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Research paper

Fate of recently fixed carbon in European beech (*Fagus sylvatica*) saplings during drought and subsequent recovery

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Drought reduces the carbon (C) assimilation of trees and decouples aboveground from belowground carbon fluxes, but little is known about the response of drought-stressed trees to rewetting. This study aims to assess dynamics and patterns of C allocation in beech saplings under dry and rewetted soil conditions. In October 2010, 5-year-old beech saplings from a forest site were transplanted into 20 l pots. In 2011, the saplings were subjected to different levels of soil drought ranging from non-limiting water supply (control) to severe water limitation with soil water potentials of less than −1.5 MPa. As a physiologically relevant measure of drought, the cumulated soil water potential (i.e., drought stress dose (DSD)) was calculated for the growing season. In late August, the saplings were transferred into a climate chamber and pulse-labeled with ^{13}C-depleted CO_2 ($\delta^{13}C$ of −47‰). Isotopic signatures in leaf and soil respiration were repeatedly measured. Five days after soil rewetting, a second label was applied using 99 atom% $^{13}CO_2$. After another 12 days, the fate of assimilated C in each sapling was assessed by calculating the ^{13}C mass balance. Photosynthesis decreased by 60% in saplings under severe drought. The mean residence time (MRT) of recent assimilates in leaf respiration was more than three times longer than under non-limited conditions and was positively correlated to DSD. Also, the appearance of the label in soil respiration was delayed. Within 5 days after rewetting, photosynthesis, MRT of recent assimilates in leaf respiration and appearance of the label in soil respiration recovered fully. Despite the fast recovery, less label was recovered in the biomass of the previously drought-stressed plants, which also allocated less C to the root compartment (45 vs 64% in the control). We conclude that beech saplings quickly recover from extreme soil drought, although transitional after-effects prevail in C allocation, possibly due to repair-driven respiratory processes.

Keywords: carbon balance, carbon fluxes, drought stress quantification, labeling, recovery.

Introduction

A key parameter for understanding the carbon (C) turnover in terrestrial ecosystems is the aboveground and belowground C allocation of plants (Horwath et al. 1994, Trumbore 2006). Up to 60% of soil respiration has been shown to be directly fueled by recently assimilated C and there is a tight temporal coupling between aboveground and belowground C fluxes (Steinmann et al. 2004, Högberg and David 2006). Changes in C allocation can therefore affect the C sequestration of ecosystems (Trumbore 2006, Carbone and Trumbore 2007).

Drought is expected to become an increasingly important climatic stressor in many regions of the earth (IPCC 2007), not only influencing physiological plant parameters such as photosynthesis and, hence, ecosystem-level gross primary production (Ciais et al. 2005, Granier et al. 2007), but also patterns

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and dynamics in plant C allocation. The tight temporal coupling of aboveground and belowground C fluxes is impaired by drought (Bréda et al. 2006, Kuzyakov and Gavrichkova 2010, Barthel et al. 2011, Dannoura et al. 2011), as evidenced by increasing mean residence times (MRTs) of recently formed assimilates in different plant compartments (Ruehr et al. 2009).

European beech (*Fagus sylvatica* L.) is an ecologically dominant tree species in Central Europe of high economic importance. Known to be drought-sensitive (Backes and Leuschner 2000, Gessler et al. 2004, 2006, Michelot et al. 2012), especially during early stages of establishment (Fotelli et al. 2001, Lendzion and Leuschner 2008), European beech has been reported to recover quickly from drought stress and to survive severe drought episodes (Gallé and Feller 2007). Such findings gave rise to controversial debates about the silvicultural consequences of climate change for European beech in Central Europe (e.g., Rennenberg et al. 2004, Ammer et al. 2005). Tognetti et al. (1995) observed a recovery of photosynthesis, leaf water potential and chlorophyll concentration of drought-stressed beech seedlings from two Italian populations within 5 days after rewetting, whereas leaf conductance did not fully recover during this period. Similar findings are reported by Gallé and Feller (2007) for beech saplings, although complete recovery of photosynthesis required 4 weeks. However, allocation dynamics of recently formed photoassimilates upon different levels of drought and subsequent rewetting remain obscure.

Comparability of studies concerning soil drought has often been restricted by the lack of stress quantification, given that most often volumetric soil water content (VWC) was considered, which does not reflect soil water availability (Vicca et al. 2012). Stomatal closure per se is questionable as a drought indicator, as beech provenances can differ in stomatal sensitivity (Peuke et al. 2002, Rose et al. 2009). In our integrated field and laboratory experiment, we made use of the soil water potential as a physiologically relevant measure of drought stress and employed the cumulated soil water potential (referred to as the drought stress dose (DSD)) as an explanatory variable (Zang et al. 2013). In doing so, we subjected planted beech saplings from a reforestation site to defined levels of drought stress and subsequent rewetting. Twofold ^{13}C labeling, before and after rewetting, was applied for every plant, allowing for the calculation of an individual C balance.

We hypothesized that increasing drought stress impedes C translocation to the belowground plant compartments as reflected by increased MRTs of recently formed photosynthates in leaves and their delayed appearance in soil respiration. We further hypothesized that, after rewetting, effects of drought on these parameters and C partitioning prevail, depending on the intensity of the preceding drought stress.

Materials and methods

Study site and experimental setup

Two-year-old beech saplings of a local provenance were planted in a mature Norway spruce forest (tree age 145 years) in the Fichtelgebirge, Bavaria, Germany (50° 8′ N, 11° 52′ E), in autumn 2008 (see Gerstberger et al. (2004) and Schulze et al. (2009) for detailed site description). The saplings were bare rooted. After two growing seasons with optimum soil water availability, 36 randomly chosen beech saplings (for leaf area, see Table 1) including the rooted soil monolith were excavated and transferred into plastic pots (diameter: 29.5 cm, height: 32 cm) that were perforated at the bottom to allow water drainage. The organic layer, which contained a large amount of herbaceous roots, was omitted and replaced by a sand layer with a thickness of 5 cm. Each pot was equipped with an frequency domain reflectometry (FDR) soil moisture sensor (EC-20, Decagon Devices, Pullman, WA, USA), which was installed vertically to integrate the VWC from a soil depth of 10–30 cm. The pots were subsequently embedded into the surrounding soil at the forest site to maintain a natural vertical temperature gradient.

Drought treatment and drought stress quantification

Prior to measurements, the plants had been randomly assigned to three groups of differing soil water availability, representing non-limited soil water availability (control (Cont)), moderate drought (mD) and severe drought (sD) corresponding to mean target soil water potentials of −0.05, −0.6 and −1.2 MPa, respectively.

A translucent roof construction (height: 2.2 m) was installed above the potted saplings in late June 2011 to exclude throughfall.

Table 1. Plant characterization and details of the labeling procedure for the three treatments.

	Cont	mD	sD
Total plant biomass (g)	135 (28)	124 (28)	119 (27)
Root/shoot ratio	0.91 (0.13)	0.83 (0.23)	0.86 (0.17)
Leaf area (m^2)	0.256 (0.084)	0.245 (0.067)	0.258 (0.095)
Labeling time (first labeling) (min)	412 (46)	421 (41)	441 (35)
CO_2 uptake during first labeling (mmol)	42.6 (11.6)	22.3 (8.6)	13.9 (6.9)
Labeling time (second labeling) (min)	247 (40)	259 (41)	255 (35)
CO_2 uptake during second labeling (mmol)	19.7 (5.3)	17.5 (6.2)	17.0 (5.2)

Plant parameters were assessed after harvest (means, SD in parentheses).

Soil water potential was measured in the soil of every pot 5–15 times during the duration of the experiment using a tensiometer for the moisture range greater than −0.3 MPa (T5 tensiometer, UMS, Munich, Germany) and a dewpoint potentiometer for soil water potentials less than −0.3 MPa (WP4 dewpoint potentiometer, Decagon Devices, Pullman, WA, USA). Adjustment of the respective target soil water potential was conducted by individual irrigation with deionized water via perforated plastic containers that were brought in to direct contact with the mineral soil. This method of water application assured slow infiltration and homogeneous distribution of water within the soil.

We correlated measured soil water potentials with the corresponding FDR sensor signal and fitted individual spline regression functions. These were used to model the time course of soil water potential based on the hourly logged FDR sensor signal. The DSD for individual beech saplings was defined as the cumulated soil water potential during the growing season, i.e.,

$$\mathrm{DSD} = -\int \Psi(t)\, dt \qquad (1)$$

where DSD is the drought stress dose (MPa day) and $\Psi(t)$ is the individual time course of soil water potential as modeled within the time period between bud burst and harvest (MPa).

First pulse label

The pots were removed from the surrounding soil and transported to a climate chamber at the University of Bayreuth on 18 August 2011. Air temperature was held constant at 18 °C and relative humidity at 80%. A light source providing photosynthetically active radiation (PAR) of a photon flux density of 500 μmol m^{-2} s^{-1} at the shoot apex height was employed for 16 h a day (MT400DL/BH-E40, Iwasaki Electric, Tokyo, Japan). The pots were arranged randomly inside the chamber and moved regularly during the experiment. Atmospheric air was continually passed through the chamber, providing a full air exchange every 1.5 h.

A first pulse label with ^{13}C-depleted CO_2 was applied individually around noon from 30 August through 2 September 2011. For this purpose, a quadratic plate (polyvinylchloride, 45 × 45 cm, 1-cm thick) with a notch was fitted around the beech stems. With its gasket on the bottom side, the plate rested flat on the container rim. The notch was made air tight with a sealant (Terostat, Henkel, Düsseldorf, Germany). A translucent labeling chamber (44 × 44 cm, height 130 cm, volume 250 l, polycarbonate) was placed over each sapling and tightly fixed on the plate. A fan inside the chambers ensured air mixing. Diffusive CO_2 loss from chambers had been quantified as <7 ppm h^{-1} for a CO_2 concentration gradient between chamber air and atmosphere of ~600 ppm. Owing to heating by the light source, chamber air was warmer than ambient air but stabilized at 21 ± 1 °C after ~1 h.

Thirty plants ($n = 10$ per treatment) were labeled during three sessions, irrespective of the drought treatment, on three consecutive days, while six plants ($n = 2$ per treatment) served as unlabeled controls. Labeling started between 9:00 and 11:00 am (for labeling times see Table 1). The target value of the CO_2 concentration inside the chambers was between 500 and 1000 ppm during the labeling period. To achieve this aim, we measured the photosynthetic CO_2 uptake of each plant within one chamber instalment prior to labeling using an infrared gas analyzer (LiCor 820, Licor, Inc., Lincoln, NE, USA, air flow rate: 1 l min^{-1}). Based on these measurements, the frequency of label injections was calculated to sustain the target CO_2 concentration range during the labeling period. Labeling gas (100% CO_2, $\delta^{13}C = -47‰$, DIN EN ISO 14175:C1, Westfalen AG, Münster, Germany) was injected with a gas-tight syringe with a maximum amount of 120 ml CO_2 per injection. Upon removal of the labeling chambers, the climate chamber was immediately flushed with atmospheric air to attenuate remaining label and prevent contamination of other plants.

CO_2 from leaf respiration was sampled for isotope-ratio mass spectrometry (IRMS) immediately before labeling (natural abundance) and at 20, 29, 44, 54, 73, 97 and 121 h after termination of labeling. To this end, on each plant a non-transparent PTFE-coated gas bag (volume of 0.5 l) was tightly fitted around one lateral branch with ~10 leaves. Gas bags were sealed and then flushed with CO_2-free air. Gas samples were taken through a septum after 30 min for isotopic assessment of recently respired C. An individual exponential model was fitted to the time course of the isotopic signature of leaf respiration for each beech sapling as follows:

$$\mathrm{atom\%}\ ^{13}C_{t_1} = \mathrm{atom\%}\ ^{13}C_{t_0} \times e^{-kt} - \mathrm{atom\%}\ ^{13}C_{NA} \qquad (2)$$

where atom% $^{13}C_{t_1}$ is the atom percentage of ^{13}C of leaf respiration at time t_1, atom% $^{13}C_{t_0}$ is the initial atom percentage of ^{13}C of leaf respiration, k is the fitted decay constant (h^{-1}), t is the time after labeling (h) and atom% $^{13}C_{NA}$ is the atom percentage of ^{13}C of leaf respiration before labeling.

The MRT of label in leaf respiration was calculated as follows:

$$\mathrm{MRT} = 1 / k \qquad (3)$$

To measure the rate of soil respiration and its $\delta^{13}C$, the bottom plates of the labeling chambers were put on the pots and made air-tight with sealant (Terostat). The increase of the CO_2 concentration in the headspace was monitored (IRGA) over an incubation time of 4 min (flow rate: 0.5 l min^{-1}). The respiration rate was calculated from the slope of the linear regression between CO_2 concentration and incubation time considering the individual head space volume according to Borken et al. (2006). Subsequently, the headspace was flushed with

CO_2-free synthetic air until no CO_2 could be detected. This procedure was repeated three times. For ^{13}C isotopic analyses of soil respiration, the soil was subsequently incubated with the incubation time depending on the soil respiration rate in order to obtain a headspace CO_2 concentration of ~1000 ppm. Again, gas samples were taken through the septum with a syringe and stored in 5 ml glass vials (Exetainer, Labco Limited, Buckinghamshire, UK) before being analyzed within 7 days for isotopic signature (GVI-Isoprime, Elementar Analysensysteme GmbH, Hanau, Germany). Soil respiration was sampled on seven time points: immediately before the labeling and at 29, 44, 54, 73, 97 and 143 h after termination of labeling.

Rewetting

Six days after the first pulse label, all saplings were irrigated to a target soil water potential of −0.05 MPa. Deionized water was applied by small portions within 5 h. Maximum irrigation per plant was 3.2 l, which corresponded to a precipitation event of 45 mm.

Second pulse label and ^{13}C mass balance

A second pulse label was applied 5 days after rewetting with enriched $^{13}CO_2$ (99 atom% ^{13}C, Eurisotop, Saint-Aubin, France). The procedure was conducted in the same way as the first labeling. Additionally, gas samples from each chamber were taken before the label application as well as at the end of the labeling period and analyzed for $\delta^{13}C$ and CO_2 concentration. The amount of ^{13}C taken up by each plant ($m^{13}C_{uptake}$) was calculated as follows:

$$m^{13}C_{uptake} = m^{13}C_{t1} - m^{13}C_{t2} + m^{13}C_{injected} \quad (4)$$

where $m^{13}C_{t1}$ is the amount of ^{13}C in the chamber air before labeling, $m^{13}C_{t2}$ is that after labeling and $m^{13}C_{injected}$ is the respective amount injected during labeling.

We calculated a ^{13}C mass balance for every beech sapling on day 12 after application of the second label as follows:

$$m^{13}C_{uptake} = m^{13}C_{AR} + m^{13}C_{SR} + m^{13}C_{Bio} + m^{13}C_{S} \quad (5)$$

where $m^{13}C_{AR}$ is the amount of ^{13}C emitted by aboveground respiration (for mainly leaf respiration ($m^{13}C_{LR}$), see below), $m^{13}C_{SR}$ is that emitted by soil respiration, $m^{13}C_{Bio}$ is that recovered in plant biomass and $m^{13}C_{S}$ is that remaining in the soil solid phase. To consider solely label-derived ^{13}C in the specific compartments, atom% excess (APE) was calculated relative to the isotopic signature of the corresponding unlabeled control plants which had been subjected to the same drought treatments as follows:

$$APE = atom\%_{Sample} - atom\%_{NA} \quad (6)$$

where $atom\%_{Sample}$ is the atom percentage of ^{13}C of the sample after labeling and $atom\%_{NA}$ is the atom percentage of ^{13}C in unlabeled control plants (natural abundance).

Abundance of ^{13}C of leaf respiration was measured at 20, 30, 45, 51, 73, 99, 121 and 296 h after termination of labeling. Light- and dark-adapted leaf respiration rates were determined on a single-leaf basis with a portable CO_2–H_2O porometer equipped with an infrared gas analyzer (LiCor 6400, Licor Inc., Lincoln, NE, USA) and a cuvette providing a red-blue LED light source. Total leaf area was determined after harvest (see below). On two occasions, light- and dark-adapted respiration rates of the total aboveground plant compartment were assessed by measuring the CO_2 accumulation rate in the labeling chambers in the absence of light. We found that foliage respiration extrapolated from single-leaf measurements accounted on average for 91 ± 4.7 (SD) % of total aboveground respiration.

We calculated the label-derived ^{13}C in leaf respiration (both light- and dark-adapted respiration) ($m^{13}C_{LR}$) within the observed time span as follows:

$$m^{13}C_{LR} = \int F_{LR} \times APE_{LR}(t) / 100\,dt \quad (7)$$

where F_{LR} is the mean of light- and dark-adapted leaf respiration rates, weighted by the set day length of 16 h in the climate chamber, and $APE_{LR}(t)$ is the APE of leaf respiration at time t.

Isotopic signature of CO_2 in soil respiration as well as soil respiration rates were measured at 10 time points: 0, 7, 20, 30, 45, 51, 73, 99, 146 and 296 h after termination of labeling. The amount of label-derived ^{13}C emitted from the soil within 12 days after labeling was calculated as follows:

$$m^{13}C_{SR} = \int F_{SR}(t) \times APE_{SR}(t) / 100\,dt \quad (8)$$

where $F_{SR}(t)$ is the soil respiration rate at time t and $APE_{SR}(t)$ is the atom percentage excess of soil respiration at time t.

Beech saplings were clipped and separated into leaves, buds, twigs (shoot parts <2 mm in diameter) and stem (shoot parts >2 mm in diameter) 296 h after labeling. Leaf area was determined through digital image evaluation (SigmaScan 5, Systat Software, Inc., Chicago, IL, USA) after foliage scanning. Six vertical soil cores along the entire soil profile (diameter = 2 cm) were randomly taken from each pot, homogenized, separated from visible root fragments and stored at −22 °C until further analyses. Samples from fine (<2 mm) and coarse roots were taken. During the following 4 weeks, the amount of live fine roots and coarse roots as well as the soil volume were quantified in each pot. All plant compartments were weighed and ground with liquid nitrogen before isotopic analyses (vario MAX, Elementar Analysensysteme, Hanau, Germany). All plant material was oven-dried at 60 °C until constant weight was achieved.

The label-derived amount of ^{13}C in total plant biomass ($m^{13}C_{Bio}$) was then calculated as follows:

$$m^{13}C_{Bio} = \Sigma m_i \times \%C_i / 100 \times APE_i / 100 \quad (9)$$

where m_i is the dry mass, $\%C_i$ is the carbon content (%) and APE_i is the atom percentage excess of ^{13}C of each plant compartment.

Owing to the considerable background of organic C in the soil (1–2% C), $m^{13}C_S$ was estimated by assessing the isotopic signature of hot water extractable soil organic carbon (SOC), which was expected to serve as a proxy for soluble carbohydrates originating from root exudates, microbial biomass or small root fractions like root hairs. For that purpose, deionized water was added to a subsample of the soil (20 g) that had been frozen directly after sampling (soil : solute mass ratio = 1 : 5). The soil was extracted for 24 h at 70 °C. The supernatant was filtered (0.45 µm) and freeze dried. The residual fraction was homogenized and analyzed for its non-purgeable organic C content with an elemental analyzer (multi N/C 2100, Analytik Jena, Jena, Germany) and its isotopic signature with an isotope-ratio mass spectrometer (delta S, Finnigan MAT, Bremen, Germany, coupled to the elemental analyzer NA 1108, CE Instruments, Hindley Green, UK). The amount of label-derived hot water-soluble organic ^{13}C was calculated for each pot in relation to individual soil volumes.

Statistical analyses

Differences between the treatments were analyzed using Tukey's HSD test after analysis of variance (ANOVA) ($n = 10$); normality was assumed when data passed the Shapiro–Wilk test ($P > 0.1$). In the case of non-normally distributed data, the non-parametric Wilcoxon test was conducted followed by the Kruskal–Wallis analysis of variance. Additionally, assessed plant parameters were subjected to a linear regression with the individual DSD; the relationship was then characterized by the P-value of the slope as well as by the adjusted r^2 and Spearman's correlation coefficient. The DSD as the explaining variable was log-transformed (log DSD) before regression analysis. All calculations involving ^{13}C abundance (calculation of the mean and standard deviation, statistical tests and regression analyses) were performed after transformation of δ‰ values to atom% or APE. Results were also displayed in the common delta notation for clarity. All statistical analyses were performed using R 2.13.0 (R Development Core Team 2009).

Results

Time course of soil water potential during the experiment

Maximum drought in treatments mD and sD was achieved 6 weeks after exclusion of precipitation. The mean soil water potential dropped to −0.82 and −1.4 MPa, respectively, at that time (Figure 1). A minimum soil water potential of −3.0 MPa occurred in treatment sD. Soil water potential in the control ranged between −0.03 and −0.06 MPa. After rewetting, a target soil water potential of −0.05 MPa was achieved within 1 day in treatments mD and sD. Thereafter, soil water potential remained above −0.06 MPa irrespective of treatment.

Plant parameters before rewetting

Photosynthesis significantly declined with decreasing soil water potential. Immediately before the first labeling, mean net photosynthesis rate (±SD) was 7.0 ± 1.1 µmol CO_2 m^{-2} s^{-1} in the control, which was reduced to 2.8 ± 3.0 µmol CO_2 m^{-2} s^{-1} in sD (Table 2). The negative correlation between photosynthesis and the individual DSD was highly significant.

Natural abundance of ^{13}C in leaf and soil respiration of control plants was significantly lower than of the stressed plants (Table 2), resulting in a significant positive correlation with DSD (Table 2, Figure 2).

The application of ^{13}C-depleted CO_2 during the first labeling pulse caused a decrease of the isotopic signature of leaf respiration (Figure 3a); its shift relative to natural abundance declined over time. The MRT of the label in leaf respiration was more than three times longer in sD than in the control (107 vs 30 h). Furthermore, MRT was positively correlated with DSD ($P = 0.014$, Table 2, see Figure 5a).

Minimum $\delta^{13}C$ in soil respiration was observed at the first sampling time (29 h after the labeling) in the control and mD. In contrast, sD achieved its minimum ^{13}C abundance at the second sampling time at 44 h after labeling (Figure 3b).

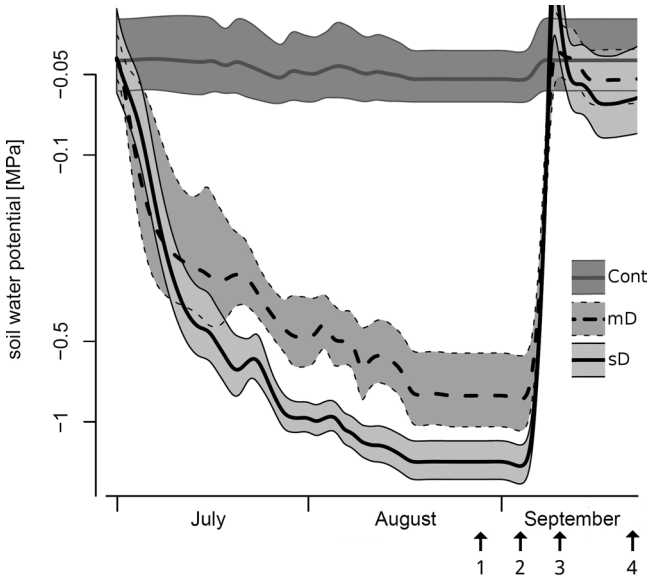

Figure 1. Time course of soil water potential for the three treatments, $n = 10$, mean ± SE. Arrows indicate the time point of the first pulse label (1), rewetting (2), second pulse label (3) and harvest (4).

Table 2. Overview of different plant parameters for the three treatments before and after rewetting (means, SD in parentheses).

	Cont	mD	sD	α	β	γ	P	r^2	ρ
Natural abundance of ^{13}C in leaf respiration (‰)	–24.3 (1.1)	–19.7 (0.8)	–19.6 (0.8)	<0.001	<0.001	0.977	<0.001	0.61	0.66
Natural abundance of ^{13}C in soil respiration (‰)	–24.3 (1.0)	–21.5 (0.8)	–20.4 (2.5)	0.018	<0.001	0.327	<0.001	0.42	0.63
Photosynthesis rate (μmol CO_2 m^{-2} s^{-1})	7.0 (1.1)	3.6 (1.6)	2.8 (3.0)	0.005	<0.001	0.653	<0.001	0.53	–0.75
MRT of label-derived ^{13}C in leaf respiration (h)	30 (12)	94 (39)	107 (58)	0.007	0.002	0.774	0.014	0.20	0.50
Photosynthesis rate (μmol CO_2 m^{-2} s^{-1})	7.0 (0.8)	6.4 (1.6)	6.5 (1.8)	0.673	0.727	0.995	0.466	0.02	–0.07
MRT of label-derived ^{13}C in leaf respiration (h)	51 (19)	48 (28)	51 (18)	0.95	0.99	0.96	0.695	0.00	–0.06
% of ^{13}C uptake recovered in									
hot water-soluble SOC	11.6 (9.4)	10.8 (9.0)	13.7 (13.6)	0.980	0.903	0.827	0.116	0.09	0.31
Soil respiration	13.2 (4.4)	12.0 (4.0)	12.2 (6.7)	0.865	0.913	0.991	0.499	0.02	–0.17
Leaf respiration	16.9 (4.6)	24.8 (11.8)	24.2 (11.7)	0.189	0.243	0.988	0.199	0.06	0.20
Total plant biomass	41.1 (14.0)	39.8 (11.9)	30.9 (3.8)	0.959	0.106	0.176	0.009	0.22	–0.42
thereof									
Leaves	5.0 (2.8)	8.0 (4.9)	8.1 (4.9)	0.275	0.255	0.987	0.114	0.09	0.33
Buds	1.9 (0.7)	2.4 (1.5)	2.5 (1.6)	0.641	0.525	0.980	0.475	0.02	0.05
Twigs	1.7 (1.0)	2.3 (1.4)	2.2 (1.1)	0.472	0.574	0.984	0.351	0.03	0.10
Stem	5.6 (4.0)	5.9 (5.0)	4.5 (2.3)	0.987	0.802	0.714	0.428	0.02	–0.21
Coarse roots	13.6 (11.0)	8.2 (10.1)	5.5 (3.0)	0.365	0.116	0.775	0.007	0.23	–0.38
Fine roots	13.3 (8.2)	13.0 (7.4)	8.0 (4.2)	0.992	0.208	0.253	0.111	0.08	–0.20
Relative to label-derived ^{13}C in total plant biomass									
Leaves	14.2 (11.1)	22.8 (16.0)	25.9 (14.4)	0.362	0.165	0.877	0.047	0.15	0.43
Buds	4.8 (1.2)	6.5 (4.1)	8.0 (4.3)	0.519	0.109	0.589	0.101	0.09	0.42
Twigs	4.0 (1.9)	5.7 (3.1)	7.1 (3.3)	0.402	0.058	0.525	0.044	0.14	0.36
Stem	13.5 (7.7)	13.3 (8.9)	14.4 (7.0)	0.990	0.960	0.950	0.928	0.00	–0.11
Coarse roots	29.7 (16.5)	18.5 (15.0)	18.1 (9.7)	0.194	0.175	0.997	0.021	0.20	–0.31
Fine roots	33.9 (17.9)	33.2 (16.6)	26.5 (14.7)	0.990	0.585	0.638	0.699	0.01	0.04

The Greek letters represent P-values yielded from multiple comparison analysis: α, difference between Cont and mD; β, difference between Cont and sD; γ, difference between mD and sD. Results of the regression analyses with DSD are represented by P (P-value of the slope), r^2 (adjusted coefficient of determination) and ρ (Spearman's correlation coefficient).

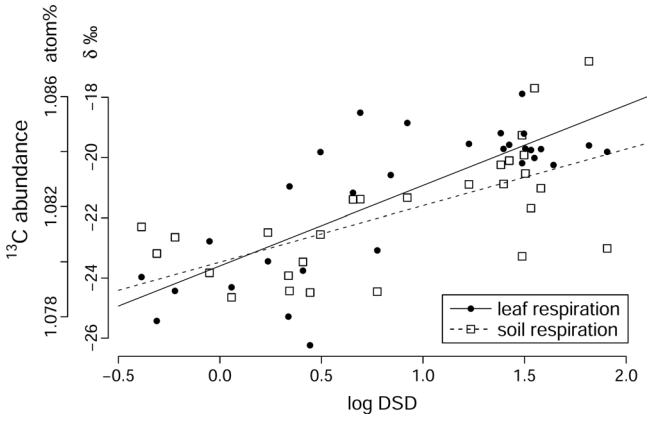

Figure 2. Correlation between DSD and natural abundance of ^{13}C in leaf respiration and soil respiration immediately before the first pulse label. For statistical information, see Table 2.

Plant parameters after rewetting

Rewetting induced fast recovery of photosynthesis within 3 days (Table 2). Before the second pulse labeling, differences in net photosynthesis between the treatments had vanished, so that correlation with DSD did not exist anymore. After the second labeling, $\delta^{13}C$ of leaf respiration rose to up to >4000‰ in any treatment before declining exponentially (Figure 4a). Mean MRT extended through ~50 h irrespective of treatment, indicating the absence of persisting drought effects upon rewetting (Table 1, Figure 5a and b). The ^{13}C peak in soil respiration occurred between 30 and 51 h after labeling in any treatment (Figure 4b) and in the absence of treatment effects.

Overall, between 81 and 88% of the applied ^{13}C label was recovered in leaf respiration, soil respiration, plant biomass and hot water-soluble SOC irrespective of treatment (Table 2, Figure 6). About 12–13% of the applied ^{13}C was released in general by soil respiration and 11–14% recovered as hot water-soluble C. In sD, less ^{13}C was incorporated into the living biomass of plants (31% of applied ^{13}C) than under the other treatments (41 and 40% in Cont and mD, respectively; Figure 6).

Consistently, the incorporation of ^{13}C in plant biomass was negatively and significantly correlated with DSD. Previously

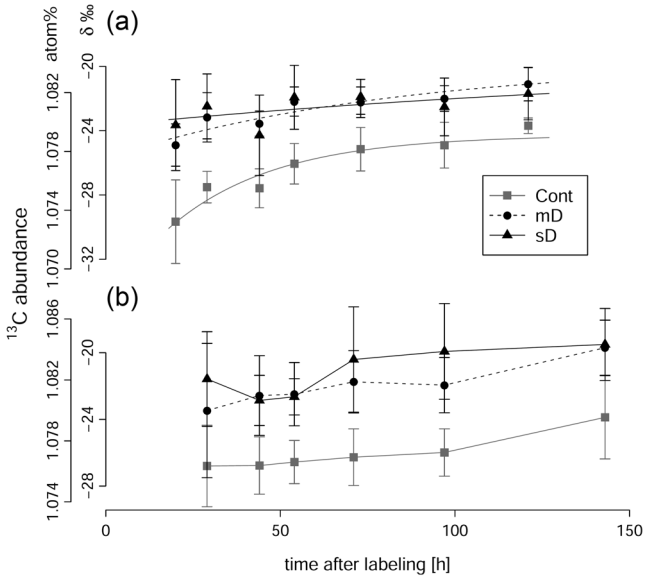

Figure 3. The ^{13}C abundance following the first pulse label (before rewetting, δ^{13}C of labeling gas = −47‰) in leaf respiration (a) and soil respiration (b) for the three treatments, $n = 10$, mean ± SD. For statistical information and MRTs, see Table 2.

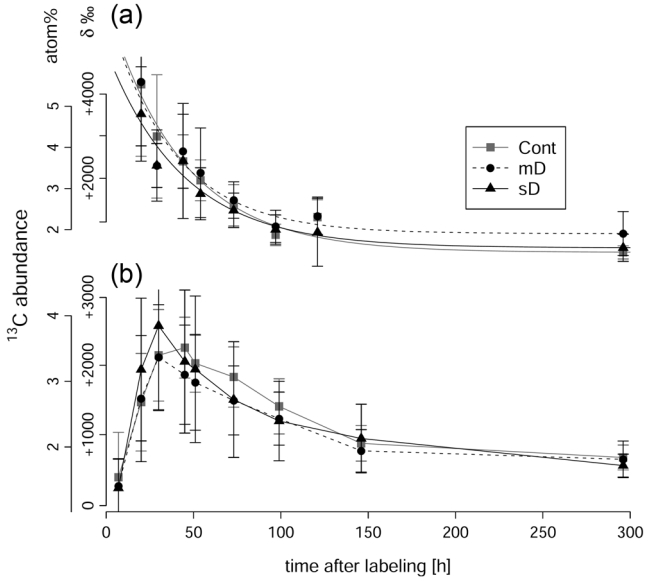

Figure 4. The ^{13}C abundance following the second pulse label (after rewetting, ^{13}C abundance in the labeling gas = 99 atom%) in leaf respiration (a) and soil respiration (b) for the three treatments, $n = 10$, mean ± SD. For statistical information and MRTs, see Table 2.

drought-stressed plants tended to exhibit higher ^{13}C release via leaf respiration than non-stressed plants (Figure 7). With increasing DSD, the proportion of label-derived ^{13}C recovered in leaves and twigs became significantly enhanced. Conversely, ^{13}C in coarse roots significantly decreased with increasing DSD. Overall, 64, 52 and 45% of biomass-bound ^{13}C was recovered in roots under Cont, mD and sD, respectively.

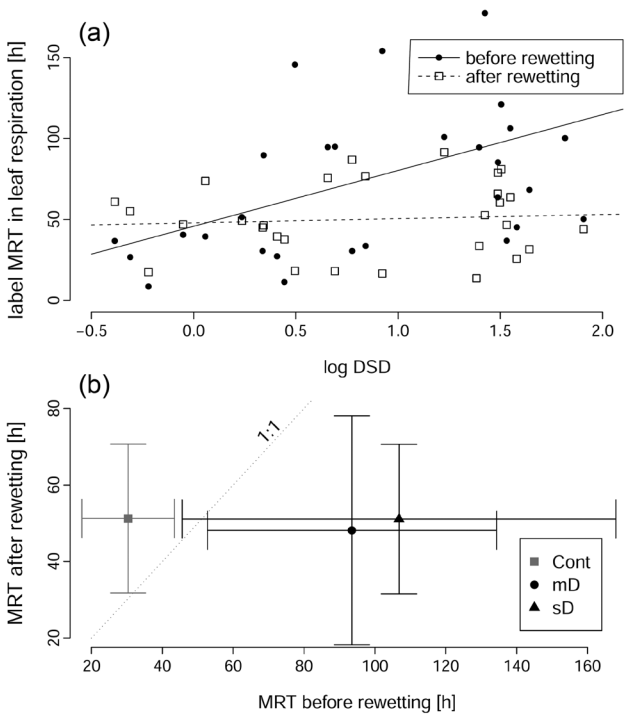

Figure 5. (a) Regression of DSD on MRT of label-derived ^{13}C in leaf respiration before and after rewetting. For statistical information see Table 2. (b) Difference in MRT before and after rewetting for the three treatments.

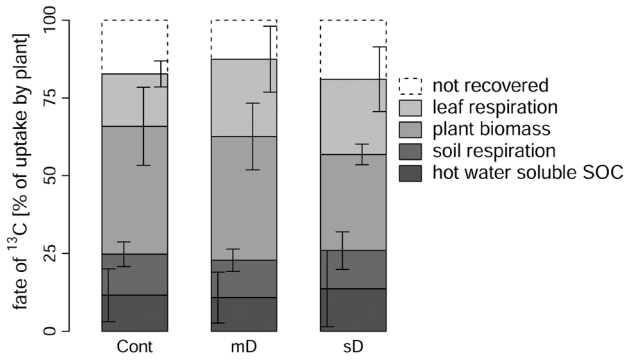

Figure 6. Fate of label-derived ^{13}C 12 days after the second pulse label, $n = 10$, mean ± SD. *P*-values (ANOVA) for treatment differences are given in Table 2.

Discussion

Drought effects

Natural abundance and photosynthesis Natural abundance of ^{13}C in leaf and soil respiration is in accordance with the 60% reduction of photosynthesis under drought conditions. Stomatal closure leads to a reduced discrimination of ^{13}C and therefore increases the isotopic signature of assimilates (Dawson et al. 2002). Thus, the observed pattern underpins the physiological response of beech saplings to soil water availability in our experiment. Furthermore, the significant correlations of natural abundance of ^{13}C both in leaf and soil respiration with the

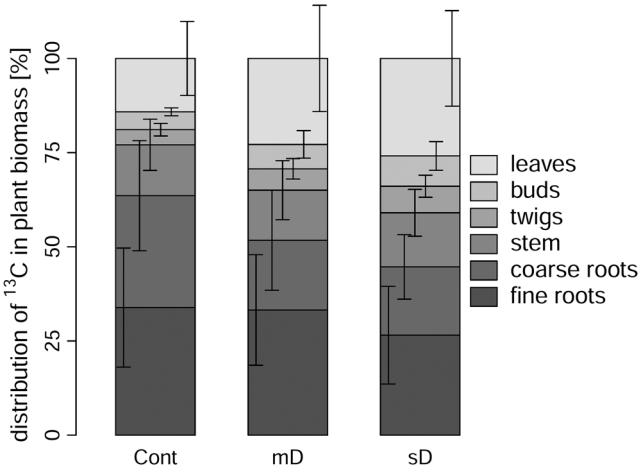

Figure 7. Relative distribution of label-derived ^{13}C in different plant compartments 12 days after the second pulse label, $n = 10$, mean ± SD. *P*-values (ANOVA) for treatment differences are given in Table 2.

individual DSD highlight the physiological relevance of cumulated soil water potential with respect to stress quantification.

However, as DSD summarizes the water availability over several months, it does not necessarily reflect single drought events in terms of their intensity and duration. In our study the individual soil water potential was monotonically lowered until the target level was reached, except for small fluctuations due to the irrigation routine. Hence, DSD correlated with the minimum soil water potential within our sample collective, effectively reflecting drought exposure at the single-tree level. The introduced concept of drought stress quantification needs to be validated at forest sites that differ in drought and precipitation patterns. Threshold ranges of drought stress may be implemented as discussed by Vicca et al. (2012), e.g., in analogy to Granier et al. (2007), who defined water stress based on a threshold value of relative extractable water.

Mean residence time of label in leaf respiration and label appearance in soil respiration Drought increased the MRT of the label in leaf respiration, indicating prolonged retention of recently formed assimilates. Ruehr et al. (2009) reported on a doubled MRT of excess ^{13}C in leaf water-soluble organic matter of drought-stressed beech saplings. This prolonged assimilate retention in the mesophyll may be associated with tissue dehydration and reduction in phloem loading due to reduced carbohydrate production rather than with increased emission of biogenic volatile organic compounds or changes in the leaf respiration rate. Given the about three times higher MRT of recently formed assimilates in sD than in control leaves, we conclude that the effective maximum drought was stronger in our experiment. Regarding the significant positive correlation between MRT of label-derived ^{13}C in leaf respiration and DSD, drought intensity does determine the dynamics of C translocation as suggested for the leaves.

The increased MRT of recently formed assimilates in the leaves coincides with the delay of the label appearance in soil respiration under drought conditions. This delay is likely linked to the reduced photosynthesis rate and points to a C-source limitation of the drought-stressed beech saplings. This may lead to slower turnover of carbohydrate pools and, induced by changes in concentration gradients, to smaller carbohydrate transfer rates (Kozlowski 1992). As soil air was not contaminated by the labeling gas, ensured by hermetic sealing of the labeling chambers, it is concluded that the label recovered in soil respiration solely originated from current photosynthesis. Although our sampling intervals do not provide a sufficient temporal resolution to pinpoint the exact time lag, we estimate that the transport of assimilates took at least 15 h longer in beech saplings under treatment sD than in non-stressed saplings. Laser spectroscopy revealed a consistent 11-h delay of the ^{13}C peak in soil respiration in drought-stressed beech saplings (Barthel et al. 2011). The greater delay in our study may be attributed to larger plants and thus to longer transport paths.

Rewetting effects

Photosynthesis Rewetting resulted in a rapid recovery of net photosynthesis, corroborating former studies on this topic (Xu et al. 2010; for beech: Tognetti et al. 1995). We attribute the recovery from photosynthetic inhibition to stomatal rather than non-stomatal limitation, as the latter might require prolonged recovery periods (Gallé and Feller 2007), unless leading to irreversible impairment.

Mean residence time of label-derived ^{13}C in leaf respiration and peak in soil respiration Previously drought-stressed beech saplings did not display persisting drought effects in the MRT of recently formed assimilates in the leaves. Presuming the C demand for aboveground respiration to be higher in such plants, the finding cannot rule out restriction in leaf-to-shoot allocation. However, as there was no delayed ^{13}C peak in soil respiration after rewetting of previously stressed plants, we deduce a fast recovery of the processes involved in assimilate transportation. As the MRT of label-derived ^{13}C in leaves of non-stressed beech saplings increased from the first to the second labeling, perhaps incipient autumnal leaf senescence retarding assimilate transport was indicated (Kuptz et al. 2011). It is open as to whether drought-stressed beech saplings behave in a similar way. Nonetheless, the decline in MRT by >50% in sD upon rewetting illustrated an appreciable recovery capacity.

^{13}C partitioning As opposed to the respiratory C dynamics and photosynthetic recovery, the ^{13}C mass balance provided evidence for after-effects of drought on C partitioning. The increased demand for recently formed assimilates in aboveground respiration of previously drought-stressed plants might be due to repair processes, e.g., repair of embolism or the photosynthetic apparatus

(Bréda et al. 2006, McDowell et al. 2008). In contrast, we did not find indications in soil respiration that drought injury extended to the belowground tree compartments. Neither did drought affect the proportion of ^{13}C recovered by soil extraction. Hence, we conclude that root exudation and root mortality did not differ shortly after the rewetting event. We did not observe differences in fine root biomass within 12 days after rewetting, yet we cannot exclude enhanced fine root production in later stages of recovery as described by Olesinski et al. (2011). The recovery rate of ^{13}C of 81–88% did not depend on the treatment, from which we conclude that it resulted from diffusive loss of CO_2 during the labeling, via drainage perforation at the bottom of the pots and from stem respiration that was not considered in the mass balance. Based on our measurements, we estimate that ^{13}C emitted by stem respiration did not exceed 2.5% of total ^{13}C uptake.

Persisting drought effects after rewetting became apparent in the partitioning of ^{13}C in the plant biomass. As there were no significant differences in total plant biomass and root : shoot ratio between the treatments after harvest (Table 1), the observed pattern of ^{13}C partitioning in different plant organs is considered to be a result of changes in C allocation and is not due to different pool sizes or tree dimensions. Smith and Paul (1988) and Epron et al. (2011) found an increasing amount of recent assimilates to become allocated to the belowground compartments toward the end of the growing season. We assume such behavior to be characteristic for non-stressed plants as they transferred >60% of recently formed assimilates (probably mostly non-structural carbohydrates) to coarse and fine roots. In contrast, previously drought-stressed individuals appear to have invested the C gain in repairing drought injury rather than in filling reserve pools (Bréda et al. 2006, McDowell 2011). Carbon partitioning and its response to drought stress may not only vary among tree species or provenances but also with tree age. It is well known that the ontogenetic stage of trees, their reached dimensions and the environment of up-growth substantially shape the responsiveness to stress (Kolb and Matyssek 2001, Hinckley et al. 2011). The micro-climatic environment of seedlings and saplings fundamentally differs from that of mature trees (Johnson et al. 2011). Further, the resource allocation—as a consequence of allometric commonalities—differs for mature and juvenile trees (Ishii 2011, King 2011, Thomas 2011). The findings here from juvenile potted trees therefore represent the first step in spatio-temporal upscaling toward stand-level scenarios of maturing trees (cf. Kolb and Matyssek 2001), i.e., principles in responsiveness are presented that await empirical validation beyond the sapling stage and reported growth conditions.

Conclusions

Drought has been found to be a stressor that can intermittently decouple aboveground from belowground C fluxes in plants. Metabolic deceleration may represent a means of extending the resistance to drought by saving assimilates under reduced photosynthesis, and hence of enhancing the chance of survival. This conclusion appears to be consistent with the fast recovery following rewetting, implying that, although drought caused C limitation to the beech saplings, essential C fluxes were maintained. Resistance to drought probably requires processes of repair, which may be one reason for the observed after-effects of drought in C partitioning. We consider the cumulated soil water potential as a proxy for drought stress that correlates with isotopic signatures in leaf and soil respiration, photosynthetic rate and MRT of recently formed assimilates in leaf respiration. Owing to its scalability, DSD might therefore serve as a reference in future drought experiments.

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Conflict of interest

None declared.

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