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

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 composition. 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.

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

1 Introduction

Artificial labelling with stable isotopes facilitates the observation of bio(geo)chemical cycling of elements or compounds with minor disturbance to the plant-soil systems. It has provided many insights into plant carbon allocation patterns (e.g. Simard et al. 1997; Keel et al. 2006; Högberg et al. 2008), water dynamics (e.g. in Plamboeck et al. 2007; Kulmatiski et al. 2010) and soil organic matter processes (e.g. in Bird and Torn 2006; Girardin et al. 2009) in terrestrial ecosystems. Only a few studies used labelling approaches with more than one stable isotope, for example to study the interactions between the carbon and nitrogen cycle (e.g. in Bird and Torn 2006; Schenck zu Schweinsberg-Mickan et al. 2010). However, to our knowledge isotopic labelling of organic matter (OM) with its three major elements, carbon (C), oxygen (O) and hydrogen (H), has never been done in ecosystem studies before, even though combined $\delta^{13}C$, $\delta^{18}O$ and $\delta^{2}H$ analyses have been widely used to study plant physiological processes and to reconstruct past climatic conditions (Hangartner et al., 2012; Roden and Farquhar, 2012; Scheidegger et al., 2000; Werner et al., 2012). Similarly, an artificial labelling with those isotopes would be useful to clarify basic mechanisms related to the plant water-use efficiency or the oxygen and hydrogen signals in tree rings, but also to study other OM dynamics in the plant-soil system such as OM decomposition in the soil.

The C, O and H contents of organic matter have been applied to distinguish major groups of compounds, by plotting the atomic ratios O/C and H/C in a van Krevelen diagram (Kim et al., 2003; Ohno et al., 2010; Sleighter and Hatcher, 2007). This approach is based on the distinct molecular composition of organic compounds. For example the glucose molecule ($C_6 H_{12} O_6$) is characterized by high O/C (= 1) and H/C (= 2) ratios and is the precursor of other compounds, such as cellulose ($(C_6 H_{10} O_5)_{[n]}$ O/C = 0.8, H/C = 1.7, Fig. 3a). Condensation or reduction reactions during biosynthesis lead to other compound groups with lower atomic ratios (e.g. lignin) or similar H/C, but lower O/C ratios (e.g. lipids, proteins) compared to glucose. Following the logic of the van Krevelen diagram, we wanted to test, if we can use the isotopic ratios $^{18}O/^{13}C$ and $^{2}H/^{13}C$ of the labels recovered in plant-soil bulk materials 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.).

2.2 Labelling chamber, procedure and environmental conditions

The labelling chambers (MICE - Multi-Isotope labelling in a Controlled Environment - facility) provide a hermetical separation of the shoots (leaves, petioles and new

stems) from the roots, rhizosphere and the soil. The plant shoots are enclosed by one large polycarbonate cuboid (volume 1.2 m^3) with a removable front plate and five 2 cm wide gaps in the bottom plate to slide in three plants in each row. Small polycarbonate pieces, Kapton tape and a malleable sealant (Terostat IX, Henkel AG & Co.) wrapped around the stem cuttings were used to seal off the upper from the lower chamber. The belowground compartments (soil and roots) are in fifteen individual pots, which are hermetically sealed from the laboratory and aerated with outdoor air. This setup ensures that all plants receive the same labelling treatment and prevents the diffusion of labelled atmospheric gases into the soil.

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.

The isotope labels (^{13}C, ^{18}O and ^{2}H) were added continuously for 14 days via gaseous phase to the plant shoots. We used CO_2 enriched in ^{13}C (10 atom% ^{13}C-CO_2, Cambridge Isotope Laboratories, Inc.), and water vapour depleted in ^{18}O and ^{2}H ($\delta^{18}O$ = - 370 ‰ and $\delta^{2}H$ = - 813 ‰, waste product from enrichment columns at the Paul Scherrer Institute). Thus the labelled gases added were enriched by 8.90 atom% ^{13}C and depleted by 0.07 atom% ^{18}O and 0.01 atom% ^{2}H relative to the ambient air.

The soil moisture was maintained at 100 % field capacity and the relative air humidity was 74 %, in order to promote the back-diffusion of water into the leaves. The light intensity was low (80 ± 25 µmol m^{-2} s^{-1} photosynthetic active radiation), and the CO_2 concentration was kept at 508 ± 22 ppm in order to maintain a high atmospheric carbon supply. The day-night cycles were twelve hours and the temperature within the labelling chamber was 31 ± 3 °C throughout the experiments.

2.3 Sample collection

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

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

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

The dried plant residues of the cryogenic vacuum extraction were used for isotopic bulk analyses (described below). The leaf water-soluble organic matter was extracted by hot water extraction. 60 mg milled leaf material was dissolved in 1.5 ml of deionised water and heated in a water bath (85 °C) for 30 min. After cooling and centrifugation (10'000 g, 2 min), the supernatant was freeze-dried and analysed for

159 $\delta^{13}C$. $\delta^{2}H$ analyses were not possible on the hot water extracts (mainly sugars), due to
160 incomplete equilibration with ambient water vapour (Filot, 2010).

161 2.4 Isotopic and elemental analyses

162 All samples were milled to a fine powder with a steel ball mill and weighed into tin
163 ($\delta^{13}C$ analyses) or silver ($\delta^{18}O$ and $\delta^{2}H$ analyses) capsules and measured by isotope-
164 ratio mass spectrometry (IRMS). The $\delta^{13}C$ samples were combusted at 1700 °C in an
165 elemental analyser (EA 1110, Carlo Erba) and the resulting CO_2 was transferred in a
166 helium stream via a variable open-split interface (ConFlo II, Finnigan MAT) to the
167 IRMS (Delta S, Thermo Finnigan; see Werner et al. 1999). The samples for $\delta^{18}O$
168 analyses were pyrolysed at 1040 °C in an elemental analyser (EA 1108, Carlo Erba)
169 and transferred via ConFlo III interface (Thermo Finnigan) to the IRMS (Delta plus
170 XL, Thermo Finnigan). The samples for $\delta^{2}H$ analyses were equilibrated with water
171 vapour of known signature prior to the IRMS measurements, to determine the isotopic
172 signature of the non-exchangeable hydrogen (as described in Filot et al. 2006;
173 Hangartner et al. 2012). After equilibration the samples were pyrolysed in a
174 thermochemical elemental analyser (TC/EA, Thermo-Finnigan) at a temperature of
175 1425 °C and the gaseous products were carried by a helium stream via a ConFlow II
176 open split interface (Thermo Finnnigan) into the IRMS (Isoprime, Cheadle). The
177 amount of exchangeable hydrogen (25-27%) and oxygen (2-3%) was measured for
178 the leaf, stem and root tissue using depleted water vapour to equilibrate the samples.
179 The measurement precisions of the solid sample analyses were 0.12 ‰ $\delta^{13}C$, 0.54 ‰
180 $\delta^{18}O$ and 1 ‰ $\delta^{2}H$ and were assessed by working standards measured frequently
181 along with the experimental samples. The precisions were lower than reported for
182 measurements of natural abundance, since highly labelled sample material was
183 analysed.
184 Elemental C, H and N content of solid samples was analysed in an elemental analyzer
185 (CHN-900, Leco Corp.) and the elemental O content by RO-478 (Leco Corp.).
186 The liquid samples from the cryogenic vacuum extraction (tissue water) were
187 pyrolysed in an elemental analyser (TC/EA, Thermo Finnigan) and the evolving CO
188 and H_2 gases were transferred via the ConFlo III interface (Thermo Finnigan) to a
189 IRMS (Delta plus XL, Thermo Finnigan) for oxygen and hydrogen isotope ratio
190 analysis (Gehre et al., 2004). The precision of the liquid sample measurement was ±
191 0.75 ‰ $\delta^{18}O$ and ± 1.59 ‰ $\delta^{2}H$.

2.5 Calculations

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

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

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

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

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

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

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

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

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

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

, where $\delta^{18}O_{stem,water}$ is the isotopic signature (in ‰) of the water extracted from the stem tissue (approximating the xylem water) and ε^{k} and ε^{*}_{atm} are the kinetic and equilibrium fractionation terms, respectively, at the specific sampling date.

The second source, thereafter called "condensation source", refers to the water vapour that diffuses from the atmosphere into the leaves and condensates at the cell walls. The contribution of this source would be maximal at 100 % relative humidity, which results in Eq. 4 when solving Eq. 1 with h = 1.

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

, where $\delta^{18}O_{atm,vap}$ is the isotopic signature of the water vapour of the chamber atmosphere and ε^{*}_{atm} is the equilibrium fractionation inside the chamber at the specific sampling date. The signature of the atmospheric water vapour was measured on its condensate ($\delta^{18}O_{atm,cond}$) collected in the peltier water trap, which was therefore corrected with the equilibrium fractionation during condensation inside the peltier-cooled water condenser (ε^{*}_{pelt}).

The kinetic fractionation due to the difference in molecular diffusivity of the water molecule species (ε^{k} = 20.7 ‰ $\delta^{18}O$ and 10.8 ‰ $\delta^{2}H$) was estimated according to Cappa et al. (2003) for a laminar boundary layer (Schmidt-number q = 2/3, Dongmann et al. 1974). The equilibrium fractionation due to the phase change during evaporation and condensation at different temperatures was calculated as in Majoube (1971) with the conditions present at the specific day. The condensation (dew point) temperature inside the peltier-cooled water condenser ($T_{pelt,DP}$) was determined based on the remaining humidity and the air pressure of the air leaving the condenser (details on the calculation are given in Appendix B). The equilibrium fractionation factors during the labelling experiment were on average ε^{*}_{atm} = 8.9 ± 0.2 ‰ for $\delta^{18}O$ and 72.7 ± 2.7 ‰ for $\delta^{2}H$ at T = 31.3 ± 2.7 °C inside the labelling chamber and ε^{*}_{pelt} = 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 water condenser.

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

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

, where $\delta^{13}C_{t=x}$ is the isotopic signature (in ‰) of the bulk tissue at sampling date x and R is the ratio of the heavier to the lighter isotope ($^{13}C/^{12}C$) of the international standard V-PDB. The atom fraction of ^{18}O and ^{2}H was calculated accordingly, but using $R_{V\text{-}SMOW}$ as reference and neglecting the ^{17}O isotope amount.

For the Van Krevelen approach we calculated the elemental ratios. The relative label distribution ($^{18}O/^{13}C$ and $^{2}H/^{13}C$) within the plant organic matter (OM) was calculated based on the excess atom fraction measured in each tissue (Eq. 6).

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

, 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 at a specific sampling date (t = x), relative to the unlabelled control (t = 0). Eq. 6 and 7 was analogously calculated for the $^{2}H/^{13}C$ ratio.

In a second step we corrected the isotopic ratios ($^{18}O/^{13}C$ and $^{2}H/^{13}C$) with the maximum label strength of the precursor of the organic matter, i.e. the maximum label strength of fresh assimilates (Eq. 7), which was assumed to be the excess atom fraction of ^{13}C in the leaf water-soluble organic matter (wsOM) and the excess atom fraction of ^{18}O and ^{2}H in the leaf water (relative to the unlabelled control).

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

3 Results

3.1 Labelling of the leaf water and water-soluble OM

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

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

312 (71 ± 4 % based on ^{18}O and 60 ± 2 % based on ^{2}H) than in the leaves at the top (66 ±
313 2 % and 55 ± 0 %, respectively) of the shoots.
314 The ^{13}C-CO_2 added (8938 ‰ $\delta^{13}C$) was assumingly also strongly diluted by respired
315 ^{12}C-CO_2, but we did not measure the isotopic signature of the CO_2 within the chamber
316 air. The leaf water-soluble OM was significantly enriched already after one day of
317 labelling and levelled off towards the end of the experiment. At the last two sampling
318 dates its isotopic signature was on average 1346 ± 162 ‰ $\delta^{13}C$.

319 ### 3.2 Labelling of the bulk organic matter

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

3.3 Atomic and isotopic ratios to characterize organic matter

The atomic ratios of the plant bulk OM were in the range of 13.7 - 115.4 C/N, 0.70 - 0.83 O/C and 1.56 - 1.72 H/C (Tab. 3). The leaf OM was characterized by the lowest C/N and O/C ratios and concurrently by highest H/C ratios (Fig. 3a). The other plant tissues indicated a linear trend in decreasing O/C and H/C and increasing C/N ratios in the order of stems, petioles, roots and cuttings.

The recovery of the three isotopes varied between the leaf, stem and root tissue, while they were similar between the sampling dates (Fig. 3b). The isotopic ratios of the 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 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 $1.0 \pm 0.2 \times 10^{-3}$ $^{18}O/^{13}C$ and $1.0 \pm 1.4 \times 10^{-4}$ $^{2}H/^{13}C$ in the roots after the equilibrium in the leaf water and water-soluble OM labelling was reached. Thus the $^{18}O/^{13}C$ ratios were on average 2.6 (± 0.2) times lower in the stems and 3.8 (± 0.7) times lower in the roots than in the leaves (Tab. 3) and the $^{2}H/^{13}C$ ratios 1.9 (± 0.2) and 3.1 (± 0.6) times lower in the stems and roots, respectively, than in the leaves.

After correction for the maximum label strength (^{18}O, ^{2}H and ^{13}C excess atom fraction within the leaf water and the water-soluble OM, respectively), the isotopic ratios were in the range of 0.17 - 0.43 $^{18}O/^{13}C$ and 0.14 - 0.23 $^{2}H/^{13}C$. The normalized isotopic ratios were thus in the magnitude order of the atomic ratios reported for OM compounds (Tab. 3, Fig. 3c), however lower than expected for fresh organic matter (in the range characteristic for condensed hydrocarbons).

4 Discussion

4.1 Diffusion of atmospheric water vapour into the leaf

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

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

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

Nonetheless, the strong depletion of the leaf water in ^{2}H and ^{18}O proofs, that back-diffusion of atmospheric water vapour into the leaf is an important mechanisms for leaf water uptake. This supports the hypothesis that atmospheric water vapour diffusion might be as important as the flux of water from the xylem into the leaf (at least under humid conditions) and be an important mechanisms for the reversed water flow observed in the tropics (Goldsmith, 2013). 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.

4.2 Tracing organic matter?

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

and H/C ratios observed in the other tissues is in the direction of condensation reactions. This trend most likely indicates the increasing lignification of OM from shoots, to roots, to cuttings.

The same trend has been observed in the ratios of the labels added from the leaf, to the stem, to the root OM (Fig. 3b,c). The lower isotopic O/C and H/C ratios in the root and stem tissue compared to the leaf tissue could indicate the utilization of the labelled assimilates for the synthesis of more condensed compounds (e.g. lignin) in those tissues. However, other factors affecting the isotopic ratios of the OM are the maximum label strength, the exchange of hydrogen and oxygen with xylem water during transport and biosynthesis and the isotopic fractionation during metabolism.

The isotopic ratios (Fig. 3b) were around three magnitudes smaller than the expected atomic ratios of OM (Sleighter and Hatcher, 2007). This is mainly due to the different maximum label strength, which was highest for the ^{13}C and lowest for the ^{2}H. After correction for this factor, the isotopic ratios were in the range of the atomic ratios characteristic for condensed hydrocarbons (Fig. 3c). The isotopic ratios might be lower than expected due to inaccurate approximation of the maximum label strength of fresh assimilates (by the leaf water and water-soluble OM), or be the result of ^{18}O and ^{2}H label losses during transport and biosynthesis.

One reason for the label loss might be the use of other (more enriched) sources during biosynthesis. For example O_2 (enriched by 23 ‰ $\delta^{18}O$) has been identified as a further source for aromatic compounds, such as phenols and sterols (Schmidt et al., 2001). However, for hydrogen, water is the only known source (Schmidt et al., 2003) and therefore the use of other O or H sources during biosynthesis can not explain the (major) loss of the ^{18}O and ^{2}H label.

Another potential reason would be the kinetic fractionation during biosynthesis that leads to distinct isotopic signatures of different OM compounds (described in Schmidt et al. 2001, 2003; Badeck et al. 2005; Bowling et al. 2008). However, assuming constant isotopic fractionation during the experimental period (constant environmental conditions), the isotopic ratios would not be affected, since they are based on the excess atom fraction relative to the unlabelled OM.

A third reason for the loss of the ^{18}O and ^{2}H label could be the exchange of hydrogen and oxygen atoms with water. O and H exchanges with tissue water during transport and the synthesis of new compounds (as recently discussed for oxygen in phloem sugars and cellulose in Offermann et al. 2011 and Gessler et al. 2013). O of carbonyl

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

490 5 Conclusions

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

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

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675 **Tables**

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

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

686

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

693

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

698

Figures

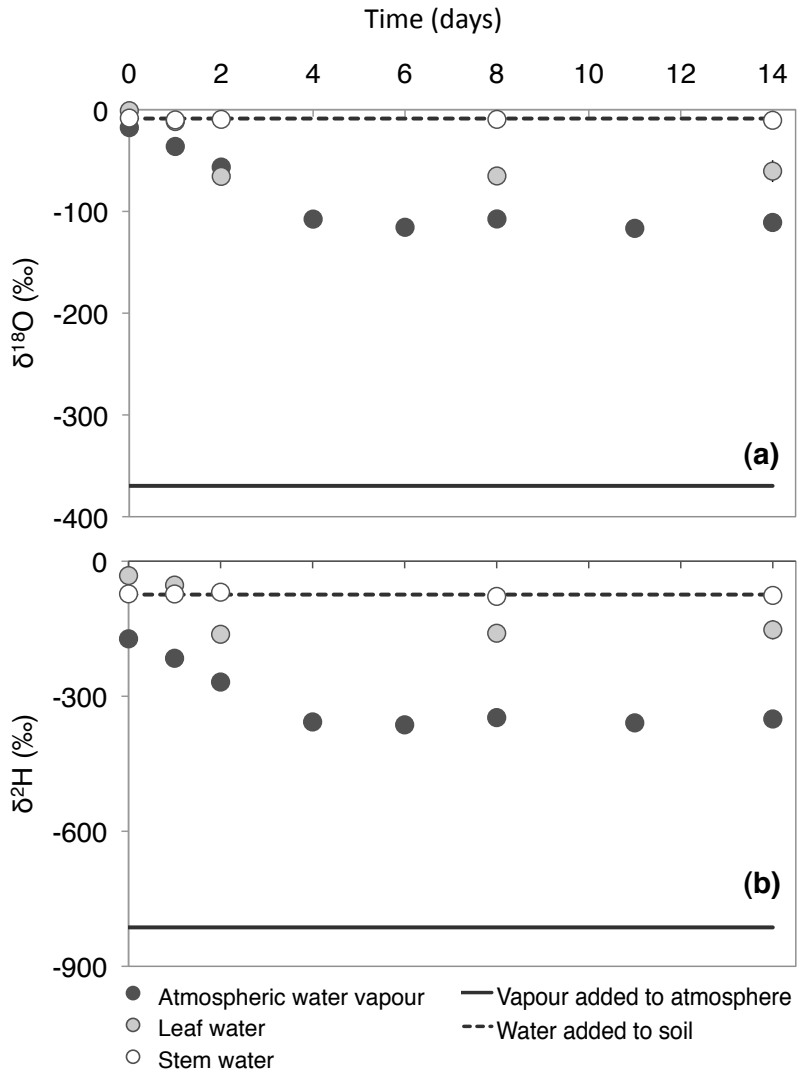
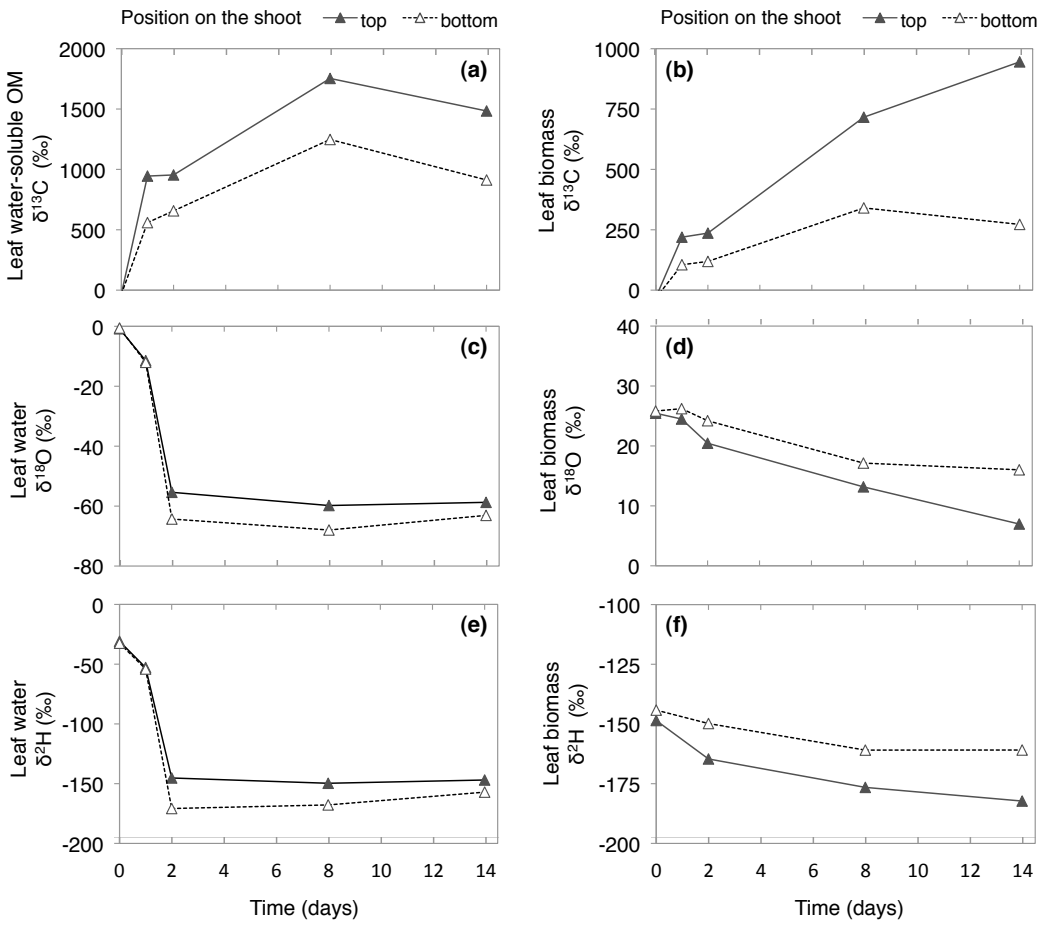
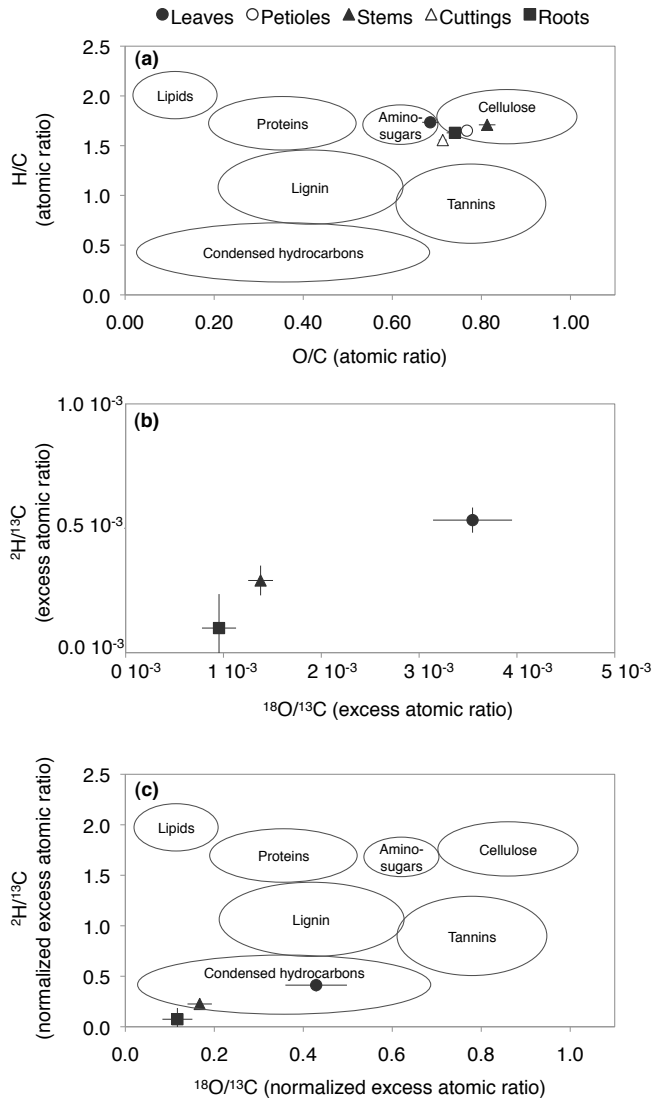


Figure 1. Temporal dynamics in the water isotopic signatures of the plant-soil-atmosphere system during continuous $^{2}H_{2}{}^{18}O$ labelling (a) $\delta^{18}O$ and (b) $\delta^{2}H$ signature (in ‰) of the depleted water label added as water vapour to the atmosphere (solid line), of the water added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



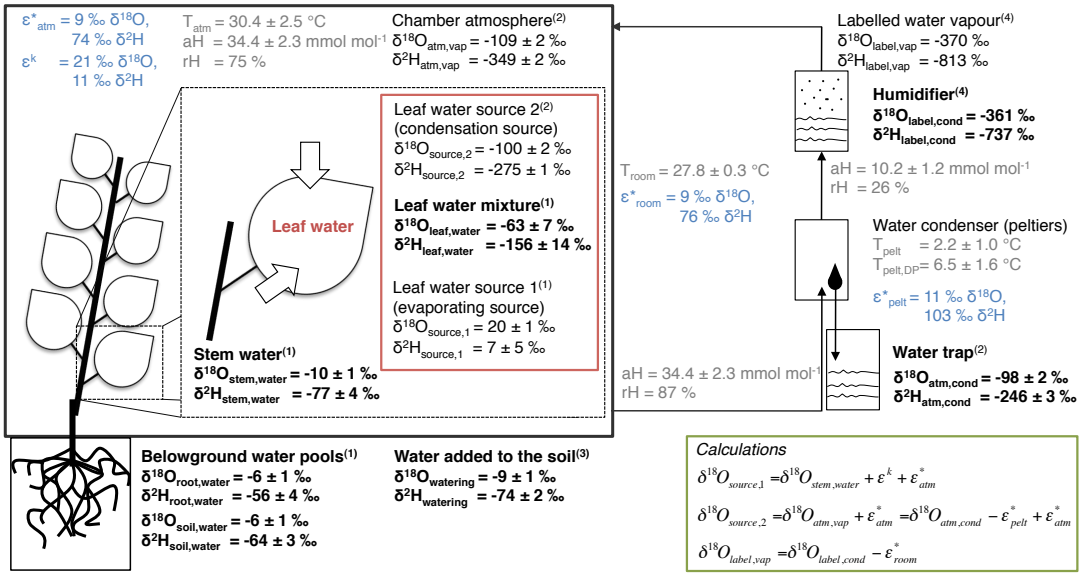
707
708 **Figure 2.** Incorporation of the gaseous labels ($^{13}CO_2$, $^{2}H_2{}^{18}O$) into the leaf water
709 water-soluble and bulk organic matter. (a,b) $\delta^{13}C$, (c,d) $\delta^{18}O$ and (e,f) $\delta^{2}H$ signature
710 (in ‰) within leaves sampled at the top (solid line, black triangles), or at the bottom
711 (dashed line, white triangles) of the shoot. Illustrated are the signatures of (a) the leaf
712 water-soluble organic matter, (b,e,f) the leaf biomass and (c,e) the leaf water



713

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

Appendix A



(1) Sampled after 3/12 hours daylight; errors represent variability between plant individuals (three plant replicates each sampling date).
(2) Integrated value over 2-3 days (water trap analysed at day 6, 8, 11 and 14), errors represent variability between sampling date 8 and 14.
(3) Average of all watering dates (day 0, 2, 6, 8, 11); errors represent variability between sampling dates.
(4) Measured at the beginning of the experiment

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

Appendix B

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

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

747 $$rH(T) = \frac{e}{e(T)} \cdot 100 \quad \text{(B1)}$$

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

751 $$e = \frac{aH}{1000} \cdot p \quad \text{(B2)}$$

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

754 $$e(T) = 0.61365 \cdot e^{\frac{17.502 \cdot T}{240.97 + T}} \quad \text{(B3)}$$

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

756 References

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