Drought effects on allocation of recent carbon: from beech leaves to soil CO₂ efflux

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Received: 24 March 2009
Accepted: 23 July 2009


Key words: ¹³C, Fagus sylvatica, isotopes, labelling, phloem, soil microbes, translocation, water stress.

Summary

• Recent studies have highlighted a direct, fast transfer of recently assimilated C from the tree canopy to the soil. However, the effect of environmental changes on this flux remains largely unknown.
• We investigated the effects of drought on the translocation of recently assimilated C, by pulse-labelling 1.5-yr-old beech tree mesocosms with ¹³CO₂. ¹³C signatures were then measured daily for 1 wk in leaves, twigs, coarse and fine root water-soluble and total organic matter, phloem organic matter, soil microbial biomass and soil CO₂ efflux.
• Drought reduced C assimilation and doubled the residence time of recently assimilated C in leaf biomass. In phloem organic matter, the ¹³C label peaked immediately after labelling then decayed exponentially in the control treatment, while under drought it peaked 4 d after labelling. In soil microbial biomass, the label peaked 1 d after labelling in the control treatment, whereas under drought no peak was measured. Two days after labelling, drought decreased the contribution of recently assimilated C to soil CO₂ efflux by 33%.
• Our study showed that drought reduced the coupling between canopy photosynthesis and belowground processes. This will probably affect soil biogeochemical cycling, with potential consequences including slower soil nitrogen cycling and changes in C-sequestration potential under future climate conditions.

Introduction

Climate models predict not only higher temperatures as a result of global warming, but also changing precipitation patterns with an increasing risk of drought periods (IPCC, 2007). Both higher temperatures and periods with limited water supply probably alter the ability of terrestrial ecosystems to act as C sinks (Schimel, 1995; IPCC, 2007). During the European 2003 summer drought, the net primary productivity of forests decreased because of a large reduction in photosynthesis and a relatively smaller decrease in plant and soil microbial CO₂ efflux (Ciais et al., 2005). Beech forests in Central Europe were among those which showed the largest reductions in net ecosystem productivity (Ciais et al., 2005). While European beech (Fagus sylvatica L.) is one of the most abundant tree species in Central Europe and at present is intensively promoted by forest management strategies, it is also known to be relatively drought-sensitive (Backes & Leuschner, 2000; Gessler et al., 2004a.)

During a drought period, plants face the dilemma ‘to lose water to gain carbon’ (Chaves et al., 2003). Under water stress conditions, increased stomatal closure and reduced mesophyll conductance are the main causes of decreased
photosynthesis rates, by reducing CO₂ diffusion from the atmosphere to the carboxylation sites (Chaves et al., 2003; Flexas et al., 2006). Whereas drought-induced regulation of assimilation is relatively well known, there is not much information available on C export from the leaf and on its transport within the plant under drought conditions. Some studies, focusing on the transport of recent assimilates under water stress within plants from leaves to sink organs (e.g. Plaut & Reinhold, 1965; Wardlaw, 1969; Deng et al., 1990; Li et al., 2003), found the translocation of newly assimilated carbon from source leaves to be delayed under severe water stress. While Wardlaw (1969) pointed out that the effects of water stress on C translocation may result indirectly from effects on growth, other studies (Plaut & Reinhold, 1965; Deng et al., 1990) have proposed that drought may directly affect the translocation pathway, for example by impaired phloem loading or by reduced transport velocity in the sieve tubes.

However, the interest in ecosystem C dynamics under future climate conditions, and particularly under drought conditions, is rising. In addition, about half of the soil CO₂ efflux is thought to originate from recent (i.e. several days old) photosynthates (Högberg & Read, 2006), thus indicating a close coupling of aboveground and belowground processes. Unfortunately, there is only little, sometimes contradictory, information about such coupling under drought conditions. In a study on wheat plants, Palta & Gregory (1997) found the allocation of recent C to roots and to soil CO₂ efflux to be higher under drought, 2 d after ¹³C pulse-labelling. By contrast, in European shrublands Gorissen et al. (2004) measured a significant decrease in the allocation of recent C to the soil and to soil microorganisms under drought, 3 d after ¹⁴C pulse-labelling. However, neither of these studies accounted for the time course of the allocation of recent C from the plants to the soil. The ¹⁴C and ¹³C pulse-labelling studies that have traced the fate of newly assimilated C over time in trees and ecosystems (e.g. Carbone et al., 2007; Högberg et al., 2008) have not yet investigated the effects of changes in water availability.

The present study examined the influence of limited water availability on the transport of recently assimilated C in beech mesocosms over time, using a ¹³C pulse-labelling approach. We hypothesized that the transport of recent assimilates (¹³C-labelled) from source leaves to the root system would be reduced in both amount and velocity under drought conditions, resulting in delayed and decreased ¹³C peaks in soil microbial biomass and soil CO₂ efflux. To test these hypotheses, we followed the pathway of recently assimilated C within the trees, to soil microorganisms and back to the atmosphere, via soil CO₂ efflux, during 6 d after having applied a ¹³C pulse to trees grown under a control treatment receiving sufficient water and under a drought treatment with limited water supply.

Materials and Methods

Experimental design and management

One-year-old beech trees (Fagus sylvatica L.) were grown in a tree nursery in Riedlingen, Germany (48°09’N, 9°30’E, 554 m above sea level (asl)). In November 2006, these trees were transplanted into pots (35.5 cm width × 25.5 cm depth × 31.5 cm height with a ground area of 0.09 m⁻², eight trees per pot) filled with sieved and homogenized soil from the field site in Tuttingen, Germany (47°59’N, 8°59’E, 750 m asl), on a layer of fleece over 2 cm of expanded clay for drainage. The soil was a leptic rendzic phaëzom derived from Weißenburg betula and gamma series with a pH of about 7.5 at 0.6 m depth (for a detailed site description see Gessler et al., 2004a, 2005). The Pots were kept outside at the Helmholtz Zentrum München, München, Germany (48°13’N, 11°35’E, 493 m asl) during the winter and transferred to a glasshouse in March 2007. The climate in the glasshouse mimicked outside conditions, and the glasshouse air temperature and relative humidity were measured every 30 min. A polyvinyl chloride (PVC) collar for soil CO₂ efflux measurements (10 cm diameter × 8 cm high) was inserted (1 cm deep) in the pots in April 2007.

Before starting the drought treatment, the pots were irrigated with 1.4 mm of demineralized water each day. Starting 1 June 2007, when the leaves were fully developed, irrigation of the control treatment (n = 6 pots) was increased, to account for increasing plant water demand, to 2.4 mm d⁻¹, which is close to the daily long-term average amount of summer precipitation measured at the field site Tuttingen (Gessler et al. 2001). By contrast, the irrigation of the drought treatment (n = 6 pots) was reduced to 0.9 mm d⁻¹ (i.e. 38% of the control). During the treatment period, soil moisture was measured every 30 min in each pot using capacitance probes (providing an integrated measure of the 0–15 cm soil depth; EC-20; Decagon Devices Inc., Pullman, WA, USA). During the end of June and in July 2007, stomatal conductance (gₛ) and maximal photosynthetic rate (Aₘₐₓ) were measured weekly between 09:00 h and 17:00 h using a portable gas-exchange analyser (GSF-3000; Walz, Effeltrich, Germany) at a photosynthetic photon flux density (PPFD) of 1600 μmol m⁻², a CO₂ concentration of 380 μmol mol⁻¹, a temperature of 26 ± 0.5°C and a vapor pressure deficit of 16 ± 2 Pa kPa⁻¹ in the cuvette.

Labelling

The trees were labelled with ¹³CO₂ on 24 July 2007, as follows. Before labelling, the soil was sealed with plastic foil and TeroStat (Henkel Teroson GmbH, Heidelberg, Germany) to minimize diffusion of the labelled CO₂ into the
soil. Inside the glasshouse, an airtight transparent plastic tent (ethylen-tetrafluoroethylen) with a volume of approx. 7 m$^3$ was erected over the pots. Before labelling, the CO$_2$ concentration in the tent atmosphere was scrubbed down to approx. 300 µmol mol$^{-1}$ by pumping the tent air through soda lime. Labelling started at 08:45 h with 2% CO$_2$ (≥ 95 atom% $^{13}$C) in 98% N$_2$ (Spectra Gases Ltd, Littleport, UK) supplied at a flow rate of approx. 1.1 min$^{-1}$ and lasted for 4 h. The use of fans ensured good mixing of the air inside the tent, and additional light was provided by metal halide lamps (HQI-TS 400W/D; Osram, Munich, Germany), supplying a canopy-height PPFD of approx. 450 µmol m$^{-2}$ s$^{-1}$. During the labelling period, the air temperature was measured and the $^{13}$CO$_2$ concentration inside the tent was monitored and its $^{13}$C signature measured. The mean air temperature was 28.9°C during labelling, slightly higher than the ambient air temperature in the glasshouse (27.1°C). During the first 2 h of labelling, the CO$_2$ concentration and the $^{13}$C signature inside the tent rose to 775 ± 16 µmol mol$^{-1}$ and 78.2 ± 1.3 atom% $^{13}$C, respectively, then remained stable during the following 2 h.

After labelling, the fumigation tent and the plastic foil covering the soil were removed and the glasshouse was flushed with outside air to allow rapid dilution of the remaining $^{13}$CO$_2$ in the glasshouse atmosphere.

Sample collection

Leaves, twigs, soil and soil CO$_2$ efflux were sampled over a 6-d period: 5, 23, 29, 46, 53, 72, 96, 120 and 144 h after the end of the labelling. At each sampling time and in each pot ($n = 5$ per treatment), one twig with its attached leaves was sampled for leaf and phloem measurements, and one soil core (2 cm in diameter) was taken over the entire depth of the pot for microbial biomass measurements (see soil microbial biomass section below). At each sampling time (starting 23 h after the end of the labelling), and in one pot per treatment, one entire tree was harvested, and fine roots (< 1 mm in diameter), coarse roots (≥ 2 mm in diameter), aboveground biomass (separated into leaves and stems plus twigs) and stem phloem exudates were sampled. Soil holes resulting from coring and entire tree sampling were filled with the original soil from the experiment. In addition, two trees per treatment were sampled 8, 10 and 12 d (i.e. 192, 240 and 288 h) after the end of the labelling, and fine and coarse roots, as well as aboveground biomass, were sampled. At the end of the experiment the total plant biomass of each pot, as well as the biomass of leaves, twigs plus stem, fine roots and coarse roots of each tree was determined. In addition, five additional pots per treatment that remained unleaved were sampled to provide the natural $^{13}$C background of plant compartments. All bulk plant biomass samples were dried (for 48 h at 65°C), weighed and ground before subsequent analyses were carried out.

Organic matter in plant compartments

Water-soluble organic matter (OM) was extracted from leaves, stems and coarse and fine roots, as follows. First, 1 ml of double-deionized water was added to 45–55 mg of dry sample material and agitated (for 1 h at 4°C). Samples were then heated (for 10 min at 95°C), cooled down to room temperature and centrifuged (for 10 min at 12 000g). The supernatant represents a mixture of sugars, organic acids and amino acids with high turnover rates (Brandes et al., 2006). A 0.15 ml sample of supernatant was transferred to tin capsules (IVA Analysetechnik, Meerbusch, Germany), water was evaporated at 60°C and the remaining OM was analysed for $\delta^{13}$C and C content. For the determination of total OM C content and $\delta^{13}$C, 0.4–0.8 mg of the dried homogenized plant material was analysed (see isotope mass spectrometry section below). $\delta^{13}$C of nonwater-soluble OM was calculated using the following mass balance equation:

$$\delta^{13}C_{\text{non soluble}}(\%o) = \frac{C_{\text{total}} \cdot \delta^{13}C_{\text{total}} - C_{\text{soluble}} \cdot \delta^{13}C_{\text{soluble}}}{C_{\text{total}} - C_{\text{soluble}}}$$

Eqn 1

($C_{\text{total}}$ and $C_{\text{soluble}}$ are the amounts of C in total organic and water-soluble OM, respectively). For validation, we compared 12 measured $\delta^{13}$C samples of water-insoluble OM, extracted according to Gessler et al. (2008), with $\delta^{13}$C values obtained according to Eqn 1. The relationship of calculated $\delta^{13}$C values with measured values of nonwater-soluble OM was highly significant ($R^2 = 0.93, P < 0.001$).

Phloem OM was extracted according to an exudation method described by Gessler et al. (2004b). Using a scalpel, 20–80 mg of active phloem tissue was removed from twigs (at the twig–stem transition) and stems (at the root–stem transition). The bark pieces were left to exude in 2 ml of polyphosphate solution (0.01 M, pH 7) for at least 5 h to obtain phloem exudates. The supernatant was decanted and kept frozen. Then, 0.3–0.4 ml of phloem exudate was transferred to tin capsules and water was evaporated by heating at 60°C before isotope analysis was carried out (see isotope ratio mass spectrometry section below). Previous studies with beech (Schneider et al., 1996) showed that contamination of phloem exudates with cellular constituents is negligible under the experimental conditions applied here.

Soil microbial biomass

The $\delta^{13}$C of soil microbial biomass C was determined at each sampling time from 5 to 144 h after labelling, using a fumigation-extraction method (Vance et al., 1987). In brief, soils were sieved (through 2-mm mesh) and two 15-g subsamples were taken from each soil sample: one was fumigated for 24 h with chloroform vapour, while the other was not fumigated. Microbial C was extracted by vigorous shaking in K$_2$SO$_4$ (0.03 M for $^{13}$C measurements, 0.5 M...
for C measurements) for 30 min, then filtered. The fumigated and nonfumigated samples of microbial C were lyophilized before isotope ratio analysis was carried out. Biomass C samples (pooled from days 1–6 after labelling) were kept frozen until required for analysis of total organic C, which was performed using an automated TOC/N-analyser (DIMA TOC-100; Dimatec, Essen, Germany). To calculate the $\delta^{13}\text{C}$ of soil microbial biomass, the following mass balance equation was used:

$$\delta^{13}\text{C}_{\text{microbes}}(\text{atom}%) = \frac{\delta^{13}\text{C}_f - \delta^{13}\text{C}_{nf} \cdot C_{nf}}{C_f - C_{nf}} \quad \text{Eqn 2}$$

($f$ and $nf$ are the extracts from the fumigated and nonfumigated subsamples, respectively (expressed as $\mu$g g$^{-1}$ of dry soil). Gravimetric soil water content was determined by comparing the mass of approx. 5 g of soil before and after drying at 105°C. Total C content in soil microbial biomass was calculated as ((total C in fumigated soil)–(total C in nonfumigated soil))/0.45 (Vance et al., 1987).

### Soil CO$_2$ efflux rate and $^{13}$C signature

A soil chamber (LI-6400-09; Li-Cor Inc., Lincoln, NE, USA) was tightly fitted on the collars installed in each pot, and the soil CO$_2$ efflux rate was measured using a closed-path infrared gas analyser (LI-6400; Li-Cor Inc.). The isotopic signature of the soil CO$_2$ efflux was estimated from the CO$_2$ concentration and $^{13}$C in air samples using a Keeling plot approach. In brief, it consists of a twoend-members mixing model that mixes the CO$_2$ emitted by the soil with the CO$_2$ of the atmospheric background (Keeling, 1958). The $^{13}$C in the chamber head space is linearly related to the inverse of the corresponding CO$_2$ concentration, and thus the intercept of the regression gives the isotopic composition of the soil CO$_2$ efflux.

At each sampling time-point five 5-ml air samples were taken from each chamber and transferred to 3.7-ml glass vials (Exetainer®; Labco Ltd, High Wycombe, UK) that had previously been evacuated and filled with N$_2$. The first sample was taken as soon as the CO$_2$ concentration started to rise linearly, approx. 30 s after closing the chamber, and the four subsequent samples were taken at approx. 20 $\mu$mol mol$^{-1}$ intervals as the CO$_2$ concentration rose inside the chambers. The samples were analysed for $^{13}$C within 2 d as described later. Additionally, the soil CO$_2$ efflux rates of each collar were measured at midday during the first 4 d after labelling.

### Isotope ratio mass spectrometry measurements

The $^{13}$C values and C contents of solid samples were measured by combustion in an elemental analyser (microbial extracts: EuroEA 3000 Series; HEKAtech, Germany; all other solid samples: NA 2500; CE Instruments, Milan, Italy), that was coupled via a Conflo II interface to an isotope ratio mass spectrometer (DeltaPlus; Finnigan MAT GmbH, Bremen, Germany).

The $^{13}$C of the soil CO$_2$ efflux was measured using a modified Finnigan Gasbench II periphery (Finnigan MAT) equipped with a custom-built cold trap and coupled to an isotope ratio mass spectrometer (DeltaPlusXP; Finnigan MAT).

Isotopic values are expressed in $\delta$ notation ($\%_\text{m}$), relative to the Vienna Pee Dee Belemnite (VPDB) standard. In addition, to estimate the amount of $^{13}$C added by pulse-labelling, $\delta$ notations were expressed, in atom%, as follows:

$$\text{atom}%) = \frac{100 \cdot 0.0111802 \cdot (\delta^{13}\text{C} + 1) + 1}{1 + 0.0111802 \cdot (\delta^{13}\text{C} + 1)} \quad \text{Eqn 3}$$

($0.0111802$ is the standard value for the isotope ratio of VPDB). To calculate excess $^{13}$C values in plant compartments, we used the following equation:

$$\text{excess}^{13}\text{C}_{\text{plant}} = \frac{\text{atom}\%_b - \text{atom}\%_0}{100} \cdot B \cdot C\% \quad \text{Eqn 4}$$

($\text{excess}^{13}\text{C}_{\text{plant}}$ (expressed as mg m$^{-2}$) is the total amount of $^{13}$C added by pulse-labelling to the DW of plant compartments and plant C pools per ground area (each pot had a ground area of 0.09 m$^2$); atom%$_b$ is the atom% of the sample; atom%$_0$ is the atom% of the natural background averaged per treatment (measured in total organic matter of unlabelled plant compartments; $n = 5$); B is the dry weight of plant biomass compartments averaged per treatment (expressed as mg dry biomass m$^{-2}$ ground area; $n = 4$); and C% is the percentage of C in the sample). We calculated the $^{13}$C excess in soil microbial biomass as follows:

$$\text{excess}^{13}\text{C}_{\text{microbes}} = \frac{\text{atom}\%_b - \text{atom}\%_0}{100} \cdot \text{TOC} \quad \text{Eqn 5}$$

($\text{excess}^{13}\text{C}_{\text{microbes}}$ (expressed as $\mu$g g$^{-1}$ of dry soil) is the total amount of $^{13}$C added by pulse-labelling to soil microbial biomass; and TOC is the total organic C content of soil microbial biomass). As measurements of the natural $^{13}$C background of soil microbial C were not available, we used the average of the $^{13}$C background value of root OM from the control treatment ($n = 5$, see Table 2) as a proxy for the atom%$_b$ of soil microbial C. To calculate excess $^{13}$C in the soil CO$_2$ efflux we used the following equation:

$$\text{excess}^{13}\text{C}_{\text{soil}} = \frac{\text{atom}\%_b - \text{atom}\%_0}{100} \cdot F \quad \text{Eqn 6}$$

($\text{excess}^{13}\text{C}_{\text{soil}}$ (expressed as mg m$^{-2}$ h$^{-1}$) is the total amount of $^{13}$C added by pulse-labelling to soil CO$_2$ efflux; and F is
the soil CO₂ efflux (expressed as mg of C m⁻² h⁻¹) averaged for each pot from daily measurements taken 1–4 d after labelling. As measurements of the natural ¹³C background of soil CO₂ efflux were not available, we used the ¹³C background values of root OM averaged per treatment (see Table 2) as a proxy for the atom%₀ value of the soil CO₂ efflux. Small changes in the ¹³C background values had only a minor influence on the ¹³C excess values of soil CO₂ efflux (e.g. a decrease in the background ¹³C of 2₀/₂₀ increased the ¹³C excess values of soil CO₂ efflux only by 0.0026%).

Statistical analysis

Overall differences between treatments in biomass, photosynthesis and soil CO₂ efflux were calculated using t-tests. The effect size of the drought treatment was calculated as %effect = 100(drought–control)/control. To test overall differences in the progression of the ¹³C label between treatments, we used linear mixed effect (lme) models with treatment and time (h after labelling) as fixed effects and pot as random effect. We also accounted for the autocorrelation structure of the time series. t-tests were applied to test for differences in treatments at single time-points. To estimate mean residence and half-life times of the ¹³C label in leaf and phloem, the following exponential decay function was used:

$$N(t) = N_0e^{-\lambda t}$$

Eqn 7

$$t$$ is the time in days after labelling; $$N_0$$ is the initial quantity of ¹³C (δ or excess) at time $$t = 0$$ (¹³C peak); λ is the decay constant; and $$N(t)$$ is the quantity of ¹³C after time $$t$$. The mean residence time ($$\tau$$) of ¹³C in the plant compartments was then calculated as $$\tau = 1/\lambda$$, and the half-life time was calculated as $$t_{1/2} = \ln(2)/\lambda$$. All statistical analyses were performed using R 2.8.0 (R Development Core Team, 2007) with the nlme package for linear mixed effect models (Pinheiro et al., 2008).

Results

The air temperature in the glasshouse followed outside air temperature and was, on average, 21.3°C during June and July 2007 (Fig. 1a). The drought treatment effectively decreased soil moisture, on average, by 65% to about 30% relative soil water content (RW, Reichstein et al., 2003) over the 2 months of treatment (Fig. 1b). Photosynthesis ($$A_{max}$$), stomatal conductance ($$g_s$$) and soil CO₂ efflux rate were significantly decreased under drought (Table 1). Drought significantly reduced overall tree biomass, but did not significantly affect leaf biomass or the root : shoot ratio (Table 2). Microbial biomass C was also left unchanged (Table 2). Drought significantly increased the C concentration in water-soluble OM in leaves by 27% and also increased the natural abundance of ¹³C in the OM of plant compartments (Table 2). In order to avoid the confounding effects of a dilution effect of the label (caused by differences in tree biomass and in the concentration of water-soluble organic C) and of differences in the natural abundance of ¹³C between treatments, the ¹³C excess values are given in addition to the ¹³C values (except for ¹³C in phloem OM, for which we have no biomass estimates).

Table 1 Effect of drought on maximum photosynthetic rate ($$A_{max}$$), stomatal conductance ($$g_s$$) and soil CO₂ efflux rate

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Drought</th>
<th>P-value</th>
<th>Percentage effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>$$A_{max}$$ (µmol CO₂ m⁻² s⁻¹)</td>
<td>7.49 ± 0.37</td>
<td>4.68 ± 0.31</td>
<td>0.001</td>
<td>-37</td>
</tr>
<tr>
<td>$$g_s$$ (mmol H₂O m⁻² s⁻¹)</td>
<td>116.54 ± 12.11</td>
<td>59.85 ± 6.47</td>
<td>0.003</td>
<td>-48</td>
</tr>
<tr>
<td>Soil CO₂ efflux rate (mg C m⁻² h⁻¹)</td>
<td>87.57 ± 3.81</td>
<td>65.58 ± 3.68</td>
<td>0.002</td>
<td>-25</td>
</tr>
</tbody>
</table>

Mean values ± 1SE are given. Treatment effect size was calculated as percentage effect = 100(drought–control)/control. The $$A_{max}$$ and $$g_s$$ values were measured on leaves of randomly selected beech trees from different pots during 26 June to 21 July (control: n = 8; drought: n = 6). Soil CO₂ efflux was measured in each pot (n = 5 per treatment) on a daily basis from 25 to 28 July.
At the first sampling time-point, 5 h after the end of labelling, the $^{13}C$ excess in the total OM of leaves ranged from 423 to 3149 mg of $^{13}C$ m$^{-2}$(i.e. 750–3644% $^{13}C$), indicating significant assimilation of $^{13}C$ by the beech trees during labelling. However, less label was taken up by trees in the drought treatment, reflecting lower assimilation rates and resulting in lower $\delta^{13}C$ and $^{13}C$ excess values in the total OM of all plant compartments (Fig. 2a–h) and in leaf water-soluble OM (by up to 50%, Fig. 3f). In both treatments, the $^{13}C$ and $^{13}C$ excess in total and water-soluble leaf OM decreased exponentially with time (Figs 2a,e and 3a,f). However, the mean residence time (i.e. the average time for which the $^{13}C$ measured 5 h after labelling remained in leaf and phloem OM) of $^{13}C$ excess in leaf OM was higher under drought: about 1 d longer for total and nonwater-soluble leaf OM and up to 2.6 d longer in leaf water-soluble OM (Table 3). These different time courses of $^{13}C$ excess within leaf total and water-soluble OM are further supported by a significant time $\times$ treatment interaction in the lme model (Table 4). Differences in $^{13}C$ dynamics were especially pronounced during the first day after labelling, when only 13% of the assimilated labelled total C (excess $^{13}C$) disappeared from the leaves in the drought treatment compared with 40% from leaves in the control treatment. However, 5 d later, about the same proportions of the excess $^{13}C$ label were absent from leaves of the drought-treatment (65%) and the control-treatment (69%) plants.

Drought significantly affected the dynamics of $^{13}C$ in twig and trunk phloem water-soluble OM over time. In twig phloem water-soluble OM, the exponential decrease in the control treatment (following the exponential decay function $N(t) = 2710e^{−0.022t}$; $R^2 = 0.77$, $P = 0.003$) contrasted with the bell-shaped pattern under drought, which peaked 4 d after the end of labelling (Fig. 3b; Table 4). The time course of $^{13}C$ in the drought treatment was best explained by a second-order polynomial function ($y = −63.73(t^2) + 466.39t + 464.15$; $R^2 = 0.95$, $P < 0.001$) during the measurement period. The time pattern of the drought effect was similar for stem phloem water-soluble OM with the highest $^{13}C$ value measured occurring 6 d after the end of labelling (Fig. 3c) (i.e. 2 d after having peaked in the twig phloem water-soluble OM).

The drought effects on the dynamics of $\delta^{13}C$ and $^{13}C$ excess in coarse and fine root water-soluble OM were consistent with the patterns observed for phloem water-soluble OM. Within 1 d after the end of labelling, $^{13}C$ excess was detected in roots of the control treatment, whereas it only started increasing after 8 d in the drought treatment (i.e. 2 d after having reached its maximum in the stem phloem water-soluble OM) (Fig. 3g,h).

While in the drought treatment the $\delta^{13}C$ and $^{13}C$ excess values of soil microbial biomass remained relatively stable during the measurement period after labelling (−10.78 ± 5.76%; 0.16 ± 0.05$^{13}C$ g$^{-1}$), a peak of about 40% was measured in microbial biomass of the control treatment 1 d after the end of labelling (Fig. 4a,c). Moreover, the observed differences between treatments in time dynamics of the $^{13}C$ excess values in soil microbial biomass were significant (Table 4).

The Keeling plot regressions used to calculate the $^{13}C$ and subsequently $^{13}C$ excess of soil CO$_2$ efflux were all significant ($R^2 > 0.90$ and $P < 0.01$). We measured very large $^{13}C$ values in soil CO$_2$ efflux during the first 24 h after the end of labelling (averaging about 7800%), these were probably caused by the back-diffusion of labelled CO$_2$ that had diffused into the soil pore spaces during labelling. This phenomenon was obviously more pronounced in the drought treatment than in the control treatment (Fig. 4b), presumably because of more air-filled soil pore space in the drier soil and therefore, these data were excluded from further analysis. However, after this initial phase (after day 2), the $^{13}C$ of soil CO$_2$ efflux was, on average, 599% (0.45 mg $^{13}C$ excess m$^{-2}$ h$^{-1}$) in the drought treatment and 844% (0.89 mg $^{13}C$ excess m$^{-2}$ h$^{-1}$) in the control treatment over

### Table 2: Biomass of beech tree compartments per ground area

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Drought</th>
<th>$P$-value</th>
<th>Percentage effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total tree (g m$^{-2}$)</td>
<td>934.18 ± 34.42</td>
<td>616.89 ± 19.31</td>
<td>$&lt; 0.001$</td>
<td>−34</td>
</tr>
<tr>
<td>Leaf (g m$^{-2}$)</td>
<td>106.25 ± 4.5</td>
<td>84.16 ± 8.6</td>
<td>0.063</td>
<td>−21</td>
</tr>
<tr>
<td>Twig and stem (g m$^{-2}$)</td>
<td>348.00 ± 18.16</td>
<td>241.75 ± 4.23</td>
<td>$0.001$</td>
<td>−30</td>
</tr>
<tr>
<td>Root (g m$^{-2}$)</td>
<td>480.13 ± 28.30</td>
<td>291.40 ± 14.76</td>
<td>$0.001$</td>
<td>−39</td>
</tr>
<tr>
<td>R : S ratio</td>
<td>1.07 ± 0.08</td>
<td>0.90 ± 0.05</td>
<td>0.141</td>
<td>−16</td>
</tr>
<tr>
<td>Leaf water-soluble OM ($\mu$g C mg$^{-1}$)</td>
<td>70.30 ± 5.30</td>
<td>90.50 ± 6.67</td>
<td>$&lt; 0.001$</td>
<td>+27</td>
</tr>
<tr>
<td>Microbial C ($\mu$g g$^{-1}$ dry soil)</td>
<td>718 ± 16</td>
<td>773 ± 52</td>
<td>0.335</td>
<td>+8</td>
</tr>
<tr>
<td>$\delta^{13}C$ leaf (%)</td>
<td>−29.31 ± 0.11</td>
<td>−27.81 ± 0.16</td>
<td>$&lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>$\delta^{13}C$ twig and stem (%)</td>
<td>−28.62 ± 0.23</td>
<td>−27.39 ± 0.13</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>$\delta^{13}C$ root (%)</td>
<td>−29.39 ± 0.44</td>
<td>−27.20 ± 0.11</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

Significant values ($P \leq 0.05$) of $t$-tests are shown in bold. Mean values ± 1SE are given. Treatment effect size was calculated as percentage effect = 100(drought−control)/control. Biomass is expressed as dry weight, root : shoot (R : S) ratio ($n = 4$ per treatment), leaf water-soluble organic matter (OM) in leaf biomass and soil microbial biomass C as well as natural abundance $\delta^{13}C$ values ($n = 5$ per treatment) for the control and drought treatments are shown.
days 2 to 6 after labelling, with the $^{13}$C excess values in soil CO$_2$ efflux of the drought treatment being significantly lower by at least 50% (Fig. 4b, d and Table 4).

**Discussion**

**Leaf carbon residence time**

In our experiment, drought reduced not only $A_{\text{max}}$ and consequently the incorporation of $^{13}$C label into the leaf tissue, but also the rate of loss of $^{13}$C excess from the leaves during the first 4 d after labelling. In agreement with our findings, Wardlaw (1969); Sung & Krieg (1979); Deng *et al.* (1990) and Li *et al.* (2003) have found longer retention times of recently assimilated C in leaves of cultivated plants under water-limited conditions using $^{14}$C pulse-labelling. This temporal pattern might be caused by the emission of biogenic volatile organic compounds (BVOC), reduced leaf respiration and loss to the atmosphere and/or reduced C export from the leaf to the phloem. Regarding

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*Fig. 2* Dynamics of $\delta^{13}$C and $^{13}$C excess in European beech leaves (a, e), stem and twigs (b, f), coarse roots (c, g) and fine roots (d, h) in control (open circles) and drought (closed triangles) treatments. (a, e). The mean value ± 1SE is given for five samples for each time-point until day 6 after labelling, then only one value per treatment was available. (b, c, d, f, g, h) $n = 1$ for each time-point with the dotted lines indicating a shift to another pot. The best fit of the exponential decay function (including data until 6 d after labelling) is given for control (dashed line) and drought (solid line) treatments. The significance levels of the $t$-tests between treatments at each time-point are given (*0.05 $\geq$ $P$ $>$ 0.01; **0.01 $\geq$ $P$ $>$ 0.001; ***0.001 $\geq$ $P$) in (a) and (e).
Fig. 3 Dynamics of δ^{13}C and δ^{13}C excess in water-soluble organic matter (OM) of European beech leaves (a, f), coarse roots (d, g) and fine roots (e, h), as well as δ^{13}C values of twig phloem OM (b) and stem phloem OM (c) in control (open circles) and drought (closed triangles) treatments. (a, b, f) The mean value ± 1SE is given for five samples for each time-point until day 6 after labelling, then only one value per treatment was available. (c, d, e, g, h) n = 1 for each time-point. The best fit (including data until 6 d after labelling) is given for control (dashed line, exponential decay function) and drought (solid line, panels a and f: exponential decay function; panels b–e and g–h: second-order polynomial function) treatments. The significance levels of the t-tests between treatments at each time-point are given (*0.05 > P > 0.01; **0.01 > P > 0.001; ***0.001 > P) in (a) and (b).
Table 3  Carbon mean residence time (MRT), half-life time (HLT) and coefficient of determination ($R^2$) from the exponential decay function (Eqn 7) of $^{13}$C excess and $\delta^{13}$C in plant organic matter (OM) pools during the first 6 d after labelling in drought and control treatments ($n = 9$ time-points per treatment)

<table>
<thead>
<tr>
<th>Carbon pool</th>
<th>Control</th>
<th>Drought</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRT (d) HLT (d) $R^2$</td>
<td>MRT (d) HLT (d) $R^2$</td>
</tr>
<tr>
<td>Excess $^{13}$C leaf OM</td>
<td>2.4 (2.0, 3.1) 1.7 (1.4, 2.1)</td>
<td>0.95</td>
</tr>
<tr>
<td>Excess $^{13}$C in leaf water-soluble OM</td>
<td>2.4 (1.8, 3.6) 1.6 (1.2, 2.5)</td>
<td>0.87</td>
</tr>
<tr>
<td>$\delta^{13}$C in leaf nonwater-soluble OM</td>
<td>2.3 (1.8, 3.0) 1.6 (1.3, 2.1)</td>
<td>0.94</td>
</tr>
<tr>
<td>$\delta^{13}$C in twig phloem OM</td>
<td>4.6 (3.2, 8.2) 3.2 (2.2, 5.7)</td>
<td>0.77</td>
</tr>
</tbody>
</table>

The 95% confidence intervals are given in brackets. All values were significant ($P \leq 0.05$).

–Polynomial function, therefore not available.

Table 4  Results of the linear mixed effect (lme) models to test for overall differences between treatments, time and their interaction until day 6 after labelling for $^{13}$C excess in leaf organic matter (OM), leaf water-soluble OM, soil microbial biomass and soil CO$_2$ efflux (from day 2 after labelling) as well as $\delta^{13}$C in twig phloem OM

<table>
<thead>
<tr>
<th></th>
<th>$^{13}$C excess in leaf OM</th>
<th>$^{13}$C excess in leaf water-soluble OM</th>
<th>$\delta^{13}$C in twig phloem OM</th>
<th>$^{13}$C excess in soil microbial biomass</th>
<th>$^{13}$C excess in soil CO$_2$ efflux</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F$-value $P$-value</td>
<td>$F$-value $P$-value</td>
<td>$F$-value $P$-value</td>
<td>$F$-value $P$-value</td>
<td>$F$-value $P$-value</td>
</tr>
<tr>
<td>Treatment</td>
<td>52.56 &lt; 0.001</td>
<td>3.51 0.098</td>
<td>6.04 0.039</td>
<td>2.49 0.153</td>
<td>12.68 0.007</td>
</tr>
<tr>
<td>Time</td>
<td>39.40 &lt; 0.001</td>
<td>31.34 &lt; 0.001</td>
<td>2.38 0.026</td>
<td>2.50 0.021</td>
<td>0.29 0.882</td>
</tr>
<tr>
<td>Treatment : Time</td>
<td>10.36 &lt; 0.001</td>
<td>7.62 &lt; 0.001</td>
<td>7.00 &lt; 0.001</td>
<td>2.18 0.042</td>
<td>0.32 0.862</td>
</tr>
</tbody>
</table>

Significant values ($P \leq 0.05$) are shown in bold.

Fig. 4  Dynamics of $\delta^{13}$C and $^{13}$C excess in microbial biomass (a, c) and soil CO$_2$ efflux (b, d). The inserted smaller panel in (b) shows the $\delta^{13}$C values during the first 2 d after labelling, when the signal was masked by back-diffusion of the $^{13}$C label from the soil. Values are mean ± 1SE ($n = 5$). The significance levels of the $t$-tests between treatments at each time-point are given (*0.05 ≤ $P$ > 0.01; **0.01 ≤ $P$ > 0.001; ***0.001 ≥ $P$). Control (open circles); drought (closed triangles).
the first point, it is known that up to 10% of newly assimilated C can be lost via the emission of BVOCs (Peñuelas & Llusia, 2003). However, the emission rates of BVOCs in beech are generally low. Simpraga et al. (2008) showed that BVOC emission represents less than 0.05% of the photosynthesis flux in this species. Even though there is some information that the BVOC emissions can decrease under severe or long-term drought (Llusia et al., 2008), we might–on the background of the generally low BVOC emission of beech–not assume a large impact on the observed leaf 13C patterns. Considering the second point, leaf respiration rates are less affected by drought than photosynthesis, resulting in an increased respiration : photosynthesis ratio in water-stressed plants than in well-watered plants (see reviews by Flexas et al., 2005, 2006; Atkin & Macherel, 2009). Drought stress almost always inhibits respiration in actively growing plant tissues (Atkin & Macherel, 2009). However, in fully expanded mature leaves, the response of respiration is more variable, with some studies even reporting a continuous or transitory increase in leaf respiration during the onset of severe water stress (Atkin & Macherel, 2009). As beech trees do not exhibit continuous leaf growth and production, and all leaves were fully developed at the start of the drought treatment, we hypothesize little effect of water-stress on leaf growth respiration in our experiment. In general, the overall changes in respiration were small compared with the large reduction in photosynthetic C uptake in response to drought (Atkin & Macherel, 2009). As a consequence, and in agreement with a previous study on cacao seedlings (Deng et al., 1990), it is unlikely that a reduction in leaf respiration of newly assimilated C is the main reason for the slower decrease of 13C excess in leaves under drought. In the third point we can exclude decreased C demand by roots as explanation for reduced C export from beech leaves under drought because the root : shoot ratio remained unchanged by the drought treatment. However, several other mechanisms may underlie decreased leaf C export under drought. First, mesophyll cells of water-limited plants require a higher osmotic potential to maintain their turgor, and photosynthates are consequently actively retained in the mesophyll (Van Bel & Gamalei, 1992). Indeed, we found a longer mean residence time of 13C excess (approx. + 50%) and an increased C content (approx. + 30%) in water-soluble OM (consisting of osmotically active sugars, amino acids, organic acids and their derivatives; Brandes et al., 2006) of beech leaves under drought, which is consistent with this mechanism. Second, tissue dehydration may also reduce the movement of assimilates from mesophyll cells to phloem-loading sites, as plasmodesmata appear to close when the turgor difference across them increases (Van Bel & Gamalei, 1992). Third, phloem loading itself may have been impaired (Deng et al., 1990). An explanation for this could be down-regulation of the expression of sucrose transporters responsible for phloem loading, as found in osmotically stressed (salt-stress) leaves of Plantago major (Pommerrenig et al., 2007). It is probable that a combination of the above-mentioned mechanisms is responsible for slower C export from beech leaves under drought.

Carbon transport in the phloem

Drought decreased phloem transport velocity in our experiment, as shown by the slower build-up of label in twig phloem sap in the drought treatment. Moreover, we found a 2-d delay between the 13C label peak in the twig and the trunk phloem in the drought treatment, which represents a very low phloem transport velocity of approx. 0.01 m h\(^{-1}\). In general, phloem transport rates in trees range from 0.5 to 1 m h\(^{-1}\) (Zimmermann & Braun, 1971). Keitel et al. (2003) observed natural abundance isotope patterns in the phloem of adult nonwater-stressed beech trees, which agreed well with this range. Recently, 13CO\(_2\) pulse-label experiments with 20-yr-old beech trees sufficiently supplied with soil water revealed phloem-transport velocities of approx. 1 m h\(^{-1}\) (D. Epron, pers. comm.). In a study on bean plants, Plaut & Reinhold (1965) showed a depressive effect of water stress on carbohydrate transport in the phloem and suggested an inhibitory effect of water stress on the movement of phloem sap in the sieve tubes. Considering the pressure flow hypothesis for phloem translocation (Münch, 1930), the pressure difference between source and sink, which drives the transport, arises from osmotic pressure differences generated by the loading and unloading of assimilates into and out of sieve elements and by the water potential of the surrounding apoplast (Patrick et al., 2001). Thus, if phloem loading is decreased (as shown in our experiment by the rather slow leaf export of 13C excess) and the water potential is decreased by drought, then the flow rate in the phloem should indeed be decreasing.

Roots, soil microbial biomass and soil CO\(_2\) efflux

In the control treatment, the 13C label peak in the soil microbial biomass was detected 24 h after exposure to 13CO\(_2\) and remained high until 48 h after labelling. This timeframe for our 0.5-m-high trees is consistent with the results of Högberg et al. (2008), who found a microbial 13C peak 48–96 h after pulse-labelling 2.4-m-tall Scots pine in a boreal forest. In the drought treatment, we found no increase in soil microbial biomass 13C, even after 6 d, suggesting that C transfer from plants to soil microbes was delayed by more than 5 d under drought. Such a delay would be consistent with the drought-induced increase in residence time of recent assimilates in the leaf and the decrease in phloem transport, as discussed above. 13C excess increased simultaneously in fine and coarse root water-
soluble OM on days 8–10 after labelling, suggesting that the \(^{13}\)C peak in soil microbial C may have appeared at around the same time in the drought treatment (unfortunately no microbial measurements are available following day 6 after labelling). Our results are consistent with the findings of Gorissen et al. (2004), who used a \(^{14}\)C labelling approach in shrublands and found that drought significantly reduced the amount of recent C allocated to soil microbial biomass by more than 60% after 72 h at two northern European sites, but were unable to detect any transfer of \(^{14}\)C to soil microbial biomass at a Mediterranean site. The consequence of such reduced input rates of labile C to soil microbes under drought conditions might be a shift of soil microbial community towards increased decomposition of soil organic C (Bradford et al., 2008).

The large \(^{13}\)C values of soil CO\(_2\) efflux observed in our study during the first 2 d after labelling were very likely caused by back-diffusion of the \(^{13}\)CO\(_2\) label out of the soil (also reported in other labelling experiments, e.g. Leake et al., 2006; Högb erg et al., 2008), despite our efforts to prevent \(^{13}\)CO\(_2\) from diffusing into the soil in the first place. The fixation of labelled C in soil air through carboxylation by phosphoenolpyruvate (PEP) carboxylase in roots and/or microorganisms is possible and might contribute to soil microbial biomass C and soil CO\(_2\) efflux. However, soil incubation studies have shown that long incubation times are required for significant amounts of C to be fixed by microorganisms (e.g. 61 d; Miltner et al., 2004). It can be noted that in the drought treatment, the \(^{13}\)C values of soil CO\(_2\) efflux were higher than the \(^{13}\)C values of fine and coarse root water-soluble OM from day 2 to day 6 after labelling. This was also discussed by Leake et al. (2006) and mainly arises from the difficulty in distinguishing live and dead roots. Fine root mortality has been shown to increase in beech sappings under drought (Meier & Leuschner, 2008), thereby increasing the probability of including dead roots when sampling fine roots.

We found no significant effect of drought on the \(^{13}\)C of soil microbial biomass over days 2 to 6 after labelling, which points towards a major contribution of root respiration to the \(^{13}\)C excess in soil CO\(_2\) efflux. The \(^{13}\)C excess value in soil CO\(_2\) efflux was 50% lower in the drought treatment. In combination with a reduction in soil CO\(_2\) efflux by 25%, this resulted in a 33% reduction of the contribution of recently (2–6 d previously) assimilated C to soil CO\(_2\) efflux under drought. This clearly shows a decreased contribution of recently assimilated C to soil CO\(_2\) efflux in beech mesocosms under drought conditions.

Conclusion

We followed the isotopic signal in beech tree mesocosms from leaves to soil CO\(_2\) efflux via phloem OM to roots and soil microbial biomass. Thereby, we were able to show a tight coupling between aboveground and belowground compartments, which is consistent with recent labelling studies performed under optimal water conditions (e.g. Carbone et al., 2007; Högb erg et al., 2008). Moreover, applying a drought treatment provided evidence that under drought conditions not only was less C assimilated, but also the coupling of soil microbial C and of soil CO\(_2\) efflux with canopy photosynthesis was significantly reduced. The drought-induced decoupling between canopy photosynthesis and belowground processes will probably affect belowground biogeochemical cycling, with potential consequences including slower soil nitrogen cycling and changes in C sequestration potential. As current climate projections predict an increase in the frequency of intensive drought events during the growing season, the drought-induced reduction in amount and velocity of C transferred from plants to the soil, as observed here, will need to be considered in biogeochemical models for the long-term C budget of forest ecosystems under future climate conditions.

Acknowledgements

We would like to thank Christian Clemenz for the installation and management of the glasshouse experiment; Hans Lang, Wolfgang Graf as well as Franz Buegger for the implementation of the labelling experiment; Tatja Dopatka and Johannes Schumacher for help with sample preparation; and Wolfgang Sternad and Judy Simon for help during the experiment. The experiment was carried out in the framework of the Beech Research Group funded by the Deutsche Forschungsgemeinschaft (contract number GE 1090/5-1). We thank Hans-Rudolf Roth for statistical advice, Annika Lenz and Roland A. Werner for isotope analyses. NKR was supported by the Staatssekretariat für Bildung und Forschung (SBF, project number C04.0255, Switzerland). JPF was granted a Marie Curie IEF (6th FP, EU).

References


