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Sudden cold temperature delays plant carbon transport and shifts allocation from growth to respiratory demand

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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 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 was analysed at regular time intervals using laser spectroscopy. In addition, $\delta^{13}C$ was analysed in bulk root and shoot samples.

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. 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. While the whole plant C turnover increased under cold temperature, the turnover time of the labile C pool decreased, probably because less ^{13}C is used for growth and/or storage. That is, (almost) all recent C remained in the labile pool serving respiration under these conditions. Overall, our results highlight the importance of temperature as a driver of C transport and relative C allocation within the plant–soil system.

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 (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 (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 can 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 above- and belowground by increasing allocation of recent C to root biomass (Palta and Gregory, 1997; Sanaullah et al., 2012; Burri et al., 2013) and delays the 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 slows the belowground transfer and/or respiratory use of recent photo-assimilates in grasslands (Bahn et al., 2009), but does not affect C cycling in pine (Warren et al., 2011). 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). Considering the multitude of responses, it is important to understand interactions between different environmental drivers but also single-factor responses.

Although it is well known that temperature has direct effects on photosynthesis and respiration, it remains unclear to what extent temperature affects the short-term coupling between photosynthesis and respiration, and C allocation above- and belowground. Therefore, we investigated the influence of a sudden 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. For time-lag estimation, respired carbon isotopic composition from shoots and roots was measured in subhourly time intervals using laser spectroscopy. To estimate effects on C allocation, shoot and root bulk biomass components were analysed at certain time steps using an isotope ratio mass spectrometer. The biological, chemical and physical processes involved in plant C uptake, transport and release are generally a function of temperature. Thus we hypothesise that low temperatures delay transport and increase residence time of recent photo-assimilates within plants as biochemical and biophysical reactions will be impaired. We further hypothesise that plants under low temperatures allocate relatively more C into respiration rather than biomass since suboptimal conditions enhance the focus on maintenance processes instead of growth or storage.

2 Methods

2.1 Experimental design

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. Light intensity was ramped up at "sunrise" to ca. 800 µmol m^{-2} s^{-1} PAR (photosynthetically active radiation) at the plant level over approximately 20 min. Plants were watered often to keep soil moisture at field capacity, and regularly 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 (pretreatment) measurement took place. The cold treatment was introduced only 5 min before label start to avoid acclimation effects (Ow et al., 2010). 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 0.25 and 0.65 kPa for the cold and warm temperature treatments, respectively.

The temperature treatments were chosen to cover a range of realistic climatic conditions in pasture-growing regions, and with the expectation of a treatment effect. A 15 °C temperature change on a subdaily to daily basis is a recurring event in several climatic regions. For example, in the agricultural region of Canterbury, New Zealand, on average every year there are more than ten occasions when the difference in daily maximum temperatures between subsequent days exceeds 10 °C, and one occasion where this difference exceeds 14 °C. Associated with the passing of cold fronts, a 10 °C drop in air temperature within 1 h occurs on average at least once per growing season (Lincoln Broadfield weather station, 1999—2013, data available at www.cliflo.niwa.co.nz).

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. A tunable diode laser (see below) monitored $\delta^{13}C_{AIR}$ and [CO_2] inside the growth cabinets during labelling and post-labelling. 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 cabinets, respectively (Fig. 1a, b). Likewise, cabinet [CO_2] of warm and cold treatments increased to 512 and 490 ppm but declined (due to plant photosynthesis) to a minimum of 311 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.

Tunable diode laser adsorption spectroscopy (TGA100A; Campbell Scientific, Logan, USA) was used to analyse the carbon isotopic composition of air samples. 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. All measured $^{13}C/^{12}C$ ratios are reported relative 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} refer to the $^{13}C/^{12}C$ ratio of the sample and the standard, respectively.

2.3 Time lags and residence time

The $\delta^{13}C$ signal of shoot and root-respired CO_2 ($\delta^{13}C_{SR}$ and $\delta^{13}C_{RR}$, respectively) was measured 20 times during the course of the experiment. It was measured subhourly during label day, and subsequently reduced to at most one sample a day. In addition, $\delta^{13}C$ of bulk shoot and root material was analysed. The time courses of these signals were used to estimate the time lag between $^{13}CO_2$ uptake and its subsequent release during shoot and root respiration, and also to calculate the carbon residence time within the plants.

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 in Tedlar® bags, following Barbour et al. (2011a, b; see Supplement, Fig. S2). From the same plants, easily accessible and washable roots at the bottom of the pot were hand-washed from all soil particles, dried with a paper towel and also sealed in Tedlar® bags. A pilot study found no difference in the $\delta^{13}C$ of root-respired CO_2 between roots from different parts of the root ball (data not shown). After sample incubation, bags were sealed and repeatedly flushed and filled with CO_2-free air in order to create a CO_2-free atmosphere inside the bag, before further incubating the samples until respiration increased the CO_2 concentration within the bag to > 320 ppm (this occurred within 6–20 min for shoots, and after ca. 30 min for roots). Bags remained in complete darkness during incubation and analysis, except approximately 2 min at very low light (< 5 µmol m^{-2} s^{-1} PAR) during bag flushing and sealing, which we assumed had 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. 2.2). 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 negligible compared with the labelling intensity, hence all leaves were used for analysis. In addition, at each time step subsamples of shoots and

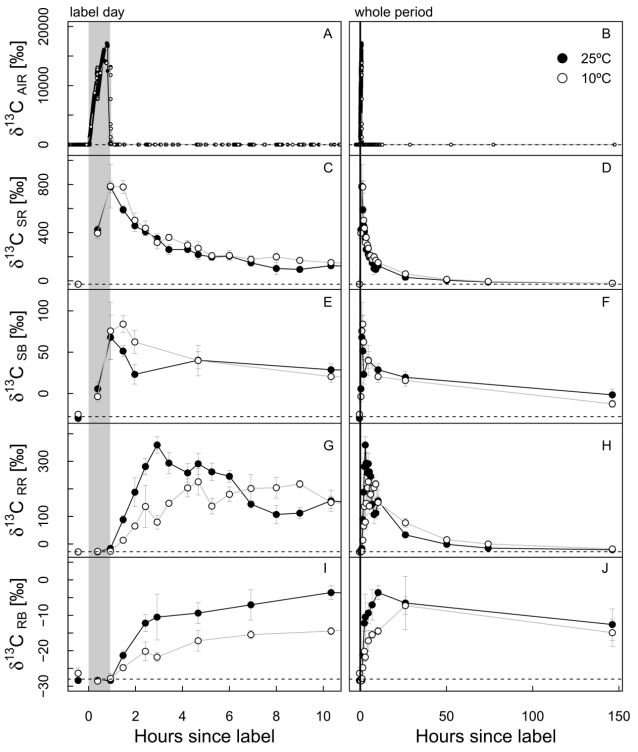

Fig. 1. Time course of $\delta^{13}C$ over the duration of the experiment, during label day (left panels) and the whole measurement period (right panels). Panels show $\delta^{13}C$ of the cabinet air ($\delta^{13}C_{AIR}$; **A**, **B**); shoot respired CO_2 ($\delta^{13}C_{SR}$; **C**, **D**); bulk 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 ($n = 3$).

roots 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 at the Waikato Stable Isotope Unit (University of Waikato, Hamilton, New Zealand; one standard deviation of the internal lab calibration standard was 0.14 ‰ and instrument error 0.3 ‰).

2.4 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 newly assimilated C, it was still possible to estimate C allocation rates between shoot and roots (Burri et al., 2013). 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. In order to correct for the dilution of ^{13}C by the existing plant 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 milligrams of ^{13}C. $^{13}C_{excess}$ was used for C budgeting and calculated following Ruehr et al. (2009):

$$^{13}C_{excess} = \frac{A_S - A_{NA}}{100} m_S f_C \quad (2)$$

with

$$A_{S,NA} = 100 \frac{R_{S,NA}}{1 + R_{S,NA}}, \quad (3)$$

where A_S and A_{NA} are the sample and natural abundance at. % values derived from the respective sample ratios. Further, m_s denotes the root or shoot biomass in milligrams and f_C the carbon fraction within the samples. Carbon fraction (f_C) was set to 0.41 for roots and shoots, as analyses of bulk material showed no difference between these components (mean ±1 standard error; roots 0.408 ± 0.125; shoots 0.413 ± 0.075).

Total root and shoot biomass was obtained from three replicates of each treatment at the end of the experiment and the resulting values applied in Eq. (2) (Table 1). For biomass measurements, 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.

2.5 Data analyses

Time lags 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 $\delta^{13}C_{SR}$, $\delta^{13}C_{RR}$, $\delta^{13}C_{SB}$, and $\delta^{13}C_{RB}$. The measurement frequency soon after labelling resulted in a minimum measurable time lag of approximately 30 min. Exponential decay functions were fitted using

$$f(t) = y_0 + ae^{-\lambda t}, \quad (4)$$

Table 1. Overview of biomass, time lags and half-life times between treatments. Decay constant (λ) of exponential decay fit using $f(t) = y_0 + ae^{-\lambda t}$, corresponding coefficient of determination for exponential decay fit (R^2), derived half-life (hlt); time lag to the first appearance of the label (t_1); time lag to the maximum induced enrichment of the label (t_2). 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} (°C)	25	10	25	10
λ (h^{-1})	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
t_1 (h)	0.38	0.38	0.93	1.47
t_2 (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

where y_0 is the asymptote, a is the initial quantity, λ denotes the decay constant and t is time. The resulting half-life time (hlt) was calculated as $\ln(2)/\lambda$. From the subhourly 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 sample t tests assuming unequal variance. All reported values are means ± standard error. Data were processed and analysed using R 2.15.1 (R Core Team, 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. 1a, b). Further, pulse labelling resulted in a similar response in the $\delta^{13}C$ signal of shoot respiration ($\delta^{13}C_{SR}$) between treatments, both in timing and magnitude (Table 1; Fig. 1c, d). There was an immediate strong increase of $\delta^{13}C_{SR}$ 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. 2.5, Table 1, Fig. 2).

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. 1g, 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

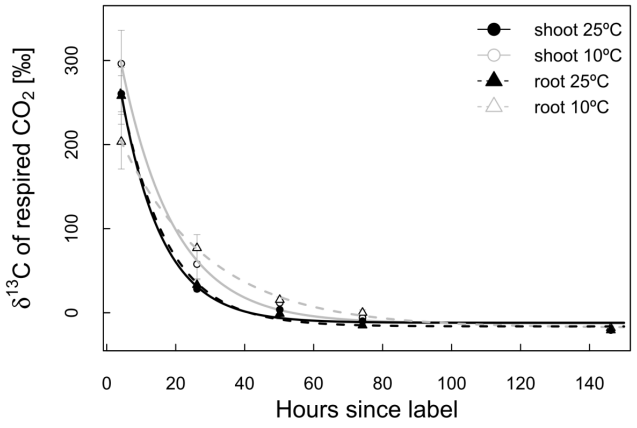

Fig. 2. Exponential decay of the δ^{13}C respiration signal in shoot and root. The data shown are means ± standard error ($n = 3$). Lines are fitted exponential decay functions (using $f(t) = y_0 + ae^{-\lambda t}$).

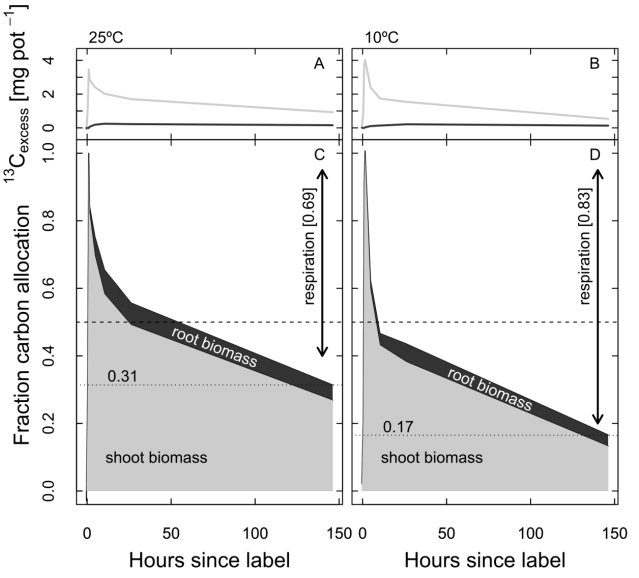

Fig. 3. (A, B) Time course of $^{13}C_{excess}$ in shoot (light grey) and root (dark grey) and **(C, D)** the derived fraction of carbon allocation relative to the initial shoot enrichment of shoot bulk material ($\delta^{13}C_{SB}$); warm treatment (25 °C, left panels); cold treatment (10 °C; right panels); final value of biomass allocated to roots and shoots (dotted line); 0.5 fraction carbon allocation is indicated for reference (dashed line).

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, Fig. 2).

The overall isotopic enrichment was always smaller in bulk compared to respired samples. However the measurements followed similar trends (Fig. 1). 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; $t = -1.32$, df = 2, $p = 0.32$) to about 1 ‰, 23 min after the introduction of the label (Fig. 1e, f). 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 bulk root material ($\delta^{13}C_{RB}$). $\delta^{13}C_{RB}$ did not differ between treatments at the initial enrichment, which occurred 1.5 h after label start (Fig. 1i, j; 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), $\delta^{13}C_{RB}$ at peak time was not significantly different (10 °C = -7.26 ± 2.46 ‰; 25 °C = -3.59 ± 1.95 ‰; $t = 1.17$, df = 4, $p = 0.30$).

3.2 Carbon allocation

Carbon allocation was estimated by assuming that the maximum ^{13}C enrichment in the bulk shoot material ($\delta^{13}C_{SB}$) accounts for all newly assimilated C (Burri et al., 2013, Sect. 2.4). 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. 3a, b). Resulting estimates of above- and belowground C allocation show that after 7 days at either temperature, more than 50 % of the initial ^{13}C allocated to shoot biomass was invested into respiration, exudation or volatile losses (Fig. 3c, d). 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 treatment, 26.8 % of the remaining 31 % C was incorporated into shoot biomass and only 4.6 % into root biomass. In the cold treatment, 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 too short to see such an effect at the whole plant scale, as no differences in biomass were observed between treatments (Table 1).

4 Discussion

There was no measurable time lag between assimilation and respiration in the shoots of *Lolium*, in either temperature treatment, indicated by the direct response of $\delta^{13}C_{SR}$ to $^{13}CO_2$ labelling. This finding suggests that recent assimilates are almost immediately (< 30 min) used 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, with recent photo-assimilates supplying 43 % of total respiration (Lehmeier et al., 2008). 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 utilisation 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.

Exposing plants to a sudden decrease in temperature from 25 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 in accordance with other environmental factors as an increased time lag was also found under drought stress for *Fagus sylvatica* saplings (Ruehr et al., 2009; Barthel et al., 2011a) and under shading in a mountain grassland (Bahn et al., 2009). Kuzyakov and Gavrichkova (2010) suggested that time lag from above- to belowground is affected by several steps including CO_2 fixation, phloem loading, phloem transport, root respiration, and diffusion out of the soil. They further state 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 biochemical and biophysical processes involved in C transport 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 cold temperature treatment is unlikely to have caused hydraulically induced stomatal limitation. Moreover, the plants were kept well watered at all times, so it is improbable that soil water was limiting. Additionally, if soil water availability was 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 than the cold treatment, which means that our results would represent 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 Q_{10} 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 biochemical and biophysical processes at the same time, it is difficult to distinguish the most prominent factor driving translocation.

Fourth, temperature also influences the CO_2 diffusion within the soil. Since we measured the $\delta^{13}C$ of root respired CO_2 directly, this temperature effect can be disregarded. Similarly, interferences due to physical back-diffusion of ^{13}C tracer from the soil, as observed by Bahn et al. (2009), could also 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., 2014; Barthel et al., 2011b; Subke et al., 2009).

Temperature influenced not only the carbon transport from above- to belowground, but also 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, half-life times of the $\delta^{13}C$ signal of respiration should also reflect the turnover of the labile C pool. Quick pool turnover is generally caused by high input (photosynthesis) or high output rates (respiration, storage, growth) or both. Since half-life times of $\delta^{13}C$ from shoot and root respiration were short at 25 °C, C turnover in the labile C pool should thus be high. Therefore, one can conclude that under cold temperatures labile C turnover is small (because of longer half-life times), hence less C is assimilated and/or used for growth and/or storage and/or respiration. This is in agreement with McGoy (1990), who found a decrease in the immobilisation flux at low temperatures in *Glycine*. Longer half-life times of the $\delta^{13}C$ signal of soil respiration under stress were 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 time range (8.3–17.7 h; Table 1).

As mentioned above, recently assimilated C can be used either for storage, growth and/or respiration. ^{13}C analysis of bulk material revealed that *Lolium* invests relatively more C into respiration and relatively less into biomass after exposure to a sudden temperature drop. In contrast to the slow C turnover of the labile pool, the higher turnover of all plant C could be explained because less ^{13}C has been used for growth and/or storage under cold conditions. That is, all recent C remained in the labile pool serving mainly respiration. This confirms our original hypothesis, where we suggested that stressed plants enhance the focus on maintenance processes rather than on growth or storage. Such maintenance of respiration was also observed in shaded *Lolium* plants where root respiration was maintained by root reserves (Schmitt el al., 2013). Further, our experiment showed that relatively more C was incorporated into root biomass under cold temperatures as indicated by the root-to-shoot ratio calculated from $^{13}C_{excess}$. The literature shows consistently that plants invest relatively more C into roots when under climatic stress, for instance during shading (Bahn et al., 2013) or under drought (Burri et al., 2013; Sanaullah et al., 2012; Palta and Gregory, 1997). The similar responses to different climatic drivers suggest that plants have a general stress response when moved from their optimum environmental range.

This short-term controlled environment study shows the importance of temperature as a driver of C transport and relative C allocation. In addition our results have also implications for the application of $\delta^{13}C$ measurements of shoot respiration to assess long-term processes. For example, while $\delta^{13}C_{SR}$ has been suggested to be related to water-use efficiency at the whole-season field scale (Barbour et al., 2011a; Werner et al., 2012), the high sensitivity of $\delta^{13}C_{SR}$ to environmental changes indicates a very fast use of recent photo-assimilates for shoot respiration and demonstrates that $\delta^{13}C_{SR}$ is not suitable for this purpose.

On a final note, it should be mentioned that significant temperature drops (such as the 15 °C applied in this study) are plausible and recurring events in typical pasture-growing climates. However, in our study, the cold and warm treatment temperatures then remained the same for 7 days, which is unlikely in this same variable field setting. Additional temperature variations during this time would buffer the clear responses found in this study. Moreover, the processes detailed here (carbon uptake, transport, allocation and release) will be affected by the interplay of temperature, light and soil moisture conditions, which are all likely to change in a field setting. Therefore, one would expect that results of a similar study under natural conditions would be more confounded. Further studies are needed to address in detail the effect of more variable temperatures and multiple interacting environmental drivers on C transport and allocation, and species-specific responses.

5 Conclusions

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 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.net/11/1425/2014/bg-11-1425-2014-supplement.pdf.

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