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Response of $\delta^{13}C$ in plant and soil respiration to a water pulse

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Abstract

Stable carbon isotopes have been used to assess the coupling between changes in environmental conditions and the response of soil or ecosystem respiration, usually by studying the time-lagged response of $\delta^{13}C$ of respired CO_2 ($\delta^{13}C_R$) to changes in photosynthetic carbon isotope discrimination (Δ_i). However, the lack of a systematic response of $\delta^{13}C_R$ to environmental changes in field studies stresses the need to better understand the mechanisms to this response.

We experimentally created a wide range of carbon allocation and respiration conditions in *Fagus sylvatica* mesocosms, by growing saplings under different temperatures and girdling combinations. After a period of drought, a water pulse was applied and the short-term responses of $\delta^{13}C$ in soil CO_2 efflux ($\delta^{13}C_{R_{soil}}$) and $\delta^{13}C$ in aboveground plant respiration ($\delta^{13}C_{R_{above}}$) were measured, as well as leaf gas exchange rates and soil microbial biomass $\delta^{13}C$ responses.

Both $\delta^{13}C_{R_{soil}}$ and $\delta^{13}C_{R_{above}}$ values of all the trees decreased immediately after the water pulse. These responses were not driven by changes in Δ_i, but rather by a fast release of C stored in roots and shoots. Changes in $\delta^{13}C_{R_{soil}}$ associated with the water pulse were significantly positively correlated with changes in stomatal conductance, showing a strong impact of the plant component on $\delta^{13}C_{R_{soil}}$. However, three days after the water pulse in girdled trees, changes in $\delta^{13}C_{R_{soil}}$ were related to changes in microbial biomass $\delta^{13}C$, suggesting that changes in the carbon source respired by soil microorganisms also contributed to the response of $\delta^{13}C_{R_{soil}}$.

Our study shows that improving our mechanistic understanding of the responses of $\delta^{13}C_R$ to changes in environmental conditions requires the understanding of not only the plant's physiological responses, but also the responses of soil microorganisms and of plant-microbial interactions.

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1 Introduction

The rate of carbon (C) allocation to respiration in terrestrial ecosystems under changing environmental conditions is a major source of uncertainty in the understanding of the terrestrial C cycle (Bowling et al., 2008). In particular, a better comprehension of the fate of recently-assimilated C – a major component of ecosystem respiration (Högberg et al., 2001) – to above- and belowground respiration is required to establish and model terrestrial C budgets (Friedlingstein et al., 1999; Landsberg, 2003; Litton et al., 2007). Stable C isotopic composition ($\delta^{13}C$) is an important tool to address C allocation and turnover in ecosystems, in particular when used as a tracer to track photosynthate flow from assimilation to respiration (Dawson et al., 2002; Högberg et al., 2008; Kodama et al., 2008; Kayler et al., 2010; Kuzyakov and Gavrichkova, 2010).

Changes in environmental conditions result in changes in leaf photosynthetic discrimination (Δ_i, Farquhar et al., 1989), which in turn imprints the $\delta^{13}C$ of newly-synthesised photoassimilates, that can then be tracked in different components of the ecosystem until they are respired. Following changes in environmental conditions (e.g., vapour pressure deficit: Bowling et al., 2002; Scartazza et al., 2004; Knohl et al., 2005; Mortazavi et al., 2005; photosynthetically active radiation: Knohl et al., 2005; air temperature: Steinmann et al., 2004), associated changes in the $\delta^{13}C$ values of ecosystem- or soil-respired CO_2 ($\delta^{13}C_{R_{system}}$ and $\delta^{13}C_{R_{soil}}$, respectively) have been typically observed after a time-lag of a few days for mature forest trees (e.g., 1 to 10 days: Bowling et al., 2002; Knohl et al., 2005). In particular, precipitations lead to decreases $\delta^{13}C_{R_{system}}$ (McDowell et al., 2004b) and increased Δ_i (Wingate et al., 2010).

However, some studies also found no changes in $\delta^{13}C_{R_{system}}$ or $\delta^{13}C_{R_{soil}}$ in response to environmental changes (e.g., Fessenden and Ehleringer, 2003; McDowell et al., 2004a; Maunoury et al., 2007), although the expected main drivers changed with a similar magnitude as in the studies which reported an isotopic shift. In a few cases, a response of $\delta^{13}C_{R_{soil}}$ to changes in environmental conditions was observed, but its timing was not compatible with realistic phloem velocity (e.g., less than a day in McDowell

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et al., 2004a). Furthermore, in a recent study, $\delta^{13}C_{R_{soil}}$ in a *Pinus sylvestris* forest responded to changes in environmental conditions, although the original ^{13}C signal due to changes in Δ_i was lost by dampening during C transport from canopy to roots (Kodama et al., 2008). These results indicate that the $\delta^{13}C_{R_{system}}$ response to environmental changes may not be driven by Δ_i alone: in addition to reflecting changes in the $\delta^{13}C$ values of phloem-transported C compounds respired in the soil, $\delta^{13}C_{R_{soil}}$ may be affected directly by changes in environmental conditions, for example, through changes in the C source respired by soil microorganisms.

In the present study, a wide range of conditions was created for aboveground and belowground respiration and carbon allocation to roots and soil microorganisms. Beech (*Fagus sylvatica*) saplings grown under controlled conditions in natural soil (the unit composed of the sapling and its soil is later referred to as mesocosm) were acclimated to different temperatures (4 °C, 12 °C and 20 °C) – an environmental factor affecting both plant and soil microorganism activity (e.g., Boone et al., 1998) – as well as subjected to a girdling treatment to stop assimilate transfer belowground. To further establish conditions in which responses of $\delta^{13}C_R$ to changes in environmental conditions should be pronounced, the saplings were exposed to a drought period followed by an irrigation pulse mimicking a rain event. Under controlled conditions, we aimed to improve the understanding of the mechanisms underlying the responses of changes in $\delta^{13}C$ of aboveground biomass respiration ($\delta^{13}C_{R_{above}}$) and soil respiration ($\delta^{13}C_{R_{soil}}$) due to the water pulse over the wide range of conditions created for C allocation and respiration. We tested the following hypotheses: (1) both $\delta^{13}C_{R_{above}}$ and $\delta^{13}C_{R_{soil}}$ should decrease in response to rewetting after a drought period; (2) Water pulse-induced changes in $\delta^{13}C_{R_{above}}$ should be negatively correlated to changes in Δ_i; (3) Changes in $\delta^{13}C$ of C allocated belowground should not alone suffice to explain water pulse-induced changes in $\delta^{13}C_{R_{soil}}$, for which microbial or physical processes may play an additional role.

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2 Material and methods

2.1 Experimental setup

Twelve beech (*Fagus sylvatica*) saplings of the same cohort (4 years old, average height of 1 m) and of similar morphological characteristics were selected in a forest stand (Laegeren, Switzerland, 47° 28′ 42.0″ N and 8° 21′ 51.8″ E at 682 m a.s.l.), growing on a cambisol (3.9 kg C m^{-2}, 0.3 kg N m^{-2}, see Heim et al., 2009, for more details). Since two companion studies have shown a large influence of ontogeny on C isotopic signatures (Salmon et al., 2011), we selected the saplings to make sure that they were all at the same developmental stage. Soil monoliths were cut around the tree roots and transferred to the laboratory in pots (18 × 18 × 17 cm height), with their roots, rhizosphere and surrounding soil being disturbed as little as possible. A PVC collar (7 cm diameter, 5 cm high) for soil CO_2 efflux measurements was inserted 2.5 cm deep in the pot surface area.

The trees were grown for five months under controlled conditions in growth chambers (PGV36, Conviron, Winnipeg, Canada) subjected to three temperature treatments: warm (day/night temperatures of 20 °C/18 °C, respectively); medium, (12 °C/10 °C) and cold (4 °C/2 °C) conditions, respectively. These temperatures represent the average temperatures the saplings would have experienced in the field in July (monthly average temperature of 20 °C), September (12 °C) and November (4 °C). Pots were rotated in the chambers to avoid position effects. Growth conditions were set to a 14 h photoperiod (photosynthetically active radiation, PAR, of ca. 400 µmol m^{-2} s^{-1}) with CO_2 concentrations maintained at ≈ 400 µmol mol^{-1}, and air humidity between 50 and 60%. Plants were watered twice a week to maintain soil water content (SWC) at 80% field capacity. Since C isotope discrimination of C_4 species is relatively constant under non-limiting conditions (Evans et al., 1986; Buchmann et al., 1996), leaves of well-watered *Zea mays* L. were used as phytometers to provide an integrated ^{13}C signature of background CO_2 in the chambers on a biweekly basis. Leaf biomass of the phytometers

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was sampled every two weeks, dried and finely ground prior to isotope ratio analysis (see below).

After five months of growth, watering was stopped for three weeks to simulate a drought, but ensuring that trees were still photosynthetically active at the end of the drought period. After three weeks, half of the trees of each temperature growth condition were girdled 5 cm above the ground to fully stop C transfer from photosynthetic organs to the belowground compartments, 48 h prior to a water pulse (see below). Paired pots, both exposed to the water pulse, were used for each treatment combination of temperature and girdling: the first pot was used to monitor $\delta^{13}C$ in soil CO_2 efflux ($\delta^{13}C_{R_{soil}}$) and in aboveground plant respiration ($\delta^{13}C_{R_{above}}$) with a time-series of on-line ^{13}C measurements, while the second one was used for auxiliary plant ecophysiological measurements. We implemented a regression approach over the wide range of environmental – and consequently physiological – conditions rather than an ANOVA approach (see statistical analysis below).

2.2 Water pulse and $\delta^{13}C$ of respired CO_2

$\delta^{13}C_{R_{soil}}$ and mesocosm $\delta^{13}C_R$ ($\delta^{13}C_{R_{mesocosm}}$: the $\delta^{13}C$ signature of CO_2 respired by both the sapling and its soil in a pot experimental unit) were measured before and after the water pulse, one pot at a time as follows. One day prior to the water pulse, the pot was inserted in a custom-built transparent air-tight PVC chamber (29 cm diameter, 72 cm high), referred to as the main chamber. The main chamber was equipped with a fan to ensure good mixing of air and with a septum to enable the sampling of chamber air (see Fig. A1 and below). CO_2 efflux in the main chamber is referred to as mesocosm-respired CO_2, i.e., the sum of both aboveground respiration and soil CO_2 efflux. A smaller chamber (0.25L PE chamber; see Fig. A1 and below), referred to as the soil chamber, was placed inside the main chamber, fitted air-tight on the PVC collar that had been previously inserted in the soil.

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The second pot of each pair (i.e., grown under the same temperature conditions and subjected to the same girdling treatment as the pot used for $\delta^{13}C_{R_{soil}}$ and $\delta^{13}C_{R_{mesocosm}}$ measurements), used for auxiliary ecophysiological measurements, was placed in a similar transparent air-tight PVC chamber and exposed to the same environmental conditions. Both PVC chambers were kept in a growth chamber during the experiment.

Both main chamber and soil chamber were connected independently to a custom-built online IRMS measurement setup (see Fig. A1 and below) and to an infra-red gas analyser (Li-840, Li-Cor Inc., Lincoln, NE, USA) to measure $\delta^{13}C_{R_{soil}}$ and $\delta^{13}C_{R_{mesocosm}}$ as well as CO_2 flux rates in both chambers.

All tubing and chambers were flushed with synthetic air until all CO_2 was removed before $\delta^{13}C_{R_{soil}}$ and $\delta^{13}C_{R_{mesocosm}}$ as well as soil CO_2 efflux and tree respiration rates were measured continuously at a frequency of one $\delta^{13}C$ measurement every 13 min and one CO_2 concentration measurement per second, during 24 h. A water pulse was applied by injecting 400 ml of water through a septum in the main chamber, to mimic a rain event that would both moisten the soil and increase relative humidity in the chamber headspace. The increase in relative humidity in the chamber was monitored using an infra-red gas analyser (Li-840, Li-Cor Inc.). The soil chamber was removed during the water pulse, so that the entire surface of the pot got homogeneously wet and then put back in place. After 15 min, the main chamber was opened, excess water discarded and the soil chamber fitted again tightly on the collar and connected to the online measurement setup. All $\delta^{13}C_R$ and respiration rate measurements were stopped during the water pulse and all tubing and chambers were flushed with synthetic air before resuming measurements and maintaining them for 3 days after the pulse. Only measurements performed at least two hours after the beginning of the water pulse were considered reliable, because of the time needed, (1) to perform the pulse, (2) to remove excess water and to ensure that no liquid water was left to enter the on-line measurement system, and (3) to remove all ambient CO_2 that entered in the chamber during these operations.

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To avoid the recycling of respired CO_2, the plants were kept in the dark for all measurements (both isotopic and physiological), except during the water pulse. However, to allow photosynthetic uptake during the water pulse, plants were lit with a greenhouse lamp for 15 min, resulting in a PAR of $\approx 400\,\mu mol\,m^{-2}\,s^{-1}$ inside the chamber. The effectiveness of plant C assimilation was controlled by monitoring CO_2 concentration inside the main chamber (see below for details).

2.3 Plant and soil sampling

Leaf, root, bulk phloem organic matter, and soil samples were taken 24 h before the water pulse (−24 h, from the second set of pots, i.e., those used for ecophysiological measurements) and three days (+72 h) after the water pulse (from the first set of pots, i.e., those used for $\delta^{13}C_R$ measurements). Additionally, phloem organic matter was sampled after the water pulse (+2 h), since it most likely carries $\delta^{13}C$ changes imprinted by changes in Δ_i faster than biomass.

Soil was sampled (5 cm diameter core over the entire pot depth), sieved (2 mm mesh) and split into two subsamples. One subsample was used for bulk $\delta^{13}C$ measurements after drying for 48 h at 60 °C and manually removing the roots that were used for root $\delta^{13}C$ measurements (see below). The other subsample was kept at 4 °C before microbial biomass C and its $\delta^{13}C$ were measured (see below).

Bulk leaf and root biomass were sampled, dried (48 h at 60 °C), ground and weighed prior to $\delta^{13}C$ measurements. Bulk phloem organic matter was collected following an exudation method (Gessler et al., 2004). One twig was sampled, the cut rinsed with ultrapure water and carefully dabbed, before inserting the twig in a tube filled with 2 ml of 0.15 M polyphosphate buffer at pH 7.5. The tube was sealed around the twig with Parafilm® and placed in the dark at 100% humidity and 4 °C to avoid evapotranspiration and microbial development in the solution. After five hours, 1.5 ml of solution was collected, freeze-dried and used for isotope ratio analysis (see below).

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2.4 Leaf gas exchange rates

Leaf gas exchange measurements were conducted on 6 plants of the second set (described above), 24 h before the water pulse, 2 h after the water pulse and 3 days after the water pulse. The following leaf gas exchange variables were measured on five of the youngest fully expanded leaves of each plant: leaf respiration rate (r_l), transpiration rate in the light (E_l), leaf conductance to H_2O in the light (g_s), CO_2 assimilation rate (A) and the ratio between internal and ambient CO_2 concentrations in the light (c_i/c_a). These measurements were conducted under standardised conditions in the growth chamber, with a portable photosynthesis system (Li-6400, Li-Cor Inc.), using a dew point generator (Li-610, Li-Cor Inc.) to ensure constant 60% relative humidity, a CO_2 source to achieve $\approx 400\,\mu mol\,mol^{-1}$ CO_2 concentration in the incoming gas flow of the Li-6400 leaf chamber, and a $400\,\mu mol\,m^{-2}\,s^{-1}$ light source (6400-02B, Li-Cor Inc.) for measurements in the light. Because the plants were kept in the dark for long periods of time prior to these measurements in the light, leaf gas exchange rates were recorded only when they reached a steady state (up to 20 min after exposure to light).

Measurements of c_i/c_a can be used to estimate photosynthetic discrimination (Δ_i; Eq. 1), based on the widely accepted simplified model developed by Farquhar et al. (1982), which assumes infinite internal conductance and neglects the effect of boundary layer resistance:

$$\Delta_i = a + (b-a)\frac{c_i}{c_a} = a + (b-a)\frac{p_i}{p_a} \quad (1)$$

where a is the discrimination occurring during CO_2 diffusion in air through the stomatal pore, equal to 4.4‰ (Craig, 1954), b is the net discrimination caused by carboxylation, c_a and c_i are the ambient and intercellular mole fractions of CO_2, respectively. p_a and p_i are the equivalent of c_a and c_i, expressed in partial pressure of CO_2. For higher C_3 plants, b results mostly from the fixation of CO_2 by Rubisco, the carboxylation enzyme, estimated at 29‰ in spinach (Roeske and O'Leary, 1984) and some PEP-carboxylase

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fixation, leading to an estimated value for b of 27‰ in ecological studies (Farquhar and Richards, 1984; Lloyd and Farquhar, 1994).

2.5 Microbial biomass C and ^{13}C

The ^{13}C signature of soil microbial biomass was determined by fumigation-extraction (Vance et al., 1987; Wu et al., 1990). From each sieved soil sample, an ≈ 10 g subsample was fumigated for 24 h with chloroform vapour before extraction, while another ≈ 10 g subsample was extracted without prior fumigation. Gravimetric soil water content was determined by comparing the mass of ≈ 10 g of soil before and after drying at 105 °C. Soil was extracted by vigorous shaking for 30 min in a 0.03 M K_2SO_4 extraction solution. All soil microbial extracts were then filtered and frozen (−20 °C). Samples were freeze-dried before isotope ratio analysis (see below). Soil microbial biomass $\delta^{13}C$ ($\delta^{13}C_{microbe}$; Eq. 2) was calculated as

$$\delta^{13}C_{microbe} = \frac{\delta^{13}C_F \cdot C_F - \delta^{13}C_{NF} \cdot C_{NF}}{C_F - C_{NF}} \quad (2)$$

where F and NF stand for fumigated and non-fumigated soil, respectively, and C for total organic C.

2.6 Isotope ratio mass spectrometry measurements

A custom-built online IRMS measurement setup, controlled by a computer and electrovalves, was used to monitor $\delta^{13}C_{R_{soil}}$ and $\delta^{13}C_{R_{leaf}}$ (Fig. A1). An IRMS circuit was connected alternatively to a soil chamber circuit or to a main chamber circuit. CO_2 and H_2O concentrations were measured with a CO_2/H_2O gas analyser (Li-840, Li-Cor Inc.) placed in the shared part of the soil and main circuits. A membrane pump ensured a flow rate of 1 l min^{-1} in the IRMS circuit. The main circuit included a synthetic air bottle and a vent to allow the flushing of the main chamber. Each circuit was independently equipped with a pump and a CO_2 scrubber (soda lime). In the IRMS circuit,

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an additional scrubbing device was installed to maintain CO_2 concentrations under 1000 µmol mol^{-1}. Before each measurement, CO_2 was removed from all the circuits and chambers. Then CO_2 concentrations were allowed to increase due to dark respiration to at least 300 µmol CO_2 mol^{-1} before the CO_2 samples were directed to the IRMS.

The $\delta^{13}C$ value of gas samples was measured with a modified Gasbench II periphery (Finnigan MAT, Bremen, Germany) equipped with a custom-built cold trap coupled to the IRMS (Delta^{plus}XP, Finnigan MAT). The $\delta^{13}C$ in bulk leaf, root, phloem organic matter and soil as well as $\delta^{13}C$ in microbial biomass extracts were measured with an elemental analyser (Flash EA 1112 Series, Thermo Italy, Rhodano, Italy) coupled to an IRMS (Delta^{plus}XP, Finnigan MAT). The long-term precision (~ 1.5 years) of the quality control standard (caffeine) was 0.09‰. C isotopic composition is expressed as the relative difference of the sample isotope abundance ratio R ($^{13}C/^{12}C$) relative to that of the international standard (VPDB). This difference is expressed in per mill (‰; Eq. 3) and defined as:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \quad (3)$$

The $\delta^{13}C$ of CO_2 respired by aboveground plant biomass ($\delta^{13}C_{R_{above}}$; Eq. 4) was not measured directly, but calculated from the mesocosm- and soil-respired CO_2 efflux ($F_{mesocosm}$ and F_{soil}, respectively, in µmol CO_2 mol^{-1} m^{-2} s^{-1}) and $\delta^{13}C_{R_{mesocosm}}$ and $\delta^{13}C_{R_{soil}}$ as follows:

$$\delta^{13}C_{R_{above}} = \frac{\delta^{13}C_{R_{mesocosm}} \cdot F_{mesocosm} - \delta^{13}C_{R_{soil}} \cdot F_{soil}}{F_{mesocosm} - F_{soil}} \quad (4)$$

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2.7 Diffusion model

A simple steady state soil diffusion model was used to establish whether any change in soil CO_2 efflux or $\delta^{13}C_{R_{soil}}$ associated with the water pulse could be due to changes in the soil's physical properties, independently of a biological response. We assumed two steady state situations regarding CO_2 fluxes: one before and one two hours after the water pulse, when the measurements were restarted, since (1) environmental conditions were kept constant during these periods, and (2) there were no data available over the water pulse.

The isotopic signature of the soil CO_2 efflux ($\delta^{13}C_F$; Eq. 5) was calculated as:

$$\delta^{13}C_F = \left(\frac{F_{^{13}CO_2} / F_{^{12}CO_2}}{R_{standard}} - 1 \right) \quad (5)$$

where $R_{standard}$ is the $^{13}C/^{12}C$ ratio of the Vienna PDB standard (0.01124, Craig, 1957).

According to Nickerson and Risk (2009), the following assumptions were made. Both $^{12}CO_2$ and $^{13}CO_2$ were treated as different gases. Furthermore, $^{12}CO_2$ was assumed to be equal to total CO_2, because of its high abundance (~ 99%). The errors associated with this assumption were estimated to be smaller than 0.01% (Amundson et al., 1998). The diffusivity of $^{13}CO_2$ was considered equal to the diffusivity of $^{12}CO_2$ divided by the theoretical difference in the diffusivity of both gases (1.0044, Cerling et al., 1991). Fluxes and $\delta^{13}C$ values obtained from measurements are referred to as experimental, while those obtained from the model are referred to as modelled. Finally, the model variables were initialized with pre-pulse conditions.

CO_2 flux through the soil was mediated by the discrete, one-dimensional form of Fick's First Law (Eq. 6; Nickerson and Risk, 2009):

$$F = -D \frac{\Delta C_{ij}}{\Delta z_{ij}} \quad (6)$$

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where D is the diffusion coefficient in the soil, ΔC_{ij} is the difference in CO_2 concentration between two layers (i and j) of the soil and Δz_{ij} is the difference of depths between the two layers. D (Eq. 7) was calculated for a depth z at a time t as follows (Moyes et al., 2010):

$$D(z,t) = -D_o(z,t) \cdot \xi(z) \tag{7}$$

where $D_o(z,t)$ is the diffusivity of CO_2 in the air and $\xi(z)$ is a tortuosity factor. $D_o(z,t)$ (Eq. 8) is calculated as follows:

$$D_o(z,t) = -D_{ao} \left(\frac{T(z,t)}{293.15} \right)^{1.75} \left(\frac{P}{101.3} \right) \tag{8}$$

where P is the atmospheric pressure (97 kPa local atmospheric pressure for Zurich) and D_{ao} is the reference value for CO_2 diffusivity in air at 293.15 K and 101.3 kPa and equals 14.7 $mm^2\,s^{-1}$, $T(z,t)$ is the temperature at depth z and time t. $\xi(z)$ (Eq. 9) is calculated based on soil air filled porosity (α) and total porosity (θ) following Millington (1959):

$$\xi(z) = \frac{\alpha(z)^{\frac{10}{3}}}{\theta^2} \tag{9}$$

Total soil porosity (θ) was estimated at 0.3 $m^3\,m^{-3}$. Air filled porosity (α) was calculated by subtracting the volumetric soil water content (VWC) from θ.

The CO_2 concentration at depth z ($C(z)$; Eq. 10) was calculated according to Cerling (1984) for a system under steady state conditions:

$$C(z) = \frac{\gamma}{D} \left(Lz - \frac{z^2}{2} \right) + C_{\text{atm}} \tag{10}$$

where γ is the CO_2 production at depth z, L is the total soil depth and C_{atm} is the ambient CO_2 concentration.

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Furthermore, the total production of CO_2 (Γ) can be related to the surface soil CO_2 efflux (Γ_{ss}) under controlled conditions (Nickerson and Risk, 2009), as $F_{ss} = \Gamma = N\gamma$ under the assumption for this work that the CO_2 production is the same in the N soil layers.

Because our experiment was performed in a pot with a homogeneous soil structure, we considered only one layer of soil, with a height (z) of 17 cm. We assumed (1) no input of CO_2 in the soil from the bottom of the pot (i.e., all CO_2 in the soil or leaving the soil was produced in the 17 cm of soil in the pot), (2) a stable and homogeneous temperature $T(z,t)$ in the pot, because the pots were maintained under controlled conditions with a stable temperature. θ, α and VWC were assumed constant throughout the soil profile. Since this model was used to test the contribution of changes in the soil's physical properties following the water pulse to changes in soil CO_2 efflux and $\delta^{13}C_{R_{soil}}$, we assumed that the soil CO_2 production (Γ) did not change with the water pulse.

2.8 Statistical analysis

Data were analysed using R 2.11.1 (R Development Core Team, 2010). For a given variable, the difference between measurements was calculated both between −24 h and +2 h and between −24 h and +72 h. These differences are referred to as water-pulse induced changes in the variable, between the respective timepoints. Linear models were used (1) to test the regression between measured variables at a given point in time, and (2) to test the regression between water pulse-induced changes in measured variables (e.g., the regression between water pulse-induced changes in variables X and Y between the −24 h and +2 h timepoints was tested as $Y_{+2h} - Y_{-24h}$ against $X_{+2h} - X_{-24h}$). Water pulse-induced changes in $\delta^{13}C_{R_{soil}}$, $\delta^{13}C_{R_{mesocosm}}$ and $\delta^{13}C_{R_{above}}$ were estimated by comparing their pre-pulse 3 h-average value to their post-pulse 3 h-average value.

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3 Results

3.1 Leaf gas exchange rates

The water pulse rewet the soil with an average increase in SWC of 16.5% (see Table 1 for details) and triggered a response of leaf gas exchange variables. Assimilation (A) increased from $0.37 \pm 0.20\,\mu mol\,m^{-2}\,s^{-1}$ at −24 h (mean ± SE) to $0.64 \pm 0.14\,\mu mol\,m^{-2}\,s^{-1}$ at +2 h and to $0.75 \pm 0.17\,\mu mol\,m^{-2}\,s^{-1}$ at +72 h (Table 1). g_s decreased from $0.12 \pm 0.08\,mmol\,m^{-2}\,s^{-1}$ at −24 h to $0.06 \pm 0.01\,mmol\,m^{-2}\,s^{-1}$ at +2 h, then increased to $0.46 \pm 0.33\,mmol\,m^{-2}\,s^{-1}$ at +72 h (Table 1). Similarly, E_l first decreased from $1.21 \pm 1.00\,mmol\,m^{-2}\,s^{-1}$ at −24 h to $0.78 \pm 0.42\,mmol\,m^{-2}\,s^{-1}$ at +2 h, and then increased to $2.45 \pm 1.22\,mmol\,m^{-2}\,s^{-1}$ at +72 h (Table 1). r_l first increased from $0.73 \pm 0.24\,\mu mol\,m^{-2}\,s^{-1}$ at −24 h to $1.20 \pm 0.58\,\mu mol\,m^{-2}\,s^{-1}$ at +2 h, and then decreased to $0.70 \pm 0.20\,\mu mol\,m^{-2}\,s^{-1}$ at +72 h (Table 1).

3.2 $\delta^{13}C$ of respired CO_2

The water pulse resulted in an average decrease of $\delta^{13}C_{R_{above}}$ by 2.94 ± 0.79‰ (mean ± SE over all trees; Fig. 1a). The largest $\delta^{13}C_{R_{above}}$ change was observed for the girdled tree at 20 °C, with a decrease of 6.0‰, while the smallest $\delta^{13}C_{R_{above}}$ change was observed for the ungirdled tree at 4 °C, with a decrease of only 0.5‰.

$\delta^{13}C_{R_{mesocosm}}$ values also decreased after the water pulse (2.99 ± 0.61‰ average decrease; Fig. 1b). The largest change in $\delta^{13}C_{R_{mesocosm}}$ was observed for the girdled tree at 4 °C, with a decrease of 5.3‰, while the smallest change in $\delta^{13}C_{R_{mesocosm}}$ was observed for the girdled tree at 20 °C, with a decrease of 1.2‰.

$\delta^{13}C_{R_{soil}}$ showed the strongest response to the water pulse, with an average decrease of 3.89 ± 0.80‰ (Fig. 1c). The girdled tree at 4 °C had the largest change (7.0‰ decrease), while the girdled tree at 20 °C showed the smallest response (1.2‰ decrease).

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Furthermore, water pulse-induced changes in $\delta^{13}C$ of respired CO_2 were related to changes in plant gas exchange rates and changes in $\delta^{13}C$ of other mesocosm C pools. Water pulse-induced changes (between −24 h and +2 h) in $\delta^{13}C_{R_{mesocosm}}$ and g_s were significantly positively related ($R^2 = 0.77$, $p = 0.021$; Fig. 2a), as well as changes in $\delta^{13}C_{R_{soil}}$ and g_s ($R^2 = 0.80$, $p = 0.017$; Fig. 2b). These regressions were mainly driven by the ungirdled trees ($R^2 = 0.99$ and $p = 0.021$ and $R^2 = 0.98$, $p = 0.091$, respectively). We found a significant positive relationship between water pulse-induced changes (between −24 h and +2 h) in $\delta^{13}C_{R_{soil}}$ and microbial biomass $\delta^{13}C$ for girdled trees ($R^2 = 0.99$, $p = 0.004$; Fig. 2c), but not across the whole physiological range of trees. Water pulse-induced changes (between −24 h and +2 h) in $\delta^{13}C_{R_{above}}$ and $\delta^{13}C_{phloem}$ values were significantly positively related ($p = 0.005$; Fig. 2d).

3.3 $\delta^{13}C$ of plant material and microbial biomass

The C_4 phytometers that were grown to identify any changes in $\delta^{13}C$ of background atmospheric CO_2 in the growth chambers had a constant foliar $\delta^{13}C$ of -11.6 ± 0.1‰ over the duration of the experiment. Thus, $\delta^{13}C$ in background atmospheric CO_2 in the growth chambers stayed constant throughout the growth period and it is valid to compare $\delta^{13}C$ values measured at different points in time in our experiment.

Furthermore, the water pulse induced a significant decrease (between −24 h and +72 h) of 2.94 ± 0.21‰ in $\delta^{13}C$ of leaf biomass ($p < 0.001$; Table 2). Neither $\delta^{13}C$ of root biomass nor $\delta^{13}C$ of phloem bulk organic matter were significantly affected by the water pulse (Table 2). Much in contrast, $\delta^{13}C$ of microbial biomass significantly increased (between −24 h and +72 h) by 4.06 ± 0.61‰ with the water pulse ($p < 0.001$; Table 2). The $\delta^{13}C$ of bulk soil was -25.5 ± 0.1‰ and remained unaffected by the water pulse.

We found a significant positive relationship between water pulse-induced changes (between −24 h and +72 h) in $\delta^{13}C$ of microbial biomass and g_s ($R^2 = 0.93$, $p = 0.002$).

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Additionally, water pulse-induced changes (between −24 h and +2 h) in phloem $\delta^{13}C$ (Table 2) and c_i/c_a were significantly positively related ($R^2 = 0.86$, $p = 0.024$; Fig. 3a). However, over a longer period (between −24 h to +72 h), a negative trend was found between water pulse-induced changes in phloem $\delta^{13}C$ and c_i/c_a ($R^2 = 0.73$, $p = 0.065$; Fig. 3b). No other significant correlations between water pulse-induced changes in leaf gas exchange variables and isotopic signatures were observed.

3.4 Diffusion model

The diffusion model was used to predict the physical effect of changes in soil air-filled porosity on soil CO_2 efflux and $\delta^{13}C_{R_{soil}}$ after the water pulse, independently of biological impacts. The model predicted an increase of both $^{12}CO_2$ and $^{13}CO_2$ flux rates after watering (at +2 h) as a result of decreased air-filled pore space ($p < 0.005$ for both, Table 3), while no changes in $\delta^{13}C_{R_{soil}}$ were predicted (Table 3). In contrast, our experiment clearly showed higher $^{12}CO_2$ and $^{13}CO_2$ flux rates as well as lower $\delta^{13}C_{R_{soil}}$ after the water pulse (at +2 h) compared to those before (at −24 h). Therefore, physical changes in soil air-filled porosity after the water pulse could not explain the changes in soil CO_2 efflux and did not appear to play a significant role in the changes in $\delta^{13}C_{R_{soil}}$.

4 Discussion

4.1 $\delta^{13}C_{R_{mesocosm}}$ response to water pulse

In agreement with our first hypothesis, we found that the water pulse triggered a decrease in $\delta^{13}C_{R_{mesocosm}}$ over the entire physiological status range of the trees, as a result of both $\delta^{13}C_{R_{above}}$ and $\delta^{13}C_{R_{soil}}$ decreasing. This response of $\delta^{13}C_R$ is in agreement with previous field studies, which found higher $\delta^{13}C_{R_{system}}$ in drier sites or conditions (e.g., Bowling et al., 2002; Fessenden and Ehleringer, 2003; Scartazza et al., 2004;

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Steinmann et al., 2004). In addition, the water pulse-induced changes in $\delta^{13}C_{R_{mesocosm}}$ were related neither to changes in c_i/c_a ratios nor to changes in photosynthetic discrimination (Δ_i, which is strongly positively related to c_i/c_a ratio, see Eq. 1 and Farquhar et al., 1989). This contrasts with several field studies, in which changes in ecosystem $\delta^{13}C_R$ were in agreement with changes in Δ_i (e.g., Bowling et al., 2002; Scartazza et al., 2004; Steinmann et al., 2004; Knohl et al., 2005; Mortazavi et al., 2005). Δ_i alone could not explain the changes in $\delta^{13}C_{R_{mesocosm}}$ that were associated with the water pulse. Our experimental design allowed monitoring simultaneously the $\delta^{13}C_{R_{above}}$ and $\delta^{13}C_{R_{soil}}$ components of $\delta^{13}C_{R_{mesocosm}}$, to deconvolute its response to a water pulse.

4.2 $\delta^{13}C_{R_{above}}$ response to water pulse

The positive regression between the $\delta^{13}C_{R_{above}}$ and $\delta^{13}C_{phloem}$ responses to the water pulse (Fig. 2d) suggests that leaf respiration was fuelled by phloem-transported C in our experiment. Previous studies have similarly been able to explain time-lagged responses of $\delta^{13}C_{R_{system}}$ to environmental change by the transfer time of recent photoassimilates, that carry an isotopic signature imprinted by these new environmental conditions from the canopy to belowground compartments of the ecosystem (e.g., Bowling et al., 2002; Knohl et al., 2005; Kuzyakov and Gavrichkova, 2010).

Since Δ_i is positively related to the c_i/c_a ratio, as leaf physiology responds to new environmental conditions, c_i/c_a and Δ_i will respond accordingly and affect the $\delta^{13}C$ of recent photoassimilates. We expected an increase in Δ_i to be associated with the water pulse, as previously measured in the field (Wingate et al. 2010), which would lead to decreased $\delta^{13}C$ of photoassimilates. Further, the isotopic signature carried by the recent photoassimilates will contribute to the signature of heterotrophic tissues that they are transported to. Thus, changes in $\delta^{13}C_{phloem}$, $\delta^{13}C_{R_{above}}$ or even $\delta^{13}C_{R_{soil}}$ (in ungirdled trees) are expected to be negatively correlated to changes in c_i/c_a and Δ_i. However, in our study, the correlation between water pulse-induced changes in phloem

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$\delta^{13}C$ and c_i/c_a was significantly positive (Fig. 3a). 2 h after the water pulse, changes in Δ_i were not yet reflected in $\delta^{13}C_{phloem}$ probably because the new assimilates had not yet been transferred to phloem organic matter in sufficient amounts to affect its overall $\delta^{13}C$ value. Thus, other mechanisms besides the changes in Δ_i seem to be responsible for the changes in phloem $\delta^{13}C$ during the first hours after the water pulse: (1) apparent fractionation during carbohydrate loading in phloem organic matter would lead to differences in $\delta^{13}C$ signatures between phloem and photoassimilates. Such fractionation has been previously observed in beech (Damesin and Lelarge, 2003; Scartazza et al., 2004). Differential fractionation factors during phloem loading in plants under different physiological statuses, could suppress the negative relationship between changes in phloem $\delta^{13}C$ and in Δ_i. (2) A second possible mechanism could be that non-structural carbohydrates that carried an isotopic signature imprinted by earlier (i.e., pre-pulse) environmental conditions may have been unloaded from the phloem and respired (Nogues et al., 2006). This hypothesis is supported by the response of $\delta^{13}C_{R_{above}}$ to the water pulse, which was very fast (within two hours) and was not related to changes in c_i/c_a or in Δ_i. Consequently, aboveground respiration after the pulse is likely using previously stored C as a substrate. (3) The preferential storage of some carbohydrates, such as fatty acids, the isotopic signature of which differs from the carbohydrate usually transported, such as sucrose (Tcherkez et al., 2003), may lead to differences in $\delta^{13}C$ between phloem and photoassimilates imprinted by Δ_i (see also point M1.4 in the recent review by Werner and Gessler, 2011).

However, the marginally significant negative regression between water pulse-induced changes in $\delta^{13}C_{phloem}$ and c_i/c_a in the longer term (between −24 h and +72 h, Fig. 3b) suggests that after an immediate response that may involve stored carbohydrates, the contribution of newly-assimilated C to the phloem C flow through the plant gained importance in driving $\delta^{13}C_{phloem}$.

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4.3 Plant contribution to $\delta^{13}C_{R_{soil}}$ upon rewetting

We found a significant positive regression between water pulse-induced changes in $\delta^{13}C_{R_{soil}}$ and stomatal conductance (g_s) in ungirdled trees, indicating a large contribution of the plant component to the soil CO_2 pulse two hours after rewetting. This contribution may have been direct, with root respiration responding to the stimulation of leaf assimilation that was triggered by the water pulse, as the recent photoassimilates were transported belowground. It may also have been indirect, as increased leaf assimilation is expected to have increased root exudation of recent photoassimilates, a source of easily metabolisable C to soil microorganisms. The contribution of the plant component to the soil CO_2 efflux pulse should however be delayed, since it is constrained by the transfer time of recent photoassimilates belowground, while the soil CO_2 efflux pulse can start within minutes after rewetting a dry soil. Our hypothesis is that under field conditions, changes in environmental conditions may be more rapid than this delay, resulting in the signal of a given discrete environmental change being lost in the noise of previous changes, especially if larger plants are considered, thereby explaining the noticeable absence of a measurable plant component in the response of $\delta^{13}C_{R_{soil}}$ in a number of studies (e.g., Fessenden and Ehleringer, 2003; Kodama et al., 2008; McDowell et al., 2004a; Maunoury et al., 2007).

Furthermore, the positive correlation between water pulse-induced changes in g_s and both $\delta^{13}C_{R_{soil}}$ and $\delta^{13}C_{R_{mesocosm}}$ – mostly due to ungirdled trees – suggests that trees that were less stressed (i.e., displaying smaller changes in g_s after the release of the drought) allocated more recent assimilates to soil respiration after the water pulse, resulting in a stronger decrease of $\delta^{13}C$ in respired CO_2 compared to trees that were more stressed. Indeed, changes in g_s have been experimentally related to soil water stress and can even be modelled as a function of soil water potential (Flexas et al., 2002; Gao et al., 2002 and references therein). Therefore, in our experiment, the magnitude of water pulse-induced changes in g_s can be related to the magnitude of soil water stress release. The larger allocation of new assimilates to belowground

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respiration in less stressed saplings is in agreement with the stronger and faster response of non-water-limited compared to water-limited young beech trees previously observed (Ruehr et al., 2009).

4.4 Drivers of $\delta^{13}C_{R_{soil}}$ values pre- and post-pulse

In our experiment, $\delta^{13}C_{R_{soil}}$ before the pulse was highly enriched compared to values generally measured in terrestrial ecosystems (Bowling et al., 2008, and references therein), with an average value of −18.5‰ across the entire physiological range. Since $\delta^{13}C_{R_{soil}}$ is on average 2 to 3‰ higher than leaf $\delta^{13}C$ (Bowling et al., 2008), $\delta^{13}C_{R_{soil}}$ would have been expected around −23‰ before the pulse. The ^{13}C enrichment of soil CO_2 efflux in our experiment could be explained by several mechanisms, including: (1) changes in Δ_i with drought: As g_s decreases with increasing drought, c_i/c_a and Δ_i also decrease, leading to ^{13}C-enriched photoassimilates, which are later transferred belowground and respired; (2) anaplerotic CO_2 fixation by the PEPc in soil microorganisms, when C supply from plant decreases and leads to ^{13}C-enriched microbial biomass and respired CO_2 (Unger et al., 2010a). However, under intense drought conditions, anaplerotic fixation by PEPc is not sufficient to sustain respiration alone. Therefore, our hypothesis is that both drought-induced changes in Δ_i and anaplerotic CO_2 fixation may contribute to the pre-pulse highly ^{13}C-enriched soil CO_2 efflux.

The CO_2 released upon rewetting in our experiment was consistently more ^{13}C-depleted than during the dry period (3.9‰ average depletion), in contrast to Unger et al. (2010b) who measured up to 7‰ more enriched $\delta^{13}C_{R_{soil}}$ following post-drought rain events in a Mediterranean savannah-type evergreen-woodland ecosystem. The depletion we measured could be due to the plant contribution to $\delta^{13}C_{R_{soil}}$ in ungirdled trees, in agreement with water pulse-induced changes in Δ_i (see above). Tree height is a most likely explanation for this difference in plant contributions (Kuzyakov and Gavrichkova, 2010): our saplings were small enough to transfer C from leaves to the

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belowground compartment within a few hours, in contrast to the mature trees studied by Unger et al. (2010b).

However, we found that $\delta^{13}C_{R_{soil}}$ also decreased after the pulse in girdled trees, indicating that mechanisms that were not related to plant C transfer were also at play, whether physical or biological. The former could be changes in the soil's physical properties, in particular air-filled pore space, which have been shown to impact soil $\delta^{13}C_{R_{soil}}$ due to the different diffusivity of $^{12}CO_2$ and $^{13}CO_2$ (Stoy et al., 2007; Nickerson and Risk, 2009; Moyes et al., 2010). However, our diffusion model showed that under steady-state conditions – similar to those before or more than 2 h after the pulse – the changes in soil physical properties alone could not explain water pulse induced-changes in $\delta^{13}C_{R_{soil}}$.

The large mineralization pulse commonly observed after rapid rewetting of a dry soil, fuelled by C compounds accumulated during the dry period and leading to a massive soil CO_2 efflux pulse (Birch, 1964; Borken and Matzner, 2009; Inglima et al., 2009) is most likely the main driver of the depletion of $\delta^{13}C_{R_{soil}}$ that we measured after the water pulse. The water pulse-induced positive correlation in girdled trees between changes in $\delta^{13}C_{R_{soil}}$ and in microbial $\delta^{13}C$ (Fig. 2c) points towards soil microbial processes being involved in the response of $\delta^{13}C_{R_{soil}}$.

The nature of the C source fuelling the mineralization flush remains elusive. Several potential organic C sources that become more abundant and available upon rewetting of dry soils have been proposed as C substrates: (1) available soil organic matter (SOM), which increases due to the shattering of soil aggregates caused by large and sudden changes in soil water content (e.g., Denef et al., 2001; Borken and Matzner, 2009); (2) dead microbial biomass which increases during the dry period, and can be consumed by living microorganisms upon rewetting (e.g., Bottner, 1985); (3) living microbial bodies that can be consumed by other microorganisms that respond faster to rewetting; (4) microbial compounds that are synthesised for drought-resistance and osmotic regulation (exopolysaccharides and compatible solutes, respectively; see Schimel et al., 2007) could also contribute to the easily metabolizable C upon rewetting.

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Our isotopic measurements of depleted $\delta^{13}C_{R_{soil}}$ upon rewetting (average −22.4‰) point first towards increased available SOM, because $\delta^{13}C$ of SOM in our experiment was on average −25.5‰. Second, since lipids are more ^{13}C-depleted than the average organic matter (DeNiro and Epstein, 1977), the consumption of microbial phospholipid cell membranes may also explain the observed ^{13}C-enrichment of microbial biomass and ^{13}C-depletion of soil CO_2 efflux. However, the relative importance of these sources or the potential role of other sources (iii and iv) cannot be further established in our experiment.

5 Conclusions

Our experiment showed that under controlled conditions, both $\delta^{13}C_{R_{above}}$ and $\delta^{13}C_{R_{soil}}$ responded to a sudden change in water availability. Both responded quickly with a ^{13}C depletion within two hours of the water pulse, however, their underlying mechanisms seemed to differ. In particular, changes in Δ_i, which is generally expected to be the main driver of both $\delta^{13}C_{R_{above}}$ and $\delta^{13}C_{R_{soil}}$ (and, thus, of $\delta^{13}C_{R_{system}}$ or $\delta^{13}C_{R_{mesocosm}}$) appear to play only a limited and delayed role in the response of $\delta^{13}C_{R_{above}}$ to the water pulse. In contrast, the immediate response of $\delta^{13}C_{R_{above}}$ seems to be driven by remobilization of stored C in the plant. Furthermore, the plant component had a strong impact on the response of $\delta^{13}C_{R_{soil}}$ to the pulse, not through Δ_i, but rather through changes in root respiration rates or changes in C supply to soil microorganism respiration. Thus, improving our mechanistic understanding of the responses of $\delta^{13}C_{R_{soil}}$, and consequently of $\delta^{13}C_{R_{system}}$, to changes in the environmental conditions requires not only to understand leaf physiological responses (controlling Δ_i), but also the responses of soil microorganisms and of plant-microbial interactions.

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Table 1. Physiological characteristics and overall average ± SE, before (−24 h) and after a water pulse (+2 h, +72 h) applied to *Fagus sylvatica* mesocosms grown under different temperatures (4 °C, 12 °C and 20 °C), combined ($n = 1$) with two girdling treatments (ungirdled and girdled). The physiological variables are CO_2 assimilation rate (A), leaf conductance for H_2O in the light (g_s), transpiration rate in the light (E_l), dark respiration (r_l), and the ratio of intrastomatal over atmospheric partial pressure of CO_2 (c_i/c_a). Gravimetric soil water content (SWC) is also given.

Temperature	Girdling	Time	SWC (%)	A (μmol $m^{-2} s^{-1}$)	g_s (mmol H_2O $m^{-2} s^{-1}$)	E_l (mmol $m^{-2} s^{-1}$)	r_l (μmol $m^{-2} s^{-1}$)	c_i/c_a
4	Ungirdled	−24 h	10.3	0.83	0.01	0.09	0.58	0.96
		+1 h	23.0	1.02	0.05	0.30	0.15	0.90
		+72 h	21.8	0.27	0.33	3.40	0.64	0.98
	Girdled	−24 h	6.0	0.06	0.47	6.19	0.79	0.98
		+1 h	16.0	0.25	0.1	0.52	0.21	0.98
		+72 h	15.3	1.10	1.89	7.32	0.31	0.98
12	Ungirdled	−24 h	11.3	1.12	0.21	0.63	1.84	0.96
		+1 h	24.9	0.46	0.08	2.87	2.68	0.95
		+72 h	23.7	n.a.	n.a.	n.a.	n.a.	n.a.
	Girdled	−24 h	6.2	0.13	0.01	0.06	0.40	0.94
		+1 h	29.3	0.76	0.05	0.37	3.29	0.96
		+72 h	28.6	0.70	0.01	0.35	1.44	0.78
20	Ungirdled	−24 h	5.4	0.07	0.03	0.29	0.56	0.98
		+1 h	25.7	1.03	0.05	0.40	0.71	0.89
		+72 h	25.0	0.42	0.02	0.72	0.22	0.93
	Girdled	−24 h	4.5	0.00	0.00	0.02	0.19	0.97
		+1 h	23.6	0.29	0.03	0.24	0.16	0.94
		+72 h	22.9	1.25	0.03	0.47	0.91	0.79
Average ± SE		−24 h	7.3 ± 1.5	0.37 ± 0.20	0.12 ± 0.08	1.21 ± 1.00	0.73 ± 0.24	0.97 ± 0.01
		+1 h	23.8 ± 4.0	0.66 ± 0.14	0.06 ± 0.01	0.78 ± 0.42	1.20 ± 0.58	0.94 ± 0.01
		+72 h	22.8 ± 3.9	0.75 ± 0.17	0.46 ± 0.33	2.45 ± 1.22	0.70 ± 0.20	0.89 ± 0.04

n.a.: data not available

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Table 2. $\delta^{13}C$ of bulk leaves, bulk roots, microbial biomass and bulk phloem organic matter (OM), before (−24 h) and after a water pulse (+72 h, and only for phloem OM: +1 h) for *Fagus sylvatica* mesocosms grown under different temperatures (4 °C, 12 °C and 20 °C), combined ($n = 1$) with two girdling treatments (ungirdled and girdled).

		Leaf $\delta^{13}C$ (‰)		Root $\delta^{13}C$ (‰)		Microbial $\delta^{13}C$ (‰)		Phloem OM $\delta^{13}C$ (‰)		
Temperature	Girdling	−24 h	+72 h	−24 h	+72 h	−24 h	+72 h	−24 h	+1 h	+72 h
4	Ungirdled	−25.93	−29.06	−29.71	−30.94	−24.40	−20.63	−28.72	n.a.	−28.75
	Girdled	−25.70	−28.43	−30.01	−30.25	−21.90	−20.57	−28.11	−28.64	−28.51
12	Ungirdled	−25.81	−28.76	−28.88	−30.42	−23.75	−18.20	−28.69	−29.29	−27.91
	Girdled	−26.49	−28.71	−29.82	−30.35	−24.66	−20.79	−30.01	−29.77	−28.97
20	Ungirdled	−25.01	−28.82	−31.53	−33.08	−24.93	−20.18	−30.16	−28.88	−29.51
	Girdled	−25.75	−28.57	−30.12	−30.90	−24.20	−19.13	−27.05	−29.72	−29.63

n.a.: data not available

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Table 3. Soil CO_2 efflux (either as $^{12}CO_2$ or $^{13}CO_2$ flux) and $\delta^{13}C$ of soil CO_2 efflux ($\delta^{13}C$) before (−24 h) and after (+2 h) a water pulse of *Fagus sylvatica* mesocosms grown under different temperatures (4 °C, 12 °C and 20 °C), combined ($n = 1$) with two girdling treatments (ungirdled and girdled). Experimental values of $F_{^{12}CO_2}$ for −24 h and +2 h are assumed to be equal to soil CO_2 efflux values, while experimental $F_{^{13}CO_2}$ values are calculated from $F_{^{12}CO_2}$ and $\delta^{13}C$ of experimental soil CO_2 efflux (see material and method for detailed description). Values at −24 h were used to set up the model. Modelled fluxes after the pulse (+2 h) are based on the assumption that the only response to the water pulse was a change in soil physical properties.

		−24 h – experimental			+2 h – modelled			+2 h – experimental		
Temperature	Girdling	$F_{^{12}CO_2}$ (mmol m^{-2} min^{-1})	$F_{^{13}CO_2}$ (mmol m^{-2} min^{-1})	$\delta^{13}C$ (‰)	$F_{^{12}CO_2}$ (mmol m^{-2} min^{-1})	$F_{^{13}CO_2}$ (mmol m^{-2} min^{-1})	$\delta^{13}C$ (‰)	$F_{^{12}CO_2}$ (mmol m^{-2} min^{-1})	$F_{^{13}CO_2}$ (mmol m^{-2} min^{-1})	$\delta^{13}C$ (‰)
4	Ungirdled	15.82	0.17	−17	67.22	0.74	−17	49.31	0.54	−20.3
	Girdled	24.01	0.26	−14	102.06	1.13	−14	98.15	1.07	−21
12	Ungirdled	28.54	0.31	−17.2	121.31	1.33	−17.2	105.98	1.16	−22.2
	Girdled	48.14	0.53	−19.7	204.58	2.24	−19.7	73.57	0.8	−22.8
20	Ungirdled	22.31	0.24	−20.6	94.8	1.04	−20.6	95.61	1.04	−24.4
	Girdled	24.63	0.27	−22.4	104.67	1.14	−22.4	191.95	2.1	−23.7

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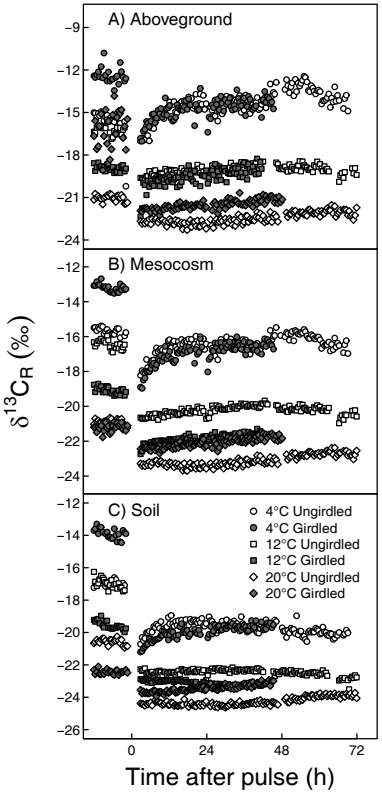

Fig. 1. $\delta^{13}C$ of aboveground respiration ($\delta^{13}C_{R_{above}}$, **A**), mesocosm respiration ($\delta^{13}C_{R_{mesocosm}}$, **B**) and soil CO_2 efflux ($\delta^{13}C_{R_{soil}}$, **C**) for beech mesocosms before and after a water pulse given at time = 0. The *Fagus sylvatica* mesocosms were grown under different temperatures (4 °C, 12 °C and 20 °C), combined ($n = 1$) with two girdling treatments (ungirdled and girdled). On-line IRMS measurements were performed in the dark, however, plants were exposed to light for 15 min starting at the water pulse (time = 0) to assimilate C immediately after the pulse.

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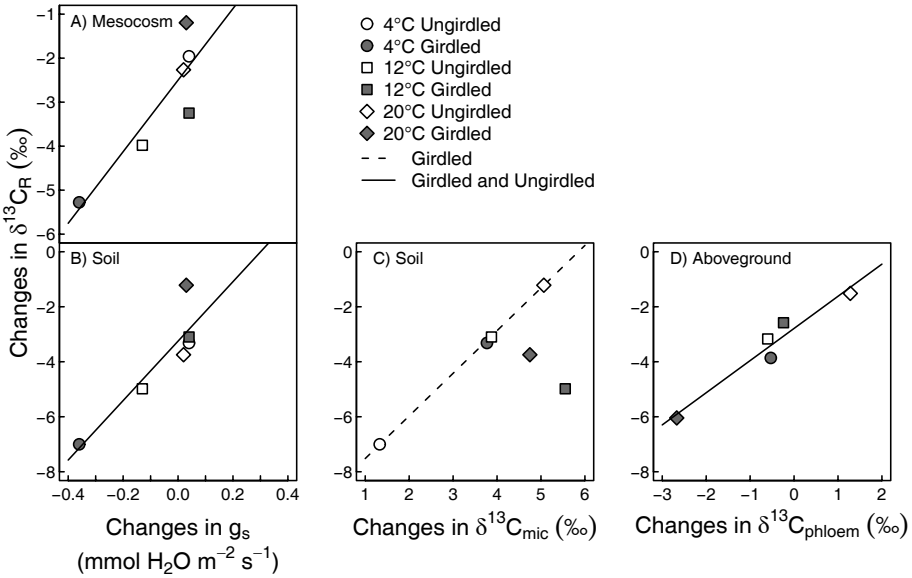

Fig. 2. Relationships between changes in $\delta^{13}C$ of respired CO_2 ($\delta^{13}C_R$; these changes were estimated by comparing $\delta^{13}C_R$ pre-pulse to $\delta^{13}C_R$ post-pulse, averaged over 3 h) and changes in other variables (between −24 h and +2 h): changes in $\delta^{13}C$ of mesocosm respiration ($\delta^{13}C_{R_{mesocosm}}$, **A**), as well as in $\delta^{13}C$ of soil CO_2 efflux ($\delta^{13}C_{R_{soil}}$, **B**), and changes in stomatal conductance (g_s); changes in $\delta^{13}C_{R_{soil}}$ and in microbial biomass $\delta^{13}C$ ($\delta^{13}C_{mic}$, **C**); changes in $\delta^{13}C$ of aboveground respiration ($\delta^{13}C_{R_{above}}$) and in $\delta^{13}C$ in phloem organic matter ($\delta^{13}C_{phloem}$, **D**) for *Fagus sylvatica* mesocosms were grown under different temperatures (4 °C, 12 °C and 20 °C), combined ($n = 1$) with two girdling treatments (ungirdled and girdled). In panel **(A)** and **(B)**, significant linear regressions for all trees: **(A)**: $y = 8.13x - 2.50$, $R^2 = 0.77$, $p = 0.021$; **(B)**: $y = 10.82x - 3.25$, $R^2 = 0.80$, $p = 0.017$; **(D)**: $y = 1.17x - 2.79$, $R^2 = 0.95$, $p = 0.005$. In panel **(C)**, only the linear regression for girdled trees was highly significant: $y = 1.55x - 9.1$, $R^2 = 0.99$, $p = 0.004$.

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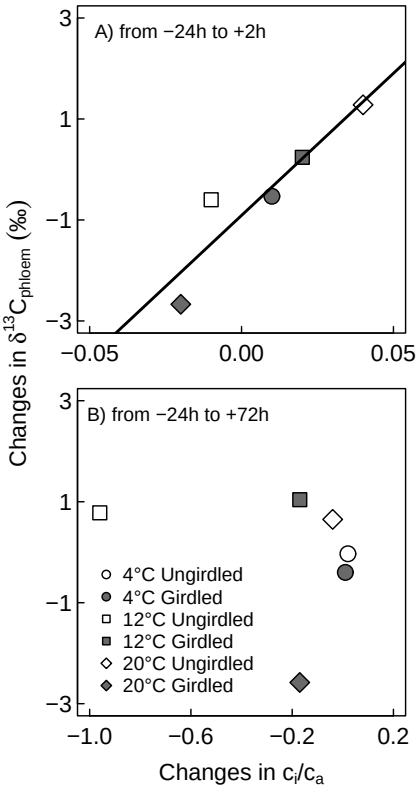

Fig. 3. Relationships between water pulse-induced changes in $\delta^{13}C$ of phloem organic matter ($\delta^{13}C_{phloem}$) and the ratio between leaf-internal and ambient CO_2 concentrations (c_i/c_a) between −24 h and +2 h (**A**, $y = 56.29x + 0.91$, $R^2 = 0.86$, $p = 0.024$) as well as between water pulse-induced changes in $\delta^{13}C_{phoem}$ and c_i/c_a between −24 h and +72 h (**B**, $y = -0.91x + 0.29$, $R^2 = 0.73$, $p = 0.065$) for *Fagus sylvatica* mesocosms were grown under different temperatures (4 °C, 12 °C and 20 °C), combined ($n = 1$) with two girdling treatments (ungirdled and girdled).

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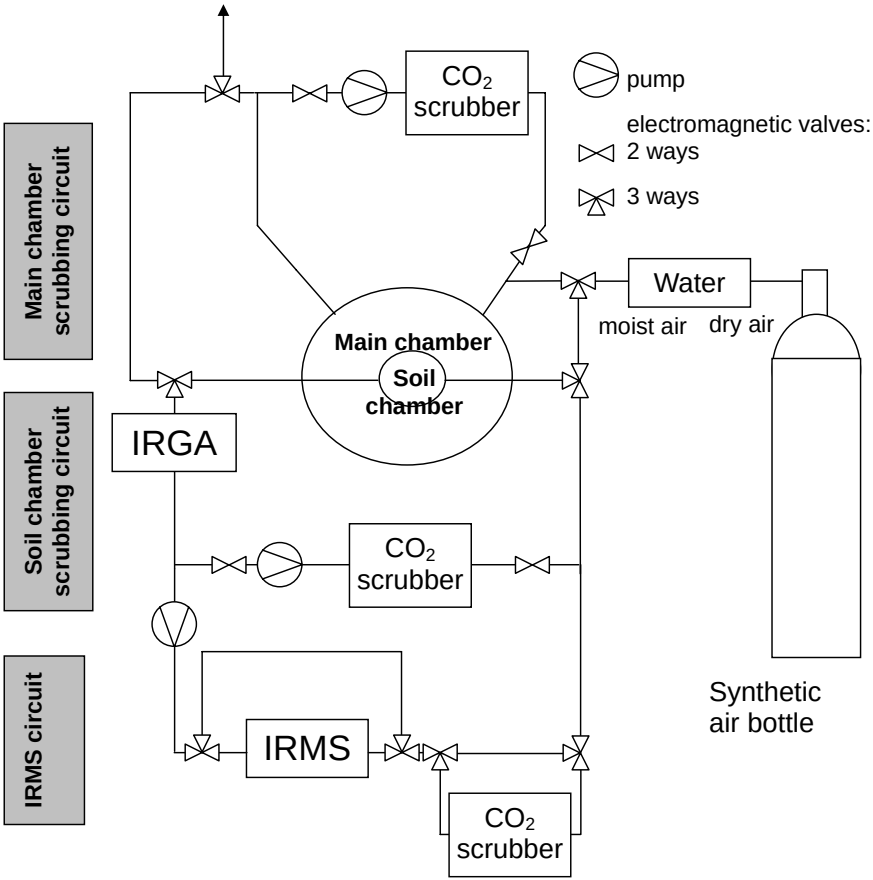

Fig. A1. Air flow for online measurements of $\delta^{13}C$ in CO_2. EV, IRGA and IRMS indicate electro-valves, infra-red gas analyser and isotope ratio mass spectrometer, respectively. The soil chamber is located inside the main chamber; both are independently connected to the IRMS and IRGA circuits.

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