Sudden cold temperature regulates the time-lag between plant CO2 uptake and release

Author(s):
Barthel, Matti; Cieraad, Ellen; Zakharova, Elena A.; Hunt, J.E.

Publication date:
2013-11-18

Permanent link:
https://doi.org/10.3929/ethz-b-000078667

Originally published in:
Biogeosciences Discussions 10(11), https://doi.org/10.5194/bgd-10-17939-2013

Biogeosciences Discuss., 10, 17939–17964, 2013
www.biogeosciences-discuss.net/10/17939/2013/
doi:10.5194/bgd-10-17939-2013

Biogeosciences Discussions
Open Access

Sudden cold temperature regulates the time-lag between plant CO_2 uptake and release

M. Barthel[1,*], E. Cieraad[1,*], A. Zakharova[1,2], and J. E. Hunt[1]

[1]Landcare Research, P.O. Box 69040, Lincoln 7640, New Zealand
[2]School of Biological Sciences, University of Canterbury, Christchurch, New Zealand
[*]These authors contributed equally to this work.

Received: 28 October 2013 – Accepted: 12 November 2013 – Published: 18 November 2013

Correspondence to: E. Cieraad (cieraade@landcareresearch.co.nz)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Since substrates for respiration are supplied mainly by recent photo-assimilates, there is a strong but time-lagged link between short-term above- and belowground carbon (C) cycling. However, regulation of this coupling by environmental variables is poorly understood. Whereas recent studies focussed on the effect of drought and shading on the link between above and belowground short-term C cycling, the effect of temperature remains unclear.

We used a $^{13}CO_2$ pulse-chase labelling experiment to investigate the effect of a sudden temperature change from 25 °C to 10 °C on the short-term coupling between assimilatory C uptake and respiratory loss. The study was done in the laboratory using two month old perennial rye-grass plants (*Lolium perenne* L.). After label application, the $\delta^{13}C$ signal of respired shoot and root samples were analysed at regular time intervals using laser spectroscopy. In addition, $\delta^{13}C$ was also analysed in bulk root and shoot samples using IRMS.

Cold temperature (10 °C) reduced the short-term coupling between shoot and roots by delaying belowground transfer of recent assimilates and its subsequent respiratory use, as indicated by the $\delta^{13}C$ signal of root respiration ($\delta^{13}C_{RR}$). That is, the time-lag from the actual shoot labelling to the first appearance of the label in $^{13}C_{RR}$ was about 1.5 times longer under cold temperature (time-lags of 1 h and 1.5 h in the warm and cold treatments, respectively). Moreover, analysis of bulk shoot and root material revealed that plants at cold temperature invest relatively more carbon into respiration compared to growth or storage.

These results increase our understanding of environmental controls on the link between short-term above- and belowground C cycling.

BGD
10, 17939–17964, 2013

1 Introduction

Whether terrestrial ecosystems are a source or sink for atmospheric CO_2 depends on the relative strength of two opposing fluxes: photosynthesis (CO_2 uptake) and respiration (CO_2 release). At the same time, these fluxes are tightly linked, with photosynthesis providing the substrate for respiration. So while there is a strong short-term coupling between above- and belowground C cycling (Högberg et al., 2001; Lehmeier et al., 2008), the transport time of newly assimilated C from shoots to roots creates a time-lag between the two fluxes (Kuzyakov and Gavrichkova, 2010). Although a tight interplay of multiple biophysical drivers are likely to control plant C transport speed and allocation (Vargas et al., 2011; Martin et al., 2012), few studies have examined these drivers. Hence detailed experiments are required to elucidate the different effect of environmental controls on plant C allocation (e.g. see Brüggemann et al., 2011; Epron et al., 2012). Stable C isotopes provide a useful tool in these experiments, as they have successfully been used to trace the fate of newly assimilated plant C within the atmosphere-plant-soil continuum (e.g. see review by Brüggemann et al., 2011).

The fate of newly assimilated plant C and its link to respiration is particularly important as terrestrial ecosystems might experience a pronounced increase in year-to-year climate variability because of continued greenhouse gas emissions (Schär et al., 2004). Small changes in either respiratory or assimilatory components in response to environmental drivers have the potential to change ecosystem C cycling, hence affecting net ecosystem exchange. As soil respiration is the largest source of ecosystem respiration it has a key role in ecosystem C budgets. The understanding of above-ground environmental controls on the short-term belowground carbon allocation is crucial since recent photo-assimilates contribute > 60 % of total soil respiration (Bhupinderpal-Singh et al., 2003).

Water availability, irradiance and temperature are probably the most important environmental drivers affecting plant C cycling, since they directly influence respiration and photosynthesis. Drought, for instance, influences the short-term coupling between

BGD
10, 17939–17964, 2013

above- and belowground by increasing allocation of recent C to root biomass (Palta and Gregory, 1997; Sanaullah et al., 2012; Burri et al., 2013a). Drought also slowed transport of newly assimilated C from above- to belowground (Ruehr et al., 2009; Barthel et al., 2011a). Shading (irradiance reduction) can show similar effects on C allocation and transport: it slowed the belowground transfer and/or respiratory use of recent photo-assimilates in grasslands (Bahn et al., 2009), but did not affect C cycling in pine (Warren et al., 2012). However, in beech, a combination of low irradiance and low temperature resulted in a reduction in the rate of C transport from above- to belowground (Plain et al., 2009). It has further been shown that shaded plants maintain belowground C allocation at the expense of aboveground C status (Bahn et al., 2013) or compensate low assimilation rates from shading with an increased use of stored C for belowground respiration (Schmitt et al., 2013). Despite existing literature on environmental effects on the short-term coupling between above- and belowground processes, only one study known to us directly examined the effect of temperature on C allocation and transport (Hawkes et al., 2008, investigated the effect of soil temperature on C transport from plant to fungus).

Although it is well known that temperature has direct effects on photosynthesis and respiration, the extent by which temperature affects the short-term coupling between photosynthesis and respiration and its effects on C allocation above- and belowground remain unclear. Therefore, we investigated the influence of a change in air temperature on C allocation, transport time and residence time within the atmosphere-plant-soil system in a laboratory study using rye-grass (*Lolium perenne* L.). In order to trace C from above- (shoot) to belowground (roots), we used a $^{13}CO_2$ pulse-labelling approach. The respired carbon isotopic composition from shoots and roots was measured using laser spectroscopy, and the bulk biomass components of shoot and roots were analysed using an isotope ratio mass spectrometer (IRMS). Since temperature directly affects biological and physical processes, we hypothesised that lower temperatures would increase the transport time between above- and belowground.

BGD
10, 17939–17964, 2013

2 Methods

2.1 Experimental design

The experiment described here is based on a preliminary study, which used natural abundance isotopic variations of $\delta^{13}C_{AIR}$ to assess the effect of a sudden decrease in air temperature on C transport from above- to belowground in rye-grass (see Supplement). However, we found that the $\delta^{13}C$ signal of shoot respiration was very sensitive to changes in $\delta^{13}C_{AIR}$, impeding the interpretation of the results. In order to overcome the sensitivity of the $\delta^{13}C$ signal of shoot respiration to small changes in $\delta^{13}C_{AIR}$ we followed up on those initial results using a ^{13}C pulse-labelling approach.

Prior to the experiment, ten perennial ryegrass seeds (*Lolium perenne* "Ultra") were sown in 2.5 L pots containing a 1 : 1 mixture of peat and perlite. After four weeks, the seedlings were thinned to five plants per pot. A total of 120 planted pots were placed inside a single controlled environment cabinet (Fitotron, Weiss Gallenkamp Ltd, Loughborough, UK) with a light/dark regime of 25/15 °C 16/8 h at 80 % relative humidity. Plants were watered often to keep soil moisture at field capacity. At regular intervals, plants were fertilised with urea 46 % N and a hydroponic nutrient mix. The labelling experiment started two months after sowing. Immediately prior to labelling (5 min), plants were randomly allocated to one of two cabinets – one with a warm treatment (25 °C; equal to the daytime growing conditions; control) and one with a cold treatment (10 °C), and the first (pre-treatment) measurement took place. The cold treatment was introduced only 5 min before label-start to avoid acclimation effects. The cold and warm treatments continued for the remainder of the experiment (7 days, "post-label"). Relative humidity was maintained at 80 % for both treatments, resulting in a daytime vapour pressure deficit (VPD) of 2.5 and 6.5 for the cold and warm temperature treatments, respectively.

BGD
10, 17939–17964, 2013

2.2 $^{13}CO_2$ pulse labelling

During the experiment, plants were grown with constant fresh air supply (CO_2 during the day = 400 ppm, −9 ‰). However, during pulse labelling, fresh air supply was temporarily stopped and 250 mL of 99 % $^{13}CO_2$ (Sigma Aldrich, St. Louis, USA) was slowly released into each growth cabinet over 55 min. The labelling resulted in an increase of $\delta^{13}C_{AIR}$ from −6.5 ‰ to 17 000 ‰ and 14 000 ‰ in the warm and cold cabinet, respectively (Fig. 2a and b). Likewise, cabinet [CO_2] of warm and cold increased to 512 ppm and 490 ppm but declined (due to plant photosynthesis) to a minimum of 311 ppm and 258 ppm at cessation of the release. After 55 min of labelling, additional fans and opening of the cabinet doors resulted in a quick drop of $\delta^{13}C_{AIR}$ to around 10 ‰ within about 10 min. The cabinets were flushed with fresh-air during the rest of the experiment. $\delta^{13}C_{AIR}$ and [CO_2] inside the growth cabinets were monitored during labelling and post-labelling using a tunable diode laser (see Sect. 2.5).

All measured $^{13}C/^{12}C$ ratios are reported relatively to the Pee Dee Belemnite scale (PDB) using the δ-notation according to:

$$\delta^{13}C = \frac{R_{sample}}{R_{PDB}} - 1, \quad (1)$$

where R_{sample} and R_{PDB} refers to the $^{13}C/^{12}C$ ratio of the sample and the standard, respectively. In order to correct for the dilution of ^{13}C by the existing carbon pool, bulk $\delta^{13}C$ samples were also expressed as $^{13}C_{excess}$. This value reflects the amount of ^{13}C added by labelling roots or shoots and is reported in mg ^{13}C. $^{13}C_{excess}$ was calculated

BGD
10, 17939–17964, 2013

following Ruehr et al. (2009):

$$^{13}C_{excess} = \frac{A_S - A_{NB}}{100} \cdot m_s \cdot f_C, \quad (2)$$

with

$$A_{S,NB} = \frac{100 \cdot 0.0111802 \cdot \left(\frac{\delta_{S,NB}}{1000} + 1\right)}{1 + 0.0111802 \cdot \left(\frac{\delta_{S,NB}}{1000} + 1\right)} \quad (3)$$

where A_S and A_{NB} are the sample and natural abundance atom % values derived from the respective $\delta^{13}C$ values. Further, m_s denotes the root or shoot biomass in mg and f_C the carbon fraction within the samples. Carbon fraction (f_C) was not different in bulk root from that in shoot samples (mean ±1 standard error; roots: 0.408 ± 0.125; shoots 0.413 ± 0.075), and was therefore set to 0.41 for both components.

2.3 $\delta^{13}C$ analysis of shoot and root respiration and bulk

2.3.1 $\delta^{13}C$ of shoot-respired CO_2 ($\delta^{13}C_{SR}$)

The $\delta^{13}C$ signal of shoot respired CO_2 ($\delta^{13}C_{SR}$) was measured 20 times during the course of the experiment. That is, during label day, samples were taken at a sub-hourly timescale and during post-label days sampling was reduced to at most one sample a day. At each sampling time, shoot samples were collected by clipping leaves (including sheaths) down to 20 mm above the soil surface from three randomly sampled pots from each treatment. After clipping, shoot samples were incubated in the dark for 2 h and then sealed in Tedlar® bags, following Barbour et al. (2011a, b). Selection of the dark incubation time was based on a preliminary experiment which showed that $\delta^{13}C_{SR}$ is highly variable after initial placement in the dark, but is stable after 2 h (Fig. 1), which was consistent with Barbour et al. (2011a). After dark incubation, the bags were repeatedly flushed and filled with CO_2-free air in order to create a CO_2-free atmosphere inside

BGD
10, 17939–17964, 2013

the bag. Subsequently, samples were incubated until leaf respiration increased the CO_2 concentration within the bag to > 320 ppm (within 6 to 20 min). During bag flushing and sealing leaves spent approximately 2 min at very low light (< 5 µmol m^{-2} s^{-1} PAR), but otherwise remained in complete darkness during incubation and analysis. The short period at very low light during bag flushing was assumed to have no effect on the respiratory biochemistry of the leaves (Barbour et al., 2011a). Finally, the respired CO_2 in the incubated bags was analysed for its carbon isotopic composition using a tunable diode laser (see Sect. 2.5). Although $\delta^{13}C$ of shoot-respired CO_2 ($\delta^{13}C_{SR}$) is dependent on the age of the leaf, this effect was assumed to be overpowered by the labelling intensity, hence all leaves were used for analysis.

In addition, at each time step subsamples of shoots from each pot were dried at 60 °C and later ground in a ball mill and analysed for their bulk $\delta^{13}C$ ($\delta^{13}C_{SB}$) and total C content using IRMS (see Sect. 2.5.2).

2.3.2 $\delta^{13}C$ of root-respired CO_2 ($\delta^{13}C_{RR}$)

A pilot study found no difference in the $\delta^{13}C$ of root-respired CO_2 ($\delta^{13}C_{RR}$) between roots from different parts of the root-ball (data not shown). To maintain the initial sub-hourly sampling frequency, only easily accessible and washable roots at the bottom of the pot were used. Roots were hand-washed from all soil particles, dried with a paper towel, sealed in Tedlar® bags and then repeatedly flushed and filled with CO_2-free air. The bags were left to incubate at room temperature until root respiration resulted in the CO_2 concentration in the bag reaching > 320 ppm (approx. 30 min). $\delta^{13}C_{RR}$ was then measured, and subsamples were collected for analysis of bulk $\delta^{13}C$ ($\delta^{13}C_{RB}$) and total C content, as described above for the shoot samples.

2.4 Biomass

At the start of the experiment, the height of the plants was 250–300 mm. After seven days, the total root and shoot biomass were measured on three replicates of each treat-

BGD
10, 17939–17964, 2013

ment. All aboveground components were cut, and all roots were hand-washed free of soil. Both components were dried at 60 °C for at least 48 h before weighing. Prior to drying, the leaf area of a subsample was determined by scanning the foliage on a flat-bed scanner and processing the images using WinFolia Pro 2004a (Regent Instruments Inc., Canada). Leaf area per pot was approximately 0.1 m^2, equating to a leaf area index (LAI) of the *Lolium* canopy of 5.6 $m^2\,m^{-2}$. This LAI however is a considerable overestimate as *all* leaves were included in the calculation (including shoots drooping beyond the edge of the 150 mm diameter pot, rather than just those exactly over the pot surface area).

2.5 Isotope measurements

Tunable diode laser adsorption spectroscopy (TGA100A; Campbell Scientific, Logan, USA) was used to analyse air samples (CO_2) for its carbon isotopic composition. The spectrometer measures concentrations of three different CO_2 isotopologues at 1 Hz, namely $^{12}C^{16}O_2$, $^{13}C^{16}O_2$ and $^{12}C^{18}O^{16}O$ from which the δ-values ($\delta^{13}C$, $\delta^{18}O$) are derived. See Bowling et al. (2003) and Barbour et al. (2007) for further details on the instrument and calibration.

Total shoot and root bulk C content and $\delta^{13}C$ were analysed at the Waikato Stable Isotope Unit (University of Waikato, New Zealand) using a Dumas elemental analyser (Europa Scientific ANCA-SL, Crewe, UK) interfaced to an isotope ratio mass spectrometer (IRMS, Europa Scientific 20–20 Stable Isotope Analyser, Crewe, UK). 3 mg of dried and ground plant material (root or shoot) was weighed into tin capsules before being placed into the autosampler. The final $\delta^{13}C$ value was referenced to an internal lab standard (sucrose), which in turn had been referenced to a certified standard from CSIRO, Canberra, Australia. The one standard deviation of the internal lab calibration standard was 0.14 ‰ and instrument error 0.3 ‰.

BGD
10, 17939–17964, 2013

2.6 Data analyses

Lag-times between the application of $^{13}CO_2$ and its use in shoot and root respiration were determined by comparing the timing of the peaks in $\delta^{13}C_{SR}$, $\delta^{13}C_{RR}$, $\delta^{13}C_{SB}$, $\delta^{13}C_{RB}$. The measurement frequency soon after labelling resulted in a minimum measureable lag-time of approximately 30 min. Exponential decay functions were fitted using $f(t) = y0 + a\, e^{(-\tau t)}$ where a is the initial quantity, τ is the time constant and t is time. The resulting half-life time (hlt) was calculated as $\ln(2)/\tau$. From the sub-hourly label day, only midday values were used for the exponential decay fit to be in line with post-label day sampling. Treatment differences in peak enrichment values were analysed using two-tailed two sample t tests assuming unequal variance. All reported values are means ± standard error. Data were processed and analysed using R 2.15.1 (R Foundation for Statistical Computing, 2012).

3 Results

3.1 Time-lags and residence time

Releasing 500 mL of 99 % $^{13}CO_2$ into the growth chambers resulted in a rapid increase of $\delta^{13}C_{AIR}$ from −6.7 ‰ to ca. 17 000 ‰ in the warm and from −6.3 ‰ to ca. 14 000 ‰ in the cold treatment (Fig. 2a and b). Further, pulse labelling resulted in a similar response in $\delta^{13}C_{SR}$ between treatments, both in timing and magnitude (Table 1; Fig. 2c and d). There was an immediate strong increase in $\delta^{13}C_{SR}$ from −28 ‰ to ca. 400 ‰ at 23 min after the start of labelling. Shortly after, $\delta^{13}C_{SR}$ reached a similar maximum 56 min after label start in both treatments (ca. 800 ‰) which was followed by a steady decay. The start of the decay period coincided with the termination of the label application. The calculated half-life time of $\delta^{13}C_{SR}$ was 8.3 h in the warm and 10.7 h in the cold treatment (see Sect. 2.6, Table 1, Fig. 3).

BGD
10, 17939–17964, 2013

The $\delta^{13}C$ signal of root respiration ($\delta^{13}C_{RR}$) followed a similar trend as that of shoot respiration, albeit with a distinct time-lag (Fig. 2g and h). The initial enrichment of $\delta^{13}C_{RR}$ in the warm treatment occurred 56 min (−16.15 ± 3.04 ‰) after label start and peaked at 2.9 h (359.28 ± 30.12 ‰). In contrast, $\delta^{13}C_{RR}$ of the cold treatment increased initially only 1.5 h after the introduction of the label (13.52 ± 8.52 ‰) and peaked at 4.7 h (225.62 ± 47.31 ‰ ; Table 1). Despite the distinct and clear overall enrichment on the day of labelling, both treatments showed irregular fluctuations around the general trend. After the label day, both $\delta^{13}C_{RR}$ curves followed an exponential decay function of which the derived half-life time was almost twice as long in the cold treatment (17.7 h) compared with the warm treatment (9.1 h; Table 1).

The $\delta^{13}C_{SR}$ and $\delta^{13}C_{RR}$ data were complemented by $\delta^{13}C$ measurements of bulk shoot and root material ($\delta^{13}C_{SB}$, $\delta^{13}C_{RB}$, respectively; Fig. 2e, f, i, and j). The overall isotopic enrichment was always smaller in bulk compared to respired samples. However the measurements followed similar trends. Bulk shoot material ($\delta^{13}C_{SB}$) of both treatments showed an immediate response to the labelling with an increase from natural abundance values (−25.13 ± 3.02 ‰ at 10 °C; = −30.03 ± 0.44 ‰ at 25 °C) to about 1 ‰, 23 min after the introduction of the label. However, while the warm treatment peaked already 56 min after label start (67.95 ± 26.80 ‰) the cold treatment reached its maximum at 1.5 h (83.93 ± 10.25 ‰) after label start. After reaching their respective maxima, $\delta^{13}C_{SB}$ of both treatments decreased thereafter. The time-lagged response of $\delta^{13}C_{RR}$ was mirrored in the $^{13}CO_2$ label induced enrichment of $\delta^{13}C_{RB}$. Bulk $\delta^{13}C_{RB}$ did not differ between treatments at the initial enrichment, which occurred 1.5 h after label start (10 °C = −24.79 ± 0.84 ‰; 25 °C = −21.32 ± 1.31 ‰; $t = 2.23$, $df = 2$, $p = 0.16$). While the timing of peak enrichment differed between treatments (10.3 h and 26.2 h for the warm and cold treatment respectively), $\delta^{13}C_{RB}$ was not significantly different (10 °C = −7.26 ± 2.46 ‰; 25 °C = −3.59 ± 1.95 ‰; $t = 1.17$, $df = 4$, $p = 0.30$).

BGD
10, 17939–17964, 2013

3.2 Carbon allocation

Full plant carbon budgeting between above- and belowground is possible only when the actual CO_2 flux densities (photosynthesis and respiration rates) are measured. Nevertheless, by assuming that the maximum ^{13}C enrichment in the bulk shoot material ($\delta^{13}C_{SB}$) accounts for all of the newly assimilated C, it was still possible to estimate C allocation rates between shoot and roots (Burri et al., 2013a). To account for the dilution of ^{13}C in plant carbon pools, $^{13}C_{excess}$ values rather than the direct δ-values were used for budgeting (Fig. 4a and b). Further, the fraction which was not recovered in biomass (storage, growth) at the end of the experiment must have been invested into plant respiration, exudation or volatile losses. The results of above- and belowground C allocation are given in Fig. 4c and d. It shows that after 6 d at either temperature, more than 50 % of the initial ^{13}C allocated to biomass was invested into respiration, exudation or volatile losses. Moreover, the cold treatment showed a stronger investment into respiration, exudation or volatile losses (83 % compared with 69 % in the warm treatment) at the end of the experiment. In the warm, 26.8 % of the remaining 31 % C was incorporated into shoot biomass and only 4.6 % into root biomass. In the cold, 13.2 % of the remaining 17 % carbon was transported into shoot biomass and only 3.3 % into root biomass. This resulted also in a higher root-to-shoot ratio under cold temperatures (0.25) compared to warm (0.17) when considering the allocation of newly photo-assimilated C only. The duration of the experiment was most likely to short to see such an effect at the whole plant scale as no differences in biomass were observed between treatments (Table 1).

4 Discussion

In the shoots, there was no measureable time-lag between assimilation and respiration in either temperature treatment, indicated by the direct response of $\delta^{13}C_{SR}$ to $^{13}CO_2$ labelling. This finding points to an almost immediate (< 30 min) utilisation of

BGD
10, 17939–17964, 2013

recent-assimilates for respiratory processes within the leaves, independent of temperature. A previous study, also on *Lolium*, found that two fast pools supply the centres of respiration where 43 % of total respiration are supplied by recent photo-assimilates (Lehmeier et al., 2008). The direct coupling of atmospheric conditions with shoot respiration (within minutes) has implications for the usage of $\delta^{13}C_{SR}$ as a proxy of whole season water use efficiency at the field scale (cf. Barbour et al., 2011a). The short-term high sensitivity of $\delta^{13}C_{SR}$ to environmental drivers demonstrates that this measurement strategy is not suitable to assess water-use efficiency at the whole-season field scale as originally proposed by Barbour et al. (2011a).

By directly analysing the $\delta^{13}C$ of root respired CO_2, interferences due to physical back-diffusion of ^{13}C tracer from the soil, as observed by Bahn et al. (2009), could be disregarded. This is important for the correct assessment of time-lags as the physical $^{13}CO_2$ flux blurs the biological flux and needs to be corrected for (Burri et al., 2013b; Barthel et al., 2011b; Subke et al., 2009). While $\delta^{13}C_{SR}$ showed a direct response to $^{13}CO_2$ labelling, label appearance was delayed in $\delta^{13}C_{RR}$ by about 1 h at 25 °C, which is similar to the lag observed by Lehmeier et al. (2008) at 20 °C. An even faster utilization of recent photo-assimilates by root respiration was measured by Domanski et al. (2001) who measured a lag of only 30 min in *Lolium* at 27 °C.

Imposing plants to a sudden decrease in temperature from 25 °C to 10 °C significantly reduced the rate of C transport from above- to belowground, hence increasing the time-lag between photosynthesis and root respiration. Plain et al. (2009) found a similar reduction in the rate of C transport from above- to belowground for 20 yr old *Fagus sylvatica* trees under colder temperatures. However, in their experiment cold temperature was accompanied with low photosynthetic active radiation, which points to a combined effect of these environmental parameters. A delay of C transport through the system under lower soil temperatures was also found in *Plantago* during an investigation of C transport from plant to fungus using ^{13}C labelling (Hawkes et al., 2008). The results presented here are also in accordance with other environmental factors as increased time-lag was also found under drought stress for *Fagus sylvatica* saplings

BGD
10, 17939–17964, 2013

(Ruehr et al., 2009; Barthel et al., 2011a) and under shading in a mountain grassland (Bahn et al., 2009).

In a review by Kuzyakov and Gavrichkova (2010) it was stated that the time-lag from above- to below-ground is affected by several steps including CO_2 fixation, phloem loading, phloem transport, root respiration, and diffusion out of the soil. They suggested that the bottle neck process determining the time-lag differs between grasses and trees. While for trees phloem transport appears to determine the time-lag, the rate-limiting step for grasses remains unclear. Temperature affects many of the biological and physical processes involved and while we cannot completely distinguish these processes, we can discuss their relative importance on the results.

First, long-distance transport is affected by the viscosity of the phloem, which generally decreases with lower temperatures (Reynolds 1886; Seeton, 2006). Thus the longer time-lag could be explained by reduced phloem viscosity under low temperatures. Viscosity varies as rapidly with temperature as metabolic processes do (Johnson and Thornley, 1985), which is in line with our immediate response to the sudden temperature change. However, the temperature dependence of phloem translocation is not consistent within the literature, ranging from maintained translocation under low temperature in *Salix* (Watson, 1975) to decreased translocation in *Sorghum* (Wardlaw and Bagnall, 1981).

Second, when changing temperature, hydraulic properties in the translocation may also be affected. This could have resulted in a sudden change in stomatal conductance, photosynthetic rate and thus assimilate transport. However, since cooling resulted in a lower vapour pressure deficit compared with the initial growing conditions and the warm treatment, the temperature treatment is unlikely to have caused hydraulically induced stomatal limitation. Moreover, the plants kept well-watered at all times, so it is unlikely that soil water was limiting. Additionally, if soil water availability would have been a factor and resulted in desiccation and subsequent hydraulic adaptations (e.g., Holloway-Phillips and Brodribb, 2011), it would have affected the warm treatment more

BGD
10, 17939–17964, 2013

than the cold treatment, which means that our results are an underestimate of the temperature effect alone.

Third, colder temperatures generally decrease enzyme activity. The reduction in *Lolium perenne* photosynthesis and respiration with temperature are approximately equal, with a Q10 of around 2 (Woledge and Dennis, 1982; Rainey et al., 1987). While rates of photosynthesis and respiration were not measured in this study, we can still assume that the C supply and demand is decreased under lower temperature. Thus, enzyme mediated reduction in photosynthesis and respiration is likely to have contributed to the decrease in the rate of translocation.

However, since temperature affects the biological and physical processes at the same time it is difficult to distinguishing the most prominent factor driving translocation.

Moreover, we showed that temperature also influences the half-life time of the $\delta^{13}C$ signal of respiration in shoots and roots. Assuming that the $\delta^{13}C$ of respiration reflects the labile C pool within plants, high half-life times reflect a quick turnover of the labile C pool. Since half-life times of $\delta^{13}C$ from shoot and root respiration were relatively higher at 25 °C, C turnover in the labile C pool should also be higher at 25 °C. Quick pool turnover is generally caused by high input (photosynthesis) or high output rates (respiration, storage, growth) or both. Therefore, one can conclude that under cold temperatures labile C turnover is small, hence less C is used for growth and/or storage. This is in agreement with McGoy (1990), who found a decrease in the immobilisation flux at low temperatures in *Glycine*. Increased half-life time of the $\delta^{13}C$ signal of soil respiration under stress was also observed for *Fagus* saplings which had been subjected to drought (Barthel et al., 2011a); however drought treatments invariably result in strong changes in hydraulic factors, which complicate the interpretation and direct comparison of such results. Lehmeier et al. (2008) performed a compartmental analysis of respiratory tracer kinetics in *Lolium* and concluded that both shoot and root respiration are supplied by three pools with half-life sizes of < 15 min, 3 h, and 33 h respectively. The observed half-life times for shoot and root respiration are well within this

BGD
10, 17939–17964, 2013

time range (8.3–17.7 h, Table 1), and suggest that under cold temperatures the labile pool turnover is slower.

As mentioned above, recent C can be used either for storage, growth or respiration. ^{13}C analysis of bulk material revealed that *Lolium* invests relatively more C into respiration after exposure to a sudden temperature drop. This is in line with the already discussed half-life time and associated labile C pool turnover: under cold temperatures relatively less C is invested into growth/storage but remains for respiration. Further, relatively more C was incorporated into root biomass under cold temperatures as indicated by the root-to-shoot ratio calculated from $^{13}C_{excess}$. This is in accordance with previous experiments on plant allocation patterns which show consistently that plants invest relatively more C into roots when under climatic stress (shading, drought). For instance, monocultures invest relatively more C into root biomass when drought stressed (Sanaullah et al., 2012). Further, under drought more C is available to the roots by a stronger reduction in shoot growth compared to root growth rates (Palta and Gregory, 1997). On the scale of plots, Burri et al. (2013a) reported an increase in the proportion of carbon allocated to roots in drought-stressed compared with well-watered grasslands, but the effect of the treatment on respiration was much less. Similar results have been found in shaded mountain grasslands where belowground allocation was maintained at the expense of reserves (Bahn et al., 2013). Moreover, in shaded *Lolium* plants root respiration was maintained by root reserves (Schmitt el al., 2013).

5 Conclusions

To conclude, plants exposed to a sudden temperature drop delay the C transport from above- to belowground and invest relatively more C into root biomass and overall plant respiration. Therefore, temperature is not solely a driver of biological processes in the plant-soil system itself, but also influences the speed at which recent photo-assimilates are made available belowground. Since belowground substrate availability is an important driver for heterotrophic and autotrophic soil respiration, our results highlight

BGD
10, 17939–17964, 2013

the importance of temperature as a crucial environmental driver for C cycling between above- and belowground and thus within terrestrial ecosystems.

Supplementary material related to this article is available online at http://www.biogeosciences-discuss.net/10/17939/2013/bgd-10-17939-2013-supplement.pdf.

Acknowledgements. The authors would like to thank Graeme Rogers for assistance with the experiment. We thank David Whitehead for helpful discussion and comments on an earlier version of this manuscript. This research was supported by funding from the Ministry of Business, Innovation and Employment.

References

Bahn, M., Schmitt, M., Siegwolf, R., Richter, A., and Brüggemann, N.: Does photosynthesis affect grassland soil-respired CO_2 and its carbon isotopic composition on a diurnal timescale?, New Phytol., 182, 451–460, 2009.

Bahn, M., Lattanzi, F. A., Hasibeder, R., Wild, B., Koranda, M., Danese, V., Brüggemann, N., Schmitt, M., Siegwolf, R., and Richter, A.: Responses of belowground carbon allocation dynamics to extended shading in mountain grassland, New Phytol., 198, 116–126, 2013.

Barbour, M. M., Farquhar, G. D., Hanson, D. T., Bickford, C. P., Powers, H., and McDowell, N. G.: A new measurement technique reveals temporal variation in $\delta^{18}O$ of leaf-respired CO_2, Plant Cell Environ., 30, 456–468, doi:10.1111/j.1365-3040.2007.01633.x, 2007.

Barbour, M. M., Tcherkez, G., Bickford, C., Mauve, C., Lamothe, M., Sinton, S., and Brown, H.: $\delta^{13}C$ of leaf-respired CO_2 reflects intrinsic water-use efficiency in barley, Plant Cell Environ., 34, 792–799, 2011a.

Barbour, M. M., Hunt, J. E., Kodama, N., Laubach, J., McSeveny, T., Rogers, G., Tcherkez, G., and Wingate, L.: Rapid changes in $\delta^{13}C$ of ecosystem-respired CO_2 after sunset are consistent with transient ^{13}C enrichment of leaf respired CO_2, New Phytol., 190, 990–1002, 2011b.

BGD
10, 17939–17964, 2013

Barthel, M., Hammerle, A., Sturm, P., Baur, T., Gentsch, L., and Knohl, A.: The diel imprint of leaf metabolism on the $\delta^{13}C$ signal of soil respiration under control and drought conditions, New Phytol., 192, 925–938, 2011a.

Barthel, M., Sturm, P., Knohl, A.: Soil matrix tracer contamination and canopy recycling did not impair $^{13}CO_2$ plant-soil pulse labelling experiments, Isot. Environ. Healt. S., 47, 359–371, doi:10.1080/10256016.2011.587610, 2011b.

Bhupinderpal-Singh, Nordgren, A., Lofvenius, M. O., Högberg, M. N., Mellander, P. E., and Högberg, P.: Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year, Plant Cell Environ., 26, 1287–1296, 2003.

Bowling, D. R., Sargent, S. D., Tanner, B. D., and Ehleringer, J. R.: Tunable diode laser absorption spectroscopy for stable isotope studies of ecosytem-atmosphere CO_2 exchange, Agr. Forest Meteorol., 118, 1–19, 2003.

Brüggemann, N., Gessler, A., Kayler, Z., Keel, S. G., Badeck, F., Barthel, M., Boeckx, P., Buchmann, N., Brugnoli, E., Esperschütz, J., Gavrichkova, O., Ghashghaie, J., Gomez-Casanovas, N., Keitel, C., Knohl, A., Kuptz, D., Palacio, S., Salmon, Y., Uchida, Y., and Bahn, M.: Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: a review, Biogeosciences, 8, 3457–3489, doi:10.5194/bg-8-3457-2011, 2011.

Burri, S., Sturm, P., Prechsl, U. E., Knohl, A., and Buchmann, N.: The impact of extreme summer drought on the short-term carbon coupling of photosynthesis to soil CO_2 efflux in a temperate grassland, Biogeosciences Discuss., 10, 11671–11704, doi:10.5194/bgd-10-11671-2013, 2013a.

Burri, S., Sturm, P., Baur, T., Barthel, M., Knohl, A., and Buchmann, N.: The effect of physical back-diffusion of $^{13}CO_2$ tracer on the coupling between photosynthesis and soil CO_2 eflux in grassland, Isot. Environ. Healt. S., in review, 2013b.

Domanski, G., Kuzyakov, Y., Siniakina, V., and Stahr, K.: Carbon flows in the rhizosphere of rygrass (*Lolium perenne*), J. Plant Nutr. Soil Sc., 164, 381–387, 2001.

Epron, D., Bahn, M., Derrien, D., Lattanzi, F. A., Pumpanen, J., Gessler, A., Högberg, P., Maillard, P., Dannoura, M., Gerant, D., and Buchmann, N.: Pulse-labelling trees to study carbon allocation dynamics: a review of methods, current knowledge and future prospects, Tree Physiol., 32, 776–798, doi:10.1093/treephys/tps057, 2012.

BGD
10, 17939–17964, 2013

Hawkes, C. V., Hartley, I. P., Ineson, P., and Fitter, A. H.: Soil temperature affects carbon allocation within arbuscular mycorrhizal networks and carbon transport from plant to fungus, Global Change Biol., 14, 1181–1190, 2010.

Johnson, I. R. and Thornley, J. H. M.: Review article: temperature dependence of plant and crop processes, Ann. Bot.-London, 55, 1–24, 1985.

Holloway-Phillips, M.-M., and Brodribb, T. J.: Minimum hydraulic safety leads to maximum water-use efficiency in a forage grass, Plant Cell Environ., 34, 302–313, 2011.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A., Ekblad, A., Högberg, M., Nyberg, G., Ottosson-Lofvenius, M., and Read, D.: Large-scale forest girdling shows that current photosynthesis drives soil respiration, Nature, 411, 789–792, 2001.

Kuzyakov, Y. and Gavrichkova, O.: Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls, Global Change Biol., 16, 3386–3406, doi:10.1111/j.1365-2486.2010.02179.x, 2010.

Lehmeier, C. A., Lattanzi, F. A., Schaeufele, R., Wild, M., and Schnyder, H.: Root and shoot respiration of perennial ryegrass are supplied by the same substrate pools: assessment by dynamic ^{13}C labeling and compartmental analysis of tracer kinetics, Plant Physiol., 148, 1148–1158, doi:10.1104/pp.108.127324, 2008.

Martin, J. G., Phillips, C. L., Schmitt, A., Irvine, J., Law, B. E.: High-frequency analysis of the complex linkage between soil CO_2 fluxes, photosynthesis and environmental variables, Tree Physiol., 32, 49–64, doi:10.1093/treephys/tpr134, 2012.

McGoy, E. L., Boersma, L., and Ekasingh, M.: Net carbon allocation in soybean seedlings as influenced by soil water stress at two soil temperatures, Bot. Gaz., 151, 497–505, 1990.

Palta, J. A. and Gregory, P. J.: Drought affects the fluxes of carbon to roots and soil in ^{13}C pulse-labelled plants of wheat, Soil Biol. Biochem., 29, 1395–1403, doi:10.1016/s0038-0717(97)00050-3, 1997.

Plain, C., Gerant, D., Maillard, P., Dannoura, M., Dong, Y., Zeller, B., Priault, P., Parent, F., and Epron, D.: Tracing of recently assimilated carbon in respiration at high temporal resolution in the field with a tuneable diode laser absorption spectrometer after in situ $^{13}CO_2$ pulse labelling of 20-year-old beech trees, Tree Physiol., 29, 1433–1445, doi:10.1093/treephys/tpp072, 2009.

R Development Core Team: R: a Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, available at: http://www.R-project.org, ISBN 3-00051-07-0, 2008.

BGD
10, 17939–17964, 2013

Rainey, D. Y., Mitton, J. B., Monson, R. K.: Associations between enzyme genotypes and dark respiration in perennial ryegrass, *Lolium perenne* L., Oecologia, 74, 335–338, 1987.
Reynolds, O.: On the theory of lubrication and its application to Mr. Beauchamp tower's experiments, including an experimental determination of the viscosity of olive oil, Philos. T. R. Soc. Lond., 177, 157–234, 1886.
Ruehr, N. K., Offermann, C. A., Gessler, A., Winkler, J. B., Ferrio, J. P., Buchmann, N., and Barnard, R. L.: Drought effects on allocation of recent carbon: from beech leaves to soil CO_2 efflux, New Phytol., 184, 950–961, doi:10.1111/j.1469-8137.2009.03044.x, 2009.
Sanaullah, M., Chabbi, A., Rumpel, C., and Kuzyakov, Y.: Carbon allocation in grassland communities under drought stress followed by ^{14}C pulse labeling, Soil Biol. Biochem., 55, 132–139, doi:10.1016/j.soilbio.2012.06.004, 2012.
Schaer, C., Vidale, P., L., Luethi, D., Frei, C., Haeberli, C., Liniger, M. A., and Appenzeller, C.: The role of increasing temperature variability in European summer heatwaves, Nature, 427, 332–336, doi:10.1038/nature02300, 2004.
Schmitt, A., Pausch, J., and Kuzyakov, Y.: Effect of clipping and shading on C allocation and fluxes in soil under ryegrass and alfalfa estimated by ^{14}C labelling, Appl. Soil Ecol., 64, 228–236, 2013.
Seeton, C. J.: Viscosity-temerature correlation for liquids, Tribol. Lett., 22, 67–78, doi:10.1007/s11249-006-9071-2, 2006.
Subke, J.-A., Vallack, H. W., Magnusson, T., Keel, S. G., Metcalfe, D. B., Högberg, P., and Ineson, P.: Short-term dynamics of abiotic and biotic soil $^{13}CO_2$ effluxes after in situ $^{13}CO_2$ pulse labelling of a boreal pine forest, New Phytol., 183, 349–357, doi:10.1111/j.1469-8137.2009.02883.x, 2009.
Vargas, R., Baldocchi, D. D., Bahn, M., Hanson, P. J., Hosman, K. P., Kulmala, L., Pumpanen, J., and Yang, B.: On the multi-temporal correlation between photosynthesis and soil CO_2 efflux: reconciling lags and observations, New Phytol., 191, 1006–1017, doi:10.1111/j.1469-8137.2011.03771.x, 2011.
Wardlaw, I. F. and Bagnall, D.: Phloem transport and the regulation of growth of *Sorghum bicolor* (Moench) at low temperature, Plant Physiol., 68, 411–414, 1981.
Warren, J. M., Iversen, C. M., Garten Jr., C. T., Norby, R. J., Childs, J., Brice, D., Evans, R. M., Gu, L., Thornton, P., and Weston, D. J.: Timing and magnitude of C partitioning through a young loblolly pine (*Pinus taeda* L.) stand using ^{13}C labeling and shade treatments, Tree Physiol., 32, 799–813, doi:10.1093/treephys/tpr129, 2011.

BGD
10, 17939–17964, 2013

Watson, B. T.: The influence of low temperature on the rate of translocation in the phloem of *Salix viminalis* L., Ann. Bot., 39, 889–900, 1975.
Woledge, J. and Dennis, W. D.: The effect of temperature on photosynthesis of ryegrass and white clover leaves, Ann. Bot., 50, 25–35, 1982.

BGD
10, 17939–17964, 2013

Table 1. Overview of time-lags and half-life times between treatments. Time constant (τ) of exponential decay fit using $f(t) = y0 + a\ e^{(-\tau t)}$, corresponding coefficient of determination for exponential decay fit (R^2), derived half-life (hlt); time-lag to the first appearance of the label (t1); time-lag to the maximum induced enrichment of the label (t2). The above- and belowground biomass at seven days after the start of the labelling and temperature treatments are also provided (mean ±1 standard error, $n = 3$).

	SHOOTS		ROOTS	
T_{air}	25 °C	10 °C	25 °C	10 °C
τ	0.0841	0.0646	0.0760	0.0392
R^2	> 0.99	> 0.99	> 0.99	> 0.99
hlt (h)	8.25	10.73	9.12	17.69
t1 (h)	0.38	0.38	0.93	1.47
t2 (h)	0.93	0.93	2.92	4.67
biomass (g)	7.57 ± 0.29	8.33 ± 0.23	2.36 ± 0.17	2.35 ± 0.15

BGD
10, 17939–17964, 2013

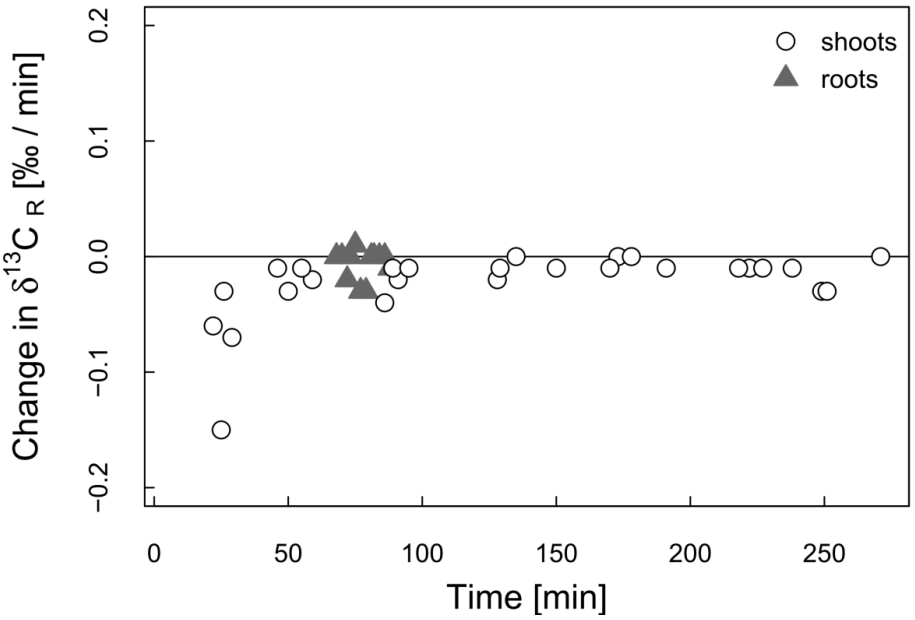

Fig. 1. Rate of change of $\delta^{13}C$ in respiration of shoots and roots ($\delta^{13}C_R$) after harvest. $\delta^{13}C_{SR}$ is highly variable after initial placement in the dark, but is stable after 2–2.5 h. Based on these results, shoot samples were left in the dark for 2 h, before being incubated (in CO_2-free air, also in the dark) for approximately 20 min, prior to measurement of $\delta^{13}C_{SR}$. Roots were measured approximately 1 h after harvesting, and showed little variation in $\delta^{13}C_{RR}$ around this time. Note, nearly all values fall within the 1σ standard deviation of the instrument's target calibration standard (0.12 ‰).

BGD
10, 17939–17964, 2013

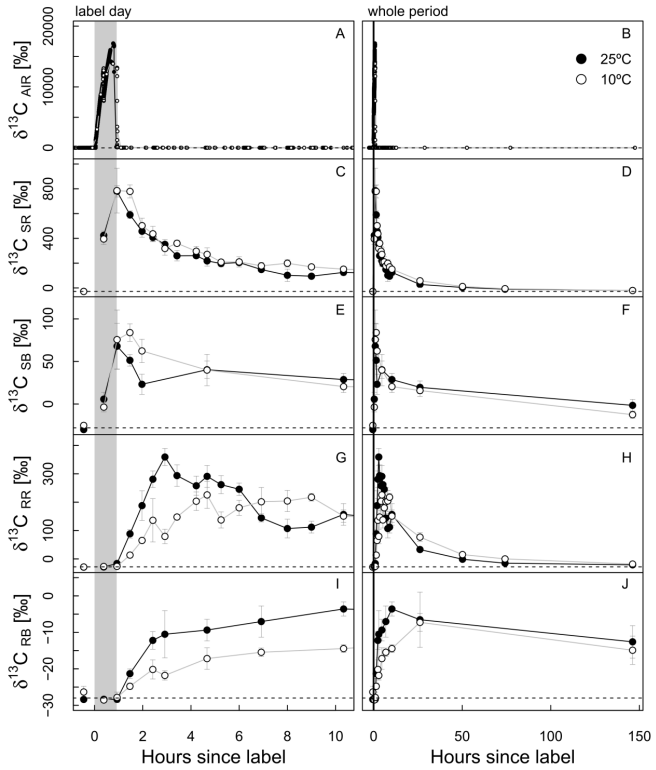

Fig. 2. Time course of $\delta^{13}C$ over the duration of the experiment, during label day (left panels) and the whole measurement period (right panels). Panels show $\delta^{13}C$ of the cabinet air ($\delta^{13}C_{AIR}$; **A, B**); shoot respired CO_2 ($\delta^{13}C_{SR}$; **C, D**); bulk shoot material ($\delta^{13}C_{SB}$; **E, F**); root respired CO_2 ($\delta^{13}C_{RR}$; **G, H**) and bulk root material ($\delta^{13}C_{RB}$; **I, J**). Duration of label application is indicated by the grey shaded area, and natural abundance by the horizontal dashed line. Closed and open symbols indicate the warm (25 °C) and cold (10 °C) treatments, respectively. Data are means ± standard error (n = 3).

BGD
10, 17939–17964, 2013

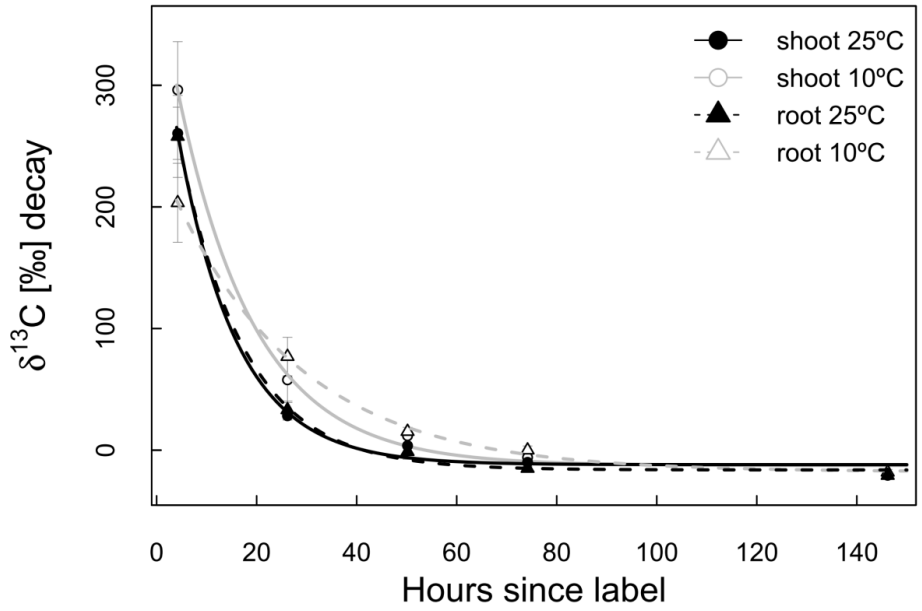

Fig. 3. Exponential decay of the $\delta^{13}C$ respiration signal in shoot and root. The data shown are means ± standard error ($n = 3$). Lines are fitted exponential decay functions (using $f(t) = y0 + a\ e^{(-\tau t)}$).

BGD
10, 17939–17964, 2013

Cold temperature regulates the time-lag between plant CO_2 uptake and release

M. Barthel et al.

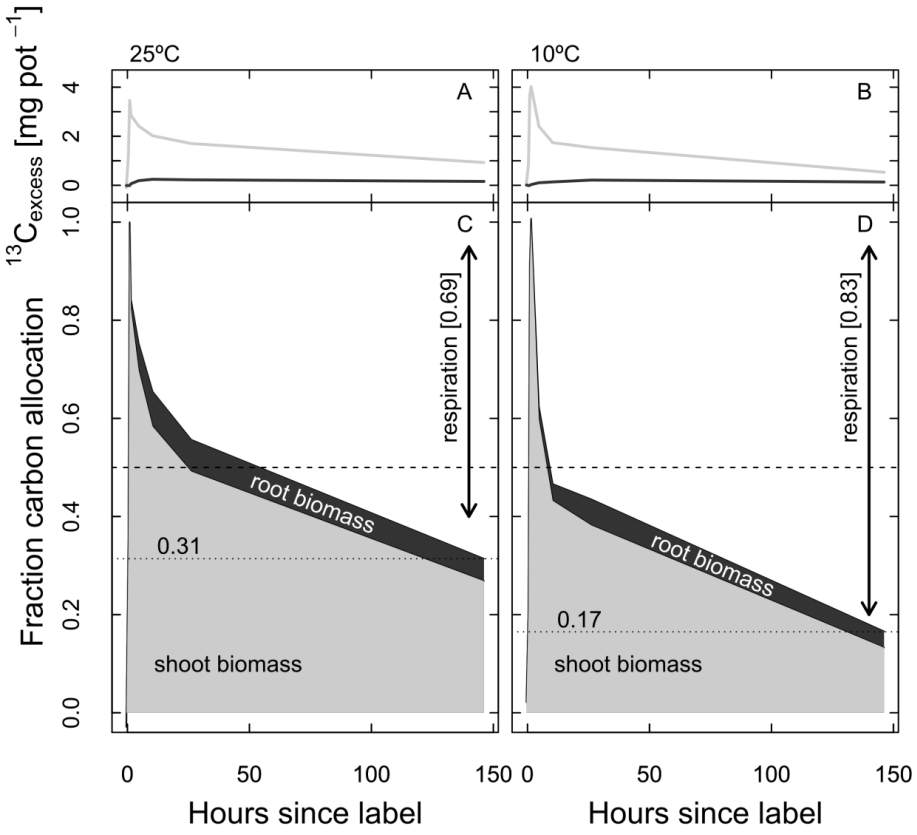

Fig. 4. (A, B) Time course of $^{13}C_{excess}$ in shoot (light grey) and root (dark grey) and **(C, D)** the derived fraction of carbon allocation relative to the initial shoot enrichment of shoot bulk material ($\delta^{13}C_{SB}$); warm treatment (25 °C, left panels); cold treatment (10 °C; right panels); 0.5 (dashed line); final value of biomass allocated to roots or shoots (dotted line).

BGD
10, 17939–17964, 2013

Cold temperature regulates the time-lag between plant CO_2 uptake and release

M. Barthel et al.