)	941.9* (±292.7)	974.7 (±292.7)
Stems	-31.4 (±0.6)	209.6* (±84.2)	281.3* (±87.6)	1093.7* (±402.2)	1119.9* (±367.6)	1151.3 (±367.6)
Cuttings	-31.2 (±0.3)	-27.0* (±1.6)	-26.9 (±1.9)	-14.6 (±15.8)	-14.5* (±2.1)	16.8 (±2.1)
Roots	-30.8 (±0.7)	98.1* (±12.5)	90.8 (±62.9)	646.5 (±335.1)	618.0* (±310.9)	648.8 (±310.9)
Bulk soil	-28.0 (±0.1)	-27.9 (±0.0)	-27.8 (±0.2)	-27.5 (±0.5)	-27.5 (±0.2)	0.5 (±0.3)
$\delta^{18}O$ (‰)	0	1	2	8	14	14 - 0 [(1)]
Leaves	25.9 (±0.8)	25.2 (±0.8)	21.9 (±2.0)	15.0* (±0.4)	9.0* (±3.0)	-16.9 (±3.2)
Petioles	21.0 (±0.2)	20.4 (±0.4)	19.5* (±0.4)	14.3* (±1.6)	12.8* (±2.3)	-8.2 (±2.3)
Stems	22.4 (±0.4)	22.2 (±0.1)	20.6* (±0.8)	14.7* (±2.4)	13.3* (±2.8)	-9.1 (±2.8)
Cuttings	21.3 (±1.5)	21.9 (±0.1)	21.8 (±0.4)	21.5 (±0.3)	21.5 (±0.4)	0.2 (±1.5)
Roots	21.2 (±0.6)	20.6 (±0.6)	20.9 (±0.4)	18.2 (±1.5)	17.5* (±1.7)	-3.7 (±1.8)
Bulk soil	14.8 (±0.4)	14.0 (±0.3)	13.8* (±0.4)	13.0* (±0.1)	13.5 (±0.8)	-1.3 (±0.9)
$\delta^{2}H$ (‰)	0	1	2	8	14	14 - 0 [(1)]
Leaves	-146.6 (±2.5)		-158.1 (±7.8)	-169.2* (±5.5)	-178.0* (±9.4)	-31.3 (±9.7)
Petioles	-138.3 (±1.8)				-150.9 (±6.7)	-12.6 (±7.3)
Stems	-129.2 (±4.2)		-136.3 (±4.7)	-153.3 (±14.8)	-152.9* (±9.4)	-23.7 (±10.3)
Cuttings	-167.3 (±2.8)				-172.8 (±6.3)	-5.5 (±6.9)
Roots	-129.7 (±6.4)		-134.0 (±12.5)	-137.0 (±6.8)	-135.9 (±7.7)	-6.2 (±10.0)
Bulk soil	-101.5 (±1.1)				-101.9 (±1.3)	0.4 (±1.7)

[(1)] Isotopic difference for the entire labelling experiment

694

694 **Table 3.** Atomic and isotopic ratios of the labelled bulk organic matter. C/N, O/C and
695 H/C atomic ratios and $^{18}O/^{13}C$ and $^{2}H/^{13}C$ isotopic ratios (of the excess atom fraction)
696 measured in different plant compartments after the equilibrium in the atmospheric
697 labelling was reached. Indicated are average values of two sampling dates (t = 8 and
698 14) with three plant replicates each (± one standard deviation)

Compartment	C/N	O/C	H/C	$^{18}O/^{13}C^{(1)}$	$^{2}H/^{13}C^{(1)}$
Leaves	13.7 (±0.4)	0.70 (±0.01)	1.72 (± 0.04)	0.43 (±0.07)	0.41 (±0.06)
Petioles	35.4 (±1.3)	0.77 (±0.01)	1.64 (±0.01)	0.18 (±0.03)	0.14 (±0.03)[2]
Stems	32.0 (±4.0)	0.83 (±0.01)	1.71 (±0.02)	0.17 (±0.03)	0.23 (±0.06)
Cuttings	115.4 (±7.2)	0.72 (±0.01)	1.56 (±0.02)	n.c.[3]	n.c.[3]
Roots	29.9 (±2.0)	0.73 (±0.02)	1.61 (±0.02)	0.12 (±0.03)	0.07 (±0.11)

[1] Ratio of excess atom fraction normalized by the maximum label strength (Eq. 7)
[2] Only the last sampling date was measured (t = 14)
[3] Not calculated (no consistent ^{18}O and ^{2}H depletion detected in the tissue)

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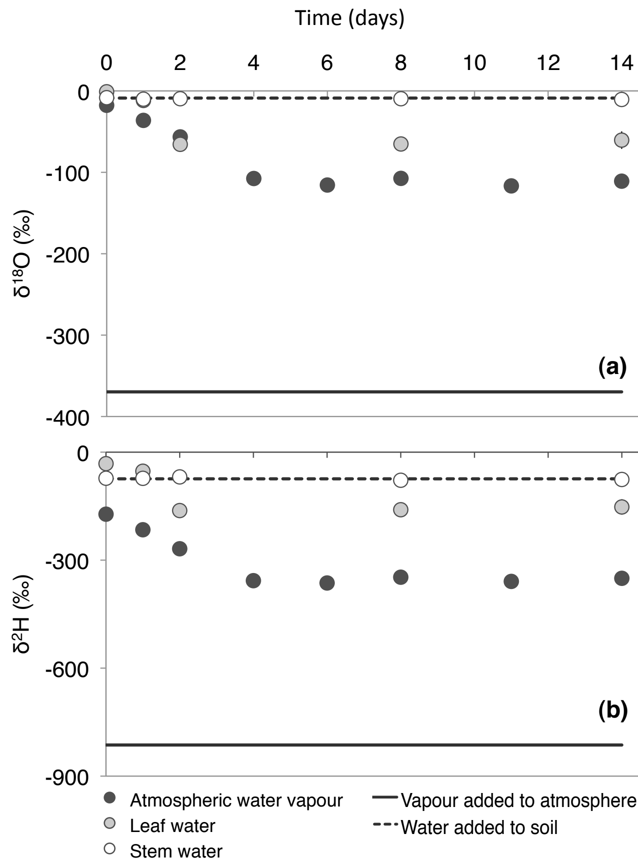
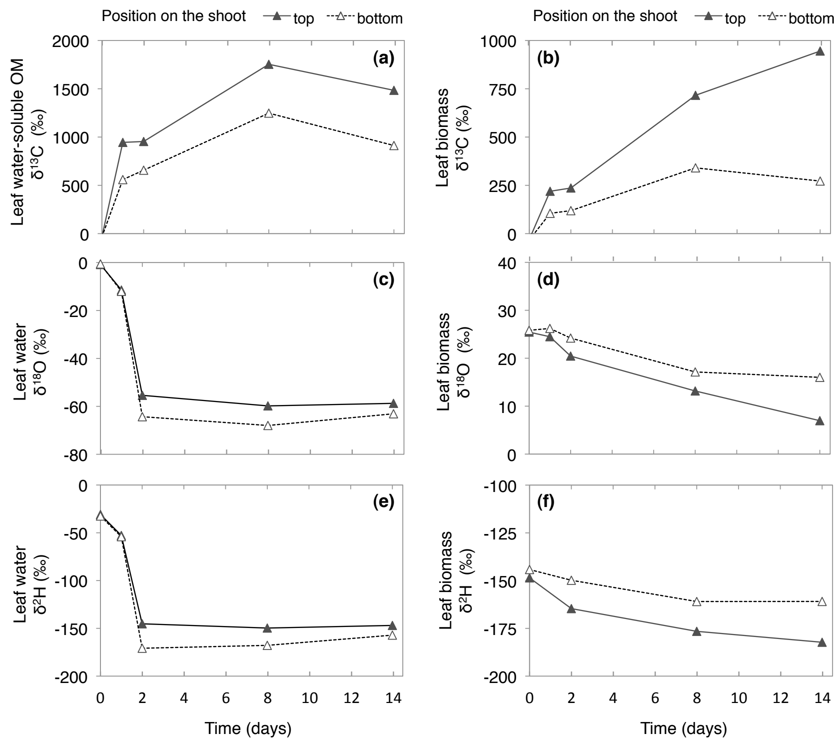
699 **Figures**



700

701 **Figure 1.** Temporal dynamics in the water isotopic signatures of the plant-soil-
702 atmosphere system during continuous $^{2}H_{2}{}^{18}O$ labelling (a) $\delta^{18}O$ and (b) $\delta^{2}H$ signature
703 (in ‰) of the depleted water label added as water vapour to the atmosphere (solid
704 line), of the water added to the soil (dashed line), of the resulting water vapour in the
705 chamber atmosphere (black dots) and of the extracted leaf (grey dots) and stem water
706 (white dots). Error bars on the leaf water indicate ± one standard deviation of three
707 plant replicates



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709 **Figure 2.** Incorporation of the gaseous labels ($^{13}CO_2$, $^{2}H_2{}^{18}O$) into the leaf water
710 water-soluble and bulk organic matter. (a,b) $\delta^{13}C$, (c,d) $\delta^{18}O$ and (e,f) $\delta^{2}H$ signature
711 (in ‰) within leaves sampled at the top (solid line, black triangles), or at the bottom
712 (dashed line, white triangles) of the shoot. Illustrated are the signatures of (a) the leaf
713 water-soluble organic matter, (b,e,f) the leaf biomass and (c,e) the leaf water

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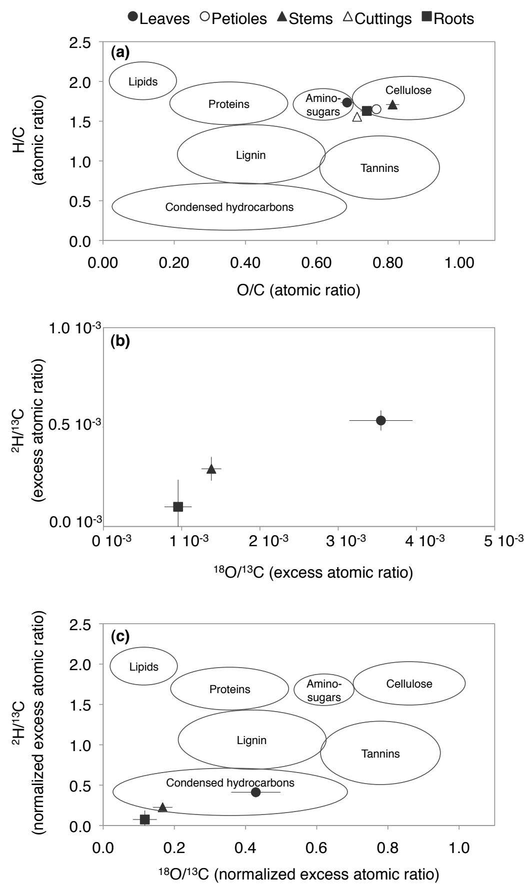
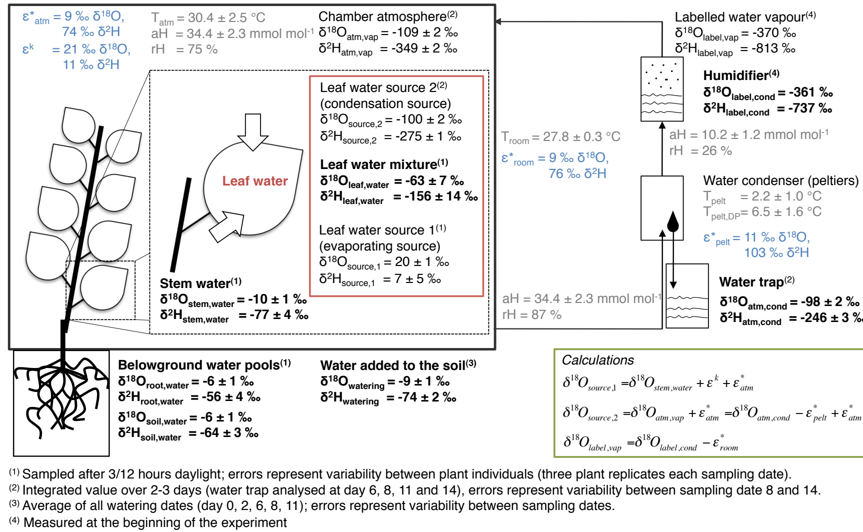


715 **Figure 3.** Atomic and isotopic ratios to illustrate change in organic matter
716 characteristics (a) Atomic and (b,c) isotopic ratios of oxygen and hydrogen to carbon
717 within the leaves (closed circles), petioles (open circles), stems (closed triangle), stem
718 cutting (open triangle) and roots (closed square). The circles overlain on the plots in
719 (a) and (c) indicate atomic ratios characteristic for different compound classes
720 (adapted from Sleighter & Hatcher, 2007). (a) illustrates the atomic ratio of all tissues
721 measured (15 replicates ± one standard deviation, (b) the isotopic ratios of the ^{13}C,
722 ^{18}O and ^{2}H excess atom fraction (relative to the unlabelled tissues) measured after
723 equilibrium in the labelling (see Fig. 1 and 2) was reached (t = 8 and 14, six replicates
724 ± one standard deviation) and **(c)** shows the isotopic ratios of after normalization with
725 the maximum label strength of the leaf water (^{18}O, ^{2}H) and water-soluble organic
726 matter (^{13}C)

727 **Appendix A**



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729 **Figure A1.** Overview on the input data of the two-source isotope mixing model. δ^{18}O
730 and δ^{2}H signatures of the water pools of the chamber system are presented as average
731 values after equilibrium in the labelling was reached (t = 8 and 14 days). The
732 monitored environmental conditions (T = temperature, aH = absolute humidity and rH
733 = relative humidity) are presented in grey. The equilibrium and kinetic fractionation
734 factors, highlighted in blue, were calculated according to Majoube (1971) and Cappa
735 et al. (2003), respectively. The fractionation factors were used for the calculations
736 (green box) of the signatures in the non-directly measured pools and the isotopic
737 signatures of the evaporating and condensation source of the leaf water (red box). The
738 equations are given for δ^{18}O, but apply for δ^{2}H analogously. Please note that the data
739 reported here are average values of the two last sampling dates, while we present in
740 the result section the data of single sampling dates or average values of the whole
741 labelling experiment (environmental conditions, equilibrium fractionation factors)

742 **Appendix B**

743 Calculation of the relative air humidity and the dew-point temperature

744 The dew-point temperature, i.e. the temperature at which the water condensed inside
745 the peltier-cooled water condenser ($T_{pelt,DP}$) was calculated by solving Equation B1
746 with the humidity measured in the air after the condenser (10 ± 1 mmol mol^{-1} aH, 26
747 % rH).

748 $$rH(T) = \frac{e}{e(T)} \cdot 100 \qquad \text{(B1)}$$

749 , where rH is the relative air humidity (in %), e is the partial pressure of water vapour
750 (calculated according to Eq. B2) and e(T) is the saturation vapour pressure (in kPa,
751 calculated according to Eq. B3).

752 $$e = \frac{aH}{1000} \cdot p \qquad \text{(B2)}$$

753 , where aH is the absolute humidity given as the mole fraction of water vapour (mmol
754 mol^{-1}) and p is the atmospheric pressure (in kPa).

755 $$e(T) = 0.61365 \cdot e^{\frac{17.502 \cdot T}{240.97 + T}} \qquad \text{(B3)}$$

756 , where T is the room air temperature (in °C).

757 References

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