RESEARCH PAPER

Soil H_2^{18}O labelling reveals the effect of drought on C^{18}OO fluxes to the atmosphere

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Abstract

Above- and belowground processes in plants are tightly coupled via carbon and water fluxes through the soil–plant–atmosphere system. The oxygen isotopic composition of atmospheric CO_2 and water vapour (H_2O) provides a valuable tool for investigating the transport and cycling of carbon and water within this system. However, detailed studies on the coupling between ecosystem components and environmental drivers are sparse. Therefore, we conducted a H_2^{18}O-labelling experiment to investigate the effect of drought on the speed of the link between below- and aboveground processes and its subsequent effect on C^{18}OO released by leaves and soils. A custom-made chamber system, separating shoot from soil compartments, allowed separate measurements of shoot- and soil-related processes under controlled conditions. Gas exchange of oxygen stable isotopes in CO_2 and H_2O, served as the main tool of investigation and was monitored in real time on Fagus sylvatica saplings using laser spectroscopy. H_2^{18}O-labelling showed that drought caused a slower transport of water molecules from soil to shoot, which was indicated by its direct derivation from independently measured concentrations and 18O/16O ratios of CO_2 and H_2O, respectively. Furthermore, drought reduced the 18O equilibrium between H_2O and CO_2 at the shoot level, resulting in less-enriched C^{18}OO fluxes from leaf to atmosphere compared with control plants. Compared with the shoot, 18O equilibrium was not instantaneous in the soil and no drought effect was apparent.

Key words: Above/belowground, C^{18}OO, coupling, drought, Fagus sylvatica, H_2^{18}O, laser spectroscopy.

Abbreviations: 18A_{leaf}, measured discrimination against C^{18}OO; 18A_{mod-simple}, modelled discrimination against C^{18}OO without varying 18; 18A_{mod-extended}, modelled discrimination against C^{18}OO including varying δ; δ_18O of CO_2 in atmosphere; δ_18O of water at the soil evaporative front; δ_{OUT-L}, δ_18O of water at the leaf evaporative front; δ_{IN-C}, δ_18O of CO_2 flux from soil to atmosphere; δ_{mod-extended}, modelled δ_18O of CO_2 flux from soil to atmosphere; δ_{mod-simple}, measured δ_18O of CO_2 flux from soil to atmosphere; δ_{BL闪耀}, δ_18O of CO_2; δ_{SR-mod}, equilibrium fractionation between liquid water and water vapour at the air–water interfaces, for leaves and soil respectively; ε_18, kinetic fractionation during H_2O diffusion from the leaf intercellular airspaces to the atmosphere; ε_{leaf}, kinetic fractionation during H_2O diffusion from the soil airspaces to the atmosphere; ε_{diff}, equilibrium fractionation between H_2O and CO_2; δ_18O of CO_2 entering the chamber compared with the photosynthetic flux; δ_{18O, net}, net photosynthesis; δ_{diff}, diffusive 18O fractionation from atmosphere to leaf evaporative front; δ_{18O, eff}, diffusive 18O fractionation from soil evaporative front to atmosphere; CA, carbonic anhydrase; C_{atm}, atmospheric CO_2 concentration; C_{CO_2}, CO_2 concentration at the site of carboxylation; C_{CO_2}, CO mole fraction at chamber inlet; C_{OUT}, CO mole fraction at chamber outlet, equal to C_{atm}; E, leaf transpiration; E_{s}, soil evaporation; g_{stom}, stomatal conductivity to H_2O; g_{v}, water vapour pressure in the atmosphere; g_{ws}, water vapour pressure in the leaf, assuming water vapour saturation in the leaf; g_{ws, water vapour pressure in the soil, assuming water vapour saturation in the soil; H_2^{18}O, water enriched in 18O label; H_2O, water vapour; r, reflux factor, i.e. factor by which leaf CO_2 refluxex exceeds photosynthetic flux; R, soil respiration; S, soil water content; Ta, leaf temperature; T_e, soil temperature; VPD, vapour pressure deficit; WIA, water vapour isotope analyser; X_{OUT}, water vapour mole fraction at chamber outlet, equivalent to atmospheric H_2O; W_{comp}, water vapour mole fraction at chamber inlet.

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Introduction

The biogeochemical cycling of carbon and water vapour between the terrestrial biosphere and the atmosphere makes terrestrial ecosystems a major player in Earth’s climate system. The predicted higher probability/frequency of climate extremes (Schär et al., 2004), such as droughts, has the potential to alter biogeochemical cycling in terrestrial ecosystems and hence to generate feedback within the climate system. In particular, soil drought is a major factor determining carbon and water fluxes through the soil–plant–atmosphere system (Granier et al., 2007). Among other factors, soil drought controls stomata regulation (e.g. Gollan et al., 1986), which in turn influences photosynthesis and transpiration. Soil drought also reduces the speed of link of carbohydrate allocation from above- to belowground ecosystem compartments, probably being limited by stomata-mediated carbon uptake and transfer (Rührt et al., 2009; Barthel et al., 2011a). Since soil drought affects the carbon-related speed of link from above- to belowground (phloem), soil drought might also affect the plant–soil coupling in the opposite direction, i.e. affecting the water-related speed of the link from below- to aboveground (xylem).

Analogous to $^{13}$C (Kayler et al., 2010), $^{18}$O in water can serve as a tool to investigate plant physiology and soil–plant coupling, i.e. the speed of the link from below- to aboveground. Moreover, the $^{18}$O-composition of soil or leaf water should immediately be passed on to CO$_2$, as dissolved CO$_2$ exchanges its oxygen isotopic signature with water. In leaves, the equilibrium reaction between CO$_2$ and H$_2$O is catalysed by the enzyme carbonic anhydrase (CA) (Silverman 1982; Gillon and Yakir 2000a, 2001), whereas in soils such CA activity is still under debate (Tans, 1998; Seibt et al., 2006; Wingate et al., 2008). As water concentrations are usually much higher than those of CO$_2$, water will always impose its oxygen isotopic ratio upon the dissolved CO$_2$, irrespective of the initial isotopic signature of CO$_2$ (Miller et al., 1999). Consequently, CO$_2$ molecules that diffuse from leaves or soils should carry the oxygen isotopic signature of leaf or soil water, respectively. Thus, any change in the soil water oxygen isotopic composition will propagate within the soil–plant–atmosphere system, and eventually affect the oxygen stable isotope composition of atmospheric CO$_2$ ($\delta_A$). $\delta_A$ in turn is one of the few tools to separate the major terrestrial ecosystem CO$_2$ gross fluxes, photosynthesis and respiration, into their net components at global (Ciais et al., 1997) or local (Kato et al., 2004; Sturm et al., 2012) scales. Similarly, Bowling et al. (2003) utilized the oxygen stable isotope difference between soil and leaves to quantify the relative contribution of soil and foliar respiration to total nocturnal ecosystem respiration. However, using $\delta_A$ in order to separate assimilatory from respiratory fluxes at subdaily time scales requires a detailed understanding of how rapid changes of soil or leaf water $^{18}$O (for instance due to rain events or strong evaporative enrichment) translate to C$^{18}$O fluxes from individual ecosystem components—especially under different environmental conditions such as drought. Yakir (2003) stated that the $\delta^{18}$O value of precipitation is the single most important environmental control on $\delta_A$, as it translates to soil water and feeds plants. He further pointed out that, at the global scale, it is the leaf and soil components that dominate the uncertainty of the global $^{18}$O mass balance. Although there are a considerable number of studies on C$^{18}$O fluxes in terrestrial ecosystems where individual components were investigated, such as the soil atmosphere C$^{18}$O flux (Miller et al., 1999; Wingate et al., 2008) or the canopy-to-atmosphere flux (Griffis et al., 2011), research on the link between components has so far been neglected.

Thus, we conducted a H$_2^{18}$O-labelling experiment to investigate the effect of drought on the speed of the link between below- and aboveground processes and its subsequent effect on C$^{18}$O released by leaves and soils. We hypothesized that $^{18}$O-labelling (watering) would result in a continuous enrichment of soil and leaf waters, which in turn would change the $^{18}$O composition of CO$_2$ released to the atmosphere (Fig. 1). We expected that H$_2^{18}$O label-induced enrichment in the shoot compartment would be time lagged, given the transport times within the plant. As stomatal conductance is generally downregulated during drought, we further hypothesized that water molecule transport from soil to shoot would be reduced under drought conditions, hence also delaying the enrichment of $^{18}$O in CO$_2$. In order to trace the $^{18}$O flux on an hourly timescale, gas exchange in the soil and shoot was measured in real time using online laser spectroscopy. To our knowledge, this is the first study reporting on measuring, concurrently and continuously, the $^{18}$O/$^{16}$O ratio in CO$_2$ and water vapour (H$_2$O) of shoot and soil gas exchange after irrigation of the soil surface with $^{18}$O-labelled water.

Materials and methods

Experimental design and set-up

The experiment was carried out in a growth cabinet using small beech saplings (Fagus sylvatica L., height approx. 1 m, n=6). The...
Effect of drought on $^{18}$O fluxes after $^{18}$O labelling

Water vapour isotope analyser
A commercially available water vapour isotope analyser (WVIA; DLT-100, Los Gatos Research, Mountain View, CA, USA), based on off-axis integrated cavity output spectroscopy, was used for the simultaneous measurement of the three water isotopologues $^{18}H_2^{16}O$, $^{18}H_2^{18}O$, and $^2H^18O$. The laser scanned over three nearby absorption lines at a wavelength of $\approx 1.389$ μm. The WVIA was calibrated using a self-made calibration system involving a piezoelectric droplet generator. The 1σ standard deviation of repeated quality-control standard measurements was ±0.23‰ for $\delta^{18}O$ and ±92 ppm for $H_2^{18}O$ concentrations. For more information on the WVIA, see Sturm and Knohl (2010).

As $H_2^{18}O$ concentration measurements are prone to condensation events, WVIA measurements at the shoot chamber outlets were verified by modelling $H_2^{18}O$ concentrations within the shoot chamber using sensors for air temperature and relative humidity (Buck, 1981). In general, very good agreement between both approaches was observed (Fig. 2). However, at very high $H_2^{18}O$ concentrations (>22 000 ppm), the linear relationship was lost, which may point to condensation events during these measurements. The effect of condensation on the $^{18}O$ measurements would have underestimated the enrichment by a maximal 2‰, which is minor considering the strong label intensity (see Supplementary information at JXB online for derived error estimation). Note that this error affected mainly control measurements, since transpiration and thus relative humidity inside the shoot chambers were higher. As air temperature and relative humidity were only measured in the shoot chambers, such independent verification could not be done for soil chamber $H_2^{18}O$ measurements.

**Watering with labelled water ($H_2^{18}O$)**
Prior to $H_2^{18}O$ application, soils of three replicates were gradually dried during approximately 20 d before the start of the experiment. Once plants achieved the desired stress level, about 30 ml of water was added daily to maintain the stress and prevent mortality. At the label day, all soils were simultaneously irrigated with 400 ml of $^{18}$O-labelled water ($\delta^{18}O=449\pm7$‰) at 11 a.m. to induce a sudden change in $\delta^{18}O$ value of soil water. For comparison, the $\delta^{18}O$ of

![Fig. 2](https://academic.oup.com/jxb/article-abstract/65/20/5783/2485043) Relationship between water vapour concentrations modelled from air temperature and the relative humidity sensor inside the shoot chamber and directly measured water vapour concentrations. Control treatment is shown by open symbols, drought treatment by closed symbols, and $x=y$ by a dashed line.

**Instrumentation**
Oxygen stable isotope ratios are reported relative to the Vienna Standard Mean Ocean Water scale (V-SMOW) using the δ notation (%):

$$\delta^{18}O = \frac{R_{\text{sample}}}{R_{\text{V-SMOW}}} - 1$$

where $R_{\text{sample}}$ and $R_{\text{V-SMOW}}$ denote the $^{18}O/^{16}O$ ratio of the sample and the standard, respectively.

$CO_2$ isotope analyser
A commercially available pulsed quantum cascade laser absorption spectrometer (Aerodyne Research, Billerica, MA, USA) was used to simultaneously measure the $CO_2$ isotopologues $^{13}C^{16}O_2$, $^{13}C^{18}O_2$, and $^{12}C^{16}O^{18}O$ at a rate of 0.5 Hz by scanning across three spectral lines near 4.3 μm (2310 cm$^{-1}$). The measurement was based on two optical multiple pass absorption cells with stabilized pressure and temperature using a spectral ratio method (Nelson et al., 2008). Furthermore, the laser system was equipped with an infrared detector cooled with liquid nitrogen. System operation was fully automated using an automated liquid nitrogen refilling device (liquid N$_2$ Microdosing system #905; Norhof, Maarssen, The Netherlands) and a self-made calibration unit. Throughout the measurement sequence, calibration was done approximately once h$^{-1}$ for 6 min in three consecutive steps. First, a dilution calibration was performed to correct for the instrument’s non-linear $CO_2$ concentration dependence of isotope ratio measurements. Secondly, two calibration gases with known mixing ratios were measured for a two-point calibration. Thirdly, a quality-control standard gas was measured to check the long-term stability of the calibrated instrument. The 1σ standard deviation of repeated quality-control standard measurements was ±0.23‰ for $\delta^{18}O$ and ±0.09 ppm for $CO_2$ concentrations. A detailed description of the quantum cascade laser absorption spectrometer calibration strategy and system operation can be found in Sturm et al. (2012).

**Absorption spectrometer calibration strategy and system operation**
Gas-exchange parameters such as photosynthesis and transpiration were calculated according to von Caemmerer and Farquhar (1981).

**Fig. 2** Relationship between water vapour concentrations modelled from air temperature and the relative humidity sensor inside the shoot chamber and directly measured water vapour concentrations. Control treatment is shown by open symbols, drought treatment by closed symbols, and $x=y$ by a dashed line.
local tap water used for regular daily irrigation was \(-11.05 \pm 0.24\%\). Subsequent to the \(^{18}\)O watering, the change in \(^{18}\)O/\(^{16}\)O of CO\(_2\) and H\(_2\)O fluxes was monitored in shoot and soil compartments of each replicate using the isotope gas-exchange data from both the WVIA and the CO\(_2\) isotope analyser.

### Data processing

Since replicates were measured successively, the data were half-hourly linearly gap filled in order to achieve consistent time intervals for averaging. By averaging the gap-filled data, a single timeline could be obtained. Because of disturbance effects during label application, isotope data for this time period (1 h) was removed from further analysis. H\(_2\)O measurements were filtered according to plausibility (\(W_{\text{OUT}} - W_{\text{IN}} > 0\) must be true, where \(W_{\text{OUT}}\) is water vapour mole fraction at the chamber outlet, equivalent to atmospheric H\(_2\)O, and \(W_{\text{IN}}\) is the water vapour mole fraction at the chamber inlet). Moreover, isotope data were filtered for daytime values only, as night-time measurements were prone to condensation in the tubing because of lower temperatures in the growth cabinets. All data were analysed and processed using the statistical software R 2.15.1 (R Development Core Team, 2011). Results are always shown as the mean ± standard error (SE) (n = 3) per treatment.

### Isotope modelling

In order to assess the \(^{18}\)O equilibrium at leaf and soil levels, directly measured \(^{18}\)O in CO\(_2\) was compared with the theoretically expected values, which can be modelled from \(^{16}\)O measurements in H\(_2\)O.

**Leaf component** The isotopic composition of leaf water \(^{18}\)O at the evaporative front (\(\delta_V\)) is generally enriched compared with the source water (Gonfiantini et al., 1965), depending on the gradient between atmospheric water vapour pressure (\(e_a\)) and the water vapour pressure within the leaf (\(e_c\)). Assuming non-steady-state conditions, leaf water enrichment at the evaporative front can be modelled as:

\[
\delta_V = \delta_T + \epsilon^* + \epsilon_K + (\delta_T - \epsilon_T - \epsilon_K) \frac{e_w}{e_a} \tag{2}
\]

The equation is based on a model of evaporative enrichment by Craig and Gordon (1965), originally developed for free water surfaces, where \(\delta_T\) and \(\delta_V\) are the isotopic composition of the transpiration flux and of atmospheric water vapour, respectively. Replacing source water \(\delta^{18}\)O with \(\delta_T\) allows the assessment of \(\delta_V\) under non-steady-state conditions (Harwood et al., 1998; Gillon and Yakir, 2000b). This assumption is essential for our set-up as the isotopic composition of source water is constantly changing after labelling within the leaf (\(e_c\)). Assuming non-steady-state conditions, leaf water enrichment at the evaporative front usually also accounts for the Peclét effect. The Peclét effect describes the convection of unenriched leaf vein water towards the evaporative sites, hence counteracting evaporative enrichment (Farquhar and Lloyd, 1993; Cernusak and Kahmen, 2013). However, since a strong \(^{18}\)O label was used for watering, we assumed the Peclét effect to be negligible in modelling \(\delta_V\). The parameter \(\epsilon_K\) in Eqn (2) denotes the kinetic fractionation during water vapour diffusion from the leaf intercellular airspaces to the atmosphere, and is obtained from the relative contributions of leaf stomatal (\(g_s\)) and leaf boundary layer conductance to H\(_2\)O (\(g_o\)), set constant to 1.42 mol m\(^{-2}\) s\(^{-1}\); Luz et al., 2009):

\[
\epsilon_K = \frac{28 g_s^{-1} + 22 g_o^{-1}}{g_s^{-1} + g_o^{-1}}. \tag{3}
\]

The equilibrium fractionation \(\epsilon^*\) between liquid water and water vapour at the air–water interfaces is expressed as a function of leaf temperature (\(T_L\), in Kelvin) according to Horita and Wesolowski (1994):

\[
\epsilon^* = -7.685 + 6.712\left(\frac{10^1}{T_L}\right) - 1.6664\left(\frac{10^6}{T_L^2}\right) + 0.35041\left(\frac{10^9}{T_L^3}\right). \tag{4}
\]

The \(\delta^{18}\)O signal of transpiration fluxes (\(\delta_T\)) was calculated using an isotopic mass balance equation:

\[
\delta_T = \frac{\delta_{\text{OUT-W}} W_{\text{OUT}} - \delta_{\text{IN-W}} W_{\text{IN}}}{W_{\text{OUT}} - W_{\text{IN}}}. \tag{5}
\]

where \(W_{\text{IN}}\) and \(W_{\text{OUT}}\) denote the respective mole fractions at chamber inlet and outlet. Similarly, \(\delta_{\text{IN-W}}\) and \(\delta_{\text{OUT-W}}\) denote the respective \(^{18}\)O isotopic compositions of H\(_2\)O at chamber inlet and outlet.

According to theory, leaf or soil water \(^{18}\)O composition is passed on to CO\(_2\) due to the following isotope equilibrium reaction of CO\(_2\) with H\(_2\)O:

\[
H_2^{18}O(l) + CO_{2(g)} \leftrightarrow H^+ + \left[\text{HCO}_3^{18}O\right]^{-} \leftrightarrow H_2O_{(g)} + CO_{18}O_{(g)}. \tag{6}
\]

In leaves, the reaction is catalysed by the enzyme CA, which facilitates CO\(_2\) hydration and \(^{18}\)O exchange. Assuming full CA-catalysed isotopic equilibrium of CO\(_2\) with leaf water, the water oxygen isotopic composition at the evaporative front (\(\delta_L\)) should correspond directly to that of dissolved CO\(_2\) when accounting for the equilibrium fractionation between H\(_2\)O and CO\(_2\). This oxygen isotopic equilibrium is expressed by:

\[
\delta_L = \delta_E + \epsilon_{W}, \tag{7}
\]

with \(\epsilon_{W} = \frac{17604}{T_T} - 17.93, \tag{8}
\]

where \(\delta_L\) is the oxygen stable isotope composition of CO\(_2\) at the evaporative front and \(\epsilon_{W}\) is the equilibrium fractionation between H\(_2\)O and CO\(_2\) (Brenninkmeijer et al., 1983). Based on this assumption, the theoretical discrimination (\(^{18}\)A\(_{\text{mod-simple}}\)) can be modelled by accounting for the weighed mean of diffusive \(^{18}\)O fractionations occurring during CO\(_2\) diffusion out of the leaf (\(a = 7\%\)) and the reflux factor following Farquhar et al. (1993):

\[
^{18}\Delta_{\text{mod-simple}} = \alpha + r(\delta_L - \delta_C) \tag{9}
\]

Farquhar et al. (1993) described the reflux factor \(r\) mathematically as \(r = C_c/(C_c - C_h)\), where \(C_c\) and \(C_h\) denote the CO\(_2\) concentration in the atmosphere and at the site of carboxylation, respectively. This equation illustrates that an estimation of \(r\) is challenging, as it requires a very good estimate of stomata as well as mesophyll conductance to CO\(_2\), since they determine the magnitude of \(C_c\) and hence \(r\). However, a precise estimate of \(C_h\) was not possible with this experimental set-up. Therefore, a sensitivity analysis was done across a range of different \(r\) values. The \(\alpha\) sensitivity analysis was based on the general assumption that only one-third of the CO\(_2\) that diffuses into the leaf is consumed by photosynthesis (Ciais et al., 1997; Tans, 1998; Yakir and Sternberg, 2000; Yakir, 2003). Therefore, the range of \(r\) was chosen to be between 1.9 and 2.1. In addition, a second sensitivity analysis was conducted where the original model from Farquhar et al. (1993) was extended to account for \(\theta\), the extent of \(^{18}\)O equilibrium between H\(_2\)O and CO\(_2\), which can range between 0 and 1, implying a 0–100% isotopic equilibrium, respectively (Yakir, 2003):

\[
^{18}\Delta_{\text{mod-extended}} = \alpha + r[\Theta_{eq}(\delta_L - \delta_C) - (1 - \Theta_{eq})\alpha/(r + 1)]. \tag{10}
\]

Finally, \(^{18}\Delta\) can be also obtained from direct measurements of \(^{18}\)O in CO\(_2\) following Evans et al. (1986):
\[
\begin{align*}
\Delta_{\text{mea}} &= \frac{\xi (\delta_{\text{OUT-C}} - \delta_{\text{IN-C}})}{1000 + \delta_{\text{OUT-C}} - \xi (\delta_{\text{OUT-C}} - \delta_{\text{IN-C}})}, \\
\text{with } \xi &= \frac{C_{\text{OUT}}}{C_{\text{IN}} - C_{\text{OUT}}},
\end{align*}
\]

where \(\xi\) is the ratio of CO\(_2\) entering the chamber compared with the photosynthetic flux with \(C_{\text{IN}}\) and \(C_{\text{OUT}}\) denoting the respective CO\(_2\) mole fractions at chamber inlet and outlet and \(\delta_{\text{IN-C}}\) and \(\delta_{\text{OUT-C}}\) the corresponding \(^{18}\)O isotopic compositions of CO\(_2\) at chamber inlet and outlet.

**Soil component** The isotopic composition of soil water at the evaporative surface (\(\delta_{\text{PS}}\)) was calculated in accordance with Eqn (2). To calculate the soil equilibrium fractionation factor (\(e_{\text{s-soil}}\)) and the saturation water vapour pressure in the soil (\(e_s\)), leaf temperature was substituted by soil temperature. Soil kinetic fractionation, \(e_{\text{k-soil}}\) was set constant at 28.5\% after Merlivat (1978). The \(\delta\) value of soil respiration (\(\delta_{\text{SR-mod}}\)) was modelled assuming a 100\% equilibration of \(^{18}\)O between soil H\(_2\)O and CO\(_2\) using \(\delta_{\text{ES}}\) and a soil kinetic fractionation (\(\delta_{\text{SR}}\)) of 8.8\% according to Miller et al. (1999):

\[
\delta_{\text{SR-mod}} = \delta_{\text{ES}} + e_{\text{ws}} - \bar{e}_s
\]

with \(e_{\text{ws}}\) calculated according to Eqn (7) using soil temperature. Finally, soil evaporation (\(\delta_{\text{EV}}\)) and soil respiration fluxes (\(\delta_{\text{SR}}\)) were calculated according to Eqn (5) using data from H\(_2\)O and CO\(_2\), respectively.

**Results**

**Experimental pre-requisites and conditions before water addition**

The analysis presented here is based upon the assumption that \(\delta_T\) reflects the \(\delta\) value of source water, which should hence be close to the \(\delta\) value used for daily irrigation. Figure 3 shows the diurnal cycle of \(\delta_T\) for control and drought treatment during the pre-label day. During the period of highest light intensity (10 a.m. to 6 p.m.), \(\delta_T\) was slightly more enriched than tap water (11.05 ± 0.24\%) with marginally more enrichment in drought treatments during the afternoon. This relatively small deviation of measured \(\delta_T\) from tap water values showed that \(\delta_T\) approximately reflected source water values. The slightly more enriched \(\delta_T\) value under drought conditions was probably caused by a stronger evaporative enrichment of soil water (source water).

Programmed diel cycles for the growth cabinets resulted in comparable diel cycles for soil and air temperatures across treatments (Fig. 4K, L). Before adding H\(_2\)\(^{18}\)O to the soil, soil water content (SWC) of drought treatments was approximately 25\% lower than that of control treatments. Withholding water reduced soil respiration (\(R_s\)) to 48\%, photosynthesis (\(A_N\)) to 30\%, evaporation (\(E_s\)) to 83\%, and transpiration (\(E\)) to 37\% compared with control values during the period of highest light intensity (Fig. 4A–D). Vapour pressure deficit (VPD) remained constantly higher in drought treatment, even after watering, but the diel cycle became less resolved (Fig. 4J).

**Effect of drought on the speed of link between below- and aboveground processes**

H\(_2\)\(^{18}\)O labelling resulted in an immediate increase in SWC (Fig. 4I). Concurrently, a distinct enrichment in \(\delta^{18}\)O values of soil evaporation (\(\delta_{\text{EV}}\); Fig. 4E) and soil respiration (\(\delta_{\text{SR}}\); Fig. 4G) was observed across treatments. The \(\delta_{\text{EV}}\) of drought treatments increased rapidly to 412\%, which closely mirrors the \(\delta\) value of the water used for labelling (\(\delta^{18}\)O=449±7\%). In contrast, the maximum \(\delta_{\text{EV}}\) of control treatments reached only ~304\%, probably due to dilution effects caused by the higher SWC. Over the course of the experiment, \(\delta_{\text{EV}}\) decreased to about 122\% (control) and 134\% (drought), probably due to mixing effects with non-labelled soil water. Furthermore, soil respiration increased above pre-labeling levels in both treatments (Fig. 4A).

In the shoot, a coincident increase in \(A_N\) and \(g_s\) was apparent in drought treatments instantly after labelling, whereas the control showed no consistent response (Fig. 5A, B). However, this initial watering response of \(A_N\) and \(g_s\) levelled out after 1.5 h and accounted only for a 12\% (\(A_N\)) and 16\% (\(g_s\)) increase compared with the control. To quantify the speed of the link between above- and belowground, \(\Delta_{\text{mea}}\) and \(\Delta_T\) were used as independent proxies, taking advantage of their direct derivation from independently measured concentrations and \(^{18}\)O/\(^{16}\)O ratios of CO\(_2\) and H\(_2\)O, respectively (Eqns 5 and 10). Both treatments displayed a delayed but exponential label-induced \(^{18}\)O enrichment in the transpirational flux (\(\delta_T\)), with a faster increase in control compared with drought treatments (Fig. 5C). Likewise, shoot discrimination against C\(_{18}\)OO (\(\Delta_{\text{mea}}\)) showed a faster exponential increase in control compared with drought after labelling (Fig. 5D).

**Effect of H\(_2\)\(^{18}\)O labelling on C\(_{18}\)OO released by soils and leaves**

Figure 6A shows that the modelled \(\delta\) value of soil respiration (\(\delta_{\text{SR-mod}}\)) was mostly overestimating measured \(\delta_{\text{SR}}\), especially during the pre-label and label day. During the pre-label day, \(\delta_{\text{SR}}\) was only 61±2\% of \(\delta_{\text{SR-mod}}\) in both treatments. During the label day, exchange constantly increased, which resulted
in a better agreement between $\delta_{\text{SR-mod}}$ and $\delta_{\text{SR}}$ from post-label d 1–3. However, also at post-label d 1–3, substantial variations around the 1:1 line were still observed, either under- or overestimating $\delta_{\text{SR}}$ by about 50‰ (Fig. 6B).

In the shoot, modelled discrimination against C\textsuperscript{18}OO, based on leaf water enrichment ($18\Delta_{\text{mod-simple}}$, $18\Delta_{\text{mod-extended}}$) predicted very well the measured discrimination against C\textsuperscript{18}OO ($18\Delta_{\text{mea}}$). A strong linear relationship between $18\Delta_{\text{mod}}$...
and $^{18}\Delta_{\text{mea}}$ was found for both treatments during the label day (Fig. 7). The slopes between $^{18}\Delta_{\text{mod}}$ and $^{18}\Delta_{\text{mea}}$ gave an indication of the extent of $^{18}$O equilibrium at the shoot level. The data suggested that drought reduced the $^{18}$O exchange between CO$_2$ and H$_2$O, as the relationship between $^{18}\Delta_{\text{mod}}$ and $^{18}\Delta_{\text{mea}}$ showed steeper slopes for the control across a given range of $r$ or $\theta$. Choosing $r=1.9$, 2.0, and 2.1 and $\theta=1$ resulted in respective slopes of 1.04, 0.99, and 0.95 in the control treatment and 0.27, 0.26, and 0.24 in the drought treatment (Fig. 7A). On the other hand, choosing $\theta=1$, 0.75, and 0.5 and $r=2.0$ resulted in respective slopes of 0.99, 1.32, and 1.99 in the control treatment and 0.26, 0.34, and 0.51 in the drought treatment (Fig. 7B). Hence, reducing $\theta$ to 0.5 with $r=2.0$ caused a doubling in slope. All regressions showed $R^2>0.97$ and

$P<0.001$. Note that a decrease of $r$ under drought conditions would produce an increase in the slope but also unrealistic results, as a certain proportion of the values would fall above the 1:1 line, hence implying an unrealistic exchange of more than 100%. On the other hand, increasing $r$ would result in even smaller slopes under drought conditions. In conclusion, under realistic conditions, slopes were always lower in the drought compared with the control treatment at any given $r$ or $\theta$.

Discussion

In soils, the increased $^{18}$O signal caused by labelling was not transferred instantaneously from H$_2$O to CO$_2$, which was reflected in the poor agreement between the modelled and measured isotopic signature of soil respiration. This discrepancy between modelled and measured values is most likely due to the simple model approach used assuming instantaneous exchange and constant kinetic fractionation. In the following, we will discuss the importance of these two model parameters on our results.

We assumed an instantaneous exchange at the soil level since recent field studies found a considerable CA activity in soils, evidenced by instantaneous $^{18}$O exchange in soils (Seibt et al., 2006; Wingate et al., 2008) and carbonyl sulfide uptake from soils (Kesselmeier et al., 1999; Kesselmeier and Hubert, 2002). Such instantaneous equilibrium in soils would be in line with the common instantaneous exchange in plant leaves. However, this subject is far from settled. In principle, $^{18}$O exchange between CO$_2$ and H$_2$O is just a function of temperature (Tans, 1998) and occurs only after hydration of the dissolved CO$_2$ to carbonic acid (Mills and Urey, 1940) with a rate constant of 0.012 s$^{-1}$ (Skirrow, 1975; Tans, 1998). Apart from soil water, Stern et al. (1999) found that the isotopic signature of soil CO$_2$ is mainly influenced by the rate constant of the isotopic exchange but also by soil-filled pore space and tortuosity. The latter two are mainly interfering with the kinetic fractionation from diffusion, and thus dry soils are likely to produce different kinetic fractionation compared with wet soils. To avoid underestimation of this effect, we assumed a maximal theoretical value of 8.8% (Miller et al., 1999) in both treatments. Several earlier works have elaborated on the correct prediction of the isotopic signature of soil CO$_2$ including effects related to the invasion flux from atmosphere to soil (Stern et al., 2001), effects from the soil water bound to soil surfaces (Miller et al., 1999), or effects from the near surface gradient of soil water $^{18}$O (Riley, 2005). Thus, predicting $\delta_{SR}$ under different environmental conditions is complex as it involves a number of physical and chemical uncertainties that are hard to quantify. The labelling approach did not result in significant differences between dry and wet soils, although a strong isotopic shift was induced. Considering the time it took until a new isotopic equilibrium was established let us suggest that $^{18}$O equilibrium was not instantaneous in soils, which in turn points to a reduced CA activity. However, the extent of CA reduction within soils cannot be deduced from the data, and additional experiments are needed.
In the shoot, H$_2^{18}$O labelling caused a rapid increase of $A_N$ and $g_s$ of drought treatments within 30 min. Such a fast ecophysiological response is in strong accordance with an irrigation experiment done in a Swiss forest with 115-year-old beech trees, where a rapid response (within 6 min) of the xylem sap flow rate was measured upon irrigation of previously drought-stressed trees (Cermak et al., 1993). A similar response has been also described for 3- to 6-month-old Eucalyptus pauciflora saplings, where a fast response of $A_N$ and $g_s$ to rewatering within 5–60 min was identified after drought (Kirschbaum, 1988). Moreover, our observed rapid reaction of $A_N$ and $g_s$ was not accompanied by the arrival of labelled water molecules, which occurred later. Furthermore, $\delta^{18}O$ and $\delta^{18}N$ showed independently that drought also delayed the transport of water from below- to aboveground, thus affecting the $^{18}$O signal propagation from soil to shoot. The observed, slightly higher stomatal conductance after H$_2^{18}$O labelling could therefore not compensate for the slower movement of molecules through the xylem. A reduced sap flow under drought conditions has also been confirmed in field studies for Fraxinus excelsior L. (Stöhr and Lösch, 2004), Betula pendula, and Picea abies (Gartner et al., 2009). According to Nadezhdina (1999), sap flow can even be used as a proxy for whole-plant water status.

Upon arrival of labelled H$_2^{18}$O molecules in the shoot, almost instantaneous $^{18}$O exchange between H$_2$O and CO$_2$ was found in both treatments as had been indicated by a strong relationship between $^{18}$Δ$_{mod}$ and $^{18}$Δ$_{mea}$. According to the theory, instantaneous $^{18}$O equilibrium between H$_2$O and CO$_2$ is facilitated by CA. CA is ubiquitous in leaves and found predominantly in leaf chloroplasts, which are generally located (to facilitate gas exchange) close to the sites of evaporative enrichment (Yakir, 2003). Furthermore, CA has a turnover rate of up to $10^6$ s$^{-1}$, which is one of the fastest known enzymatic reactions (Silverman, 1982). In the 1990s, studies on $\delta^{18}$O generally assumed a full isotopic equilibrium between CO$_2$ and H$_2$O for both leaves and soil (Francey and Tans, 1987; Farquhar et al., 1993; Yakir and Wang, 1996; Ciais et al., 1997), which was then fundamentally refuted by Gillon and Yakir (2001). They showed marked differences among different plant taxa and physiological groups, resulting in an overall global weighted mean with $\theta$=0.78. Among groups, C$_4$ plants showed the highest equilibrium rates with $\theta$>0.95 in 26 out of 39 species. This was contrasted by a $\theta$ of only 0.38 in C$_3$ grasses (Gillon and Yakir, 2000a, 2001). In C$_3$ trees, an average $\theta$ was estimated at 0.93, which is similar to the range observed in this study under control conditions for beech (0.93–0.98). After the paper by Gillon and Yakir (2001), a number of papers confirmed these findings. For instance, Cernusak et al. (2004) estimated $\theta$ in Ricinus communis during leaf dark respiration and photosynthesis at 0.8 and 1, respectively. However, Edwards et al. (2007) showed that the low CA activity in C$_4$ grasses could also be a characteristic physiological trait of the PACCAD clade of grasses, which also include C$_3$ grasses.

The data presented here suggest that drought reduces the $^{18}$O exchange between CO$_2$ and H$_2$O at the shoot level across a range of different retroflux intensities and $\theta$ values. The reduced extent of $^{18}$O equilibrium under drought is in accordance with Guliyev et al. (2008), who found a reduction in CA activity after a long drought period in wheat. Among potential environmental influences on CA activity, so far only irradiation has been shown to have an effect on the hydration efficiency in leaves, as shown by Cousins et al. (2006). Since an integrated shoot approach was chosen in our study for estimating the extent of $^{18}$O equilibrium at the shoot level, intraleaf variations of CA activity as observed in Zea mays

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**Fig. 6.** (A) Ratio between the $\delta^{18}$O of modelled soil respiration ($\delta_{SR-mod}$) based on $\delta_{EV}$ and the measured $\delta^{18}$O of soil respiration ($\delta_{SR}$) over the course of the experiment. Data shown are mean half-hourly values from each treatment. (B) Corresponding relationship between $\delta_{SR-mod}$ and $\delta_{SR}$. Control treatment is shown by open symbols, drought treatment by closed symbols, and $x=y$ by a dashed line. PRE, pre-label day; LD, label day; PLD, post-label day. Data shown are individual measurements from each treatment.
Effect of drought on $^{18}$O/CO$_2$ fluxes after H$_2$O labelling | 5791

Fig. 7. Left: Relationship between simple modelled discrimination against $^{18}$O/CO$_2$ ($\Delta_{\text{mod-simple}}$) versus measured discrimination against $^{18}$O/CO$_2$ ($\Delta_{\text{mea}}$) in the shoot. Right: Relationship between extended modelled discrimination against $^{18}$O/CO$_2$ ($\Delta_{\text{mod-extended}}$) versus measured discrimination against $^{18}$O/CO$_2$ ($\Delta_{\text{mea}}$) in the shoot. Control treatment is shown by open symbols, drought treatment by closed symbols, and x=y by dashed lines. $r$, retroflux factor. Results are shown as least squares linear regressions (solid lines, all $R^2$>0.97 and $P$<0.001). Data shown are individual measurements from the label day between 11 a.m. and 7 p.m.

Experimental work to constrain model approaches such as those of Riley et al. (2002, 2003). With the fast development of optical measurement techniques for isotope research (reviewed by Griffis, 2013), there is a promising basis for extended research in this area.

Supplementary data

Supplementary data are available at JXB online.

Supplementary information. Potential error estimation on $\delta$ value from condensation.

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References


... can be dismissed (Affek et al., 2006; Griffis et al., 2011). It should be noted that the $^{18}$O equilibrium presented here is rather an apparent equilibrium, which also summarizes effects that result from measuring an entire shoot. Thus, the measurements include effects from stem respiration, shading, etc., which are not present when measuring single leaves as done in most other laboratory studies.

In a model approach, Xiao et al. (2010) pointed out the need for a better understanding of environmental controls on CO$_2$ hydration efficiency, as they concluded that only $\theta$=0.46 was able to logically explain the disagreement between simulated and observed $^{18}$O isoflux for a soybean field. Low $\theta$ values have been confirmed during other field measurements in C$_3$ ecosystems (F. sylvatica) with a chamber approach ($\theta$=0.7; A. Hammerle, L. Gentsch, P. Sturm, M. Barthel, R. Siegwolf, N. Buchmann, and A. Knohl, unpublished data). Using eddy covariance measurements, Griffis et al. (2011) further showed for a C$_4$ ecosystem that $\theta$ can markedly differ when estimating $\theta$ for the canopy scale ($\theta$=0.2) compared with the leaf scale ($\theta$=0.7).

Overall, we showed that H$_2$O labeling in combination with laser spectroscopy can be used to investigate the speed of the link between below- and aboveground processes under drought conditions by measuring the $^{18}$O/CO$_2$ ratio in CO$_2$ and H$_2$O$_2$. We conclude that drought impairs the $^{18}$O signal propagation from below- to aboveground and reduces the $^{18}$O equilibrium between CO$_2$ and H$_2$O$_2$ at the shoot level. An instantaneous $^{18}$O exchange as observed in the shoot was not present in the soil. To summarize, drought stress affects $^{18}$O/CO$_2$ fluxes within the soil-plant-atmosphere system at different temporal and spatial scales, highlighting complex interactions between different components. Despite the existing published research body, there is need for further...


Effect of drought on C\textsuperscript{18}O fluxes after H\textsubscript{2}\textsuperscript{18}O labelling


