Biogeosciences Discuss., 8, 885–919, 2011
www.biogeosciences-discuss.net/8/885/2011/
doi:10.5194/bgd-8-885-2011

Biogeosciences Discussions

Seasonal variations of belowground carbon transfer assessed by in situ $^{13}CO_2$ pulse labelling of trees

D. Epron[1,2], J. Ngao[3,4,5], M. Dannoura[6,7], M. R. Bakker[8,9], B. Zeller[10], S. Bazot[3,4,5], A. Bosc[6], C. Plain[1,2], J. C. Lata[11], P. Priault[1,2], L. Barthes[3,4,5], and D. Loustau[6]

[1]Université Henri Poincaré, UMR 1137, 54500 Vandoeuvre-les-Nancy, France
[2]INRA, UMR 1137, 54280 Champenoux, France
[3]Université Paris-Sud, UMR 8079, Orsay, 75231 Paris, France
[4]CNRS, UMR 8079, 91190 Gif-sur-Yvette, France
[5]AgroParisTech, UMR 8079, 75231 Paris, France
[6]INRA, UR1263, 33140 Villenave d'Ornon, France
[7]Kyoto University, Laboratory of Forest Utilization, Kyoto 606-8502, Japan
[8]ENITA de Bordeaux, UMR 1220, 33883 Villenave d 'Ornon, France
[9]INRA, UMR 1220, 33883 Villenave d'Ornon, France
[10]INRA, UR 1138, Centre de Nancy, 54280 Champenoux, France
[11]UPMC-Paris6, UMR7618, 75230 Paris, France

Received: 7 January 2011 – Accepted: 17 January 2011 – Published: 2 February 2011

Correspondence to: D. Epron (daniel.epron@scbiol.uhp-nancy.fr)

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

Abstract

Soil CO_2 efflux is the main source of CO_2 from forest ecosystems and it is tightly coupled to the transfer of recent photosynthetic assimilates belowground and their metabolism in roots, mycorrhiza and rhizosphere microorganisms feeding on root-derived exudates. The objectives of our study were to assess patterns of belowground carbon allocation among tree species and along seasons. Pure $^{13}CO_2$ pulse labelling of the entire crown of three different tree species (beech, oak and pine) was carried out at distinct phenological stages. Excess ^{13}C in soil CO_2 efflux was tracked using tunable diode laser absorption spectrometry to determine time lags between the start of the labelling and the appearance of ^{13}C in soil CO_2 efflux and the amount of ^{13}C allocated to soil CO_2 efflux. Isotope composition ($\delta^{13}C$) of CO_2 respired by fine roots and soil microbes was measured at several occasions after labelling, together with $\delta^{13}C$ of bulk root tissue and microbial carbon. Time lags ranged from 0.5 to 1.3 days in beech and oak and were longer in pine (1.6–2.7 days during the active growing season, more than 4 days during the resting season), and the transfer of C to the microbial biomass was as fast as to the fine roots. The amount of ^{13}C allocated to soil CO_2 efflux was estimated from a compartment model. Seasonal patterns of carbon allocation to soil CO_2 efflux differed markedly between species, with pronounced seasonal variations in pine and beech. In beech, it may reflect competition with other sinks (aboveground growth in late spring and storage in late summer) that were not observed in oak.

1 Introduction

Soil CO_2 efflux is the major biospheric source of carbon from terrestrial ecosystems to the atmosphere and it accounts for a large fraction (40–70%) of total ecosystem respiration (Janssens et al., 2001). It includes the respiration of microorganisms and telluric meso- or macroorganisms involved in the mineralisation of soil organic matter ("heterotrophic respiration"), and the use of recently assimilated substrates that

BGD
8, 885–919, 2011

fuels root metabolism and rhizospheric microorganisms that are either associated to roots (symbionts) or that feed on root exudates ("autotrophic" respiration, Epron, 2009; Subke et al., 2006). The transfer of photosynthetic products belowground therefore fulfils energy requirements of metabolic processes occurring in the root and associated rhizosphere and mycorhizosphere.

Up to recently, temporal variations of soil CO_2 efflux were mostly ascribed to environmental drivers (e.g., Bahn et al., 2010; Epron et al., 1999), but there is now a growing amount of evidence that soil CO_2 efflux is strongly linked to plant activities (Högberg and Read, 2006; Ryan and Law, 2005). Beyond the relationships between ecosystem productivity and soil CO_2 efflux at a regional scale (Janssens et al., 2001), temporal variations in soil CO_2 efflux were recently ascribed to short-term changes in microclimatic conditions (photon flux density, vapour pressure deficit, air temperature, ...) that affect canopy photosynthesis (Craine et al., 1999; Gaumont-Guay et al., 2008). Not only the total flux of soil CO_2 efflux but also its ^{13}C composition was correlated to short-term fluctuations in microclimate confirming a close coupling between photosynthesis and soil CO_2 efflux (Bowling et al., 2008; Ekblad et al., 2005; Marron et al., 2009; Mortazavi et al., 2005).

Root and root-derived carbohydrates contribute to 50–60% of soil CO_2 efflux in forest ecosystems (Subke et al., 2006) highlighting the effect of photosynthesis on soil CO_2 efflux. Soil CO_2 efflux will therefore vary according to temporal change in canopy photosynthesis but the coupling will depend on both the velocity of carbon transfer belowground (Plain et al., 2009) and on the partitioning of photosynthates among several pools and organs within the tree, i.e. on carbon allocation (Litton et al., 2007). The contribution of recent photosynthate to soil CO_2 efflux further depends on the partitioning of carbon allocated belowground to growth, storage and respiration of both root and microorganisms feeding on root-derived carbohydrates (Ryan and Law, 2005). This will affect the residence time of carbon in the tree-soil system before it returns to the atmosphere.

BGD
8, 885–919, 2011

Total annual belowground carbon allocation can be successfully derived from soil CO_2 efflux and litterfall using a mass balance equation (Giardina and Ryan, 2002). It varied with stand age and resource availability (Litton et al., 2007). It increased with mean annual temperature at global scale (Litton and Giardina, 2008). However, there is still little information on the seasonal variations of belowground carbon allocation in forest ecosystems. Pulse labelling of field-grown trees with $^{13}CO_2$ and high frequency of measurement of ^{13}C in respiratory efflux during the chase period using laser-based infrared gas analysers is a powerful approach to estimate the effect of environment on velocity, allocation and residence time (Dannoura et al., 2011; Plain et al., 2009).

The aim of this study was to assess the belowground transfer of recently assimilated carbon in two deciduous broadleaved species (beech and oak) and one coniferous evergreen species (maritime pine) at different times in the season. $^{13}CO_2$ recovery in soil CO_2 efflux was recorded for 3 weeks by tuneable diode laser absorption spectrometers (TDLAS) after pulse labelling of 8–10 m tall trees and compared to ^{13}C recovery in both root and microbial biomass and their respective respiratory CO_2. We hypothesised that (i) the transfer time would vary between broadleaved and coniferous species due to difference in anatomy and growth rhythm, (ii) the amount of ^{13}C recovered in soil CO_2 efflux would vary along the season reflecting changes in carbon allocation related to phenology, temperature and soil water content, and (iii) ^{13}C would be first recovered in the roots and later in the microbial biomass.

2 Materials and methods

2.1 Study sites and experimental design

The study was conducted on three temperate forest stands (Dannoura et al., 2011): a 20 year-old natural beech regeneration on a luvisol (*Fagus sylvatica* L., 48°40′ N, 7°04′ E, 300 m elevation), a 15 year-old natural sessile oak regeneration on a gleyic luvisol (*Quercus petraea* Matt Liebl, 48°2′ N, 02°47′ E, 90 m elevation) and a 12 year-old

BGD
8, 885–919, 2011

maritime pine plantation on a sandy podsol (*Pinus pinaster* Ait, 44°45′ N, 0°42′ W, 60 m elevation). Tree density was 1.6, 1.5 and 0.25 trees per m^2 for beech, oak and pine, respectively.

Labelling was done three to four times covering the whole leafy season for all species. Three trees were selected for each labelling date in all sites and two of them were labelled. Tree height ranged between 8 to 10 m in all stands (DBH are given in Table 1). A 0.5–0.6 m deep trench was dug around each tree at least 5 months before labelling to limit possible confounding effects of root cutting on carbon allocation. The trench was lined with a polyethylene film and filled back. All roots and root exudates within this soil volume therefore originated from the isolated tree, and were contained in this trench volume. The areas delimited by the trench were about 3 m^2 in the beech (2.9–3.8) and oak stands (2.2–5.7), and 6 m^2 in the pine stand and were larger than the average areas per tree (inverse of tree density).

In early spring 2009 and at least three months prior to the labelling to allow enough time for ingrowth of mycorrhizal hyphae, root exclusion cores were installed in those trench plots that were selected for the labelling experiment. A root auger (⌀ 8 cm; depth of corer 15 cm) was used to extract intact soil cores to a depth of 15 cm. Upon removal of the auger, the soil was gently pushed out with a crank and wrapped in a 30 µm nylon mesh bag of similar dimensions (BUISINE, Clermont de l'Oise, France) and then put back in the core hole. The 30 µm mesh permits to exclude root growth in the cores so that the only carbon passage into these root exclusion cores is by hyphae of mycorrhizal fungi. During the chase period, the root exclusion cores were retrieved and compared to normal cores collected freshly with the same auger.

In April 2009, rainfall exclusion roofs, made with polyethylene film and supported by a woody frame (3 × 3 m), were installed 1.5 m above the forest floor on two beech trees to divert rainfall from the soil delimited by the trench. Predawn leaf water potential was measured once on 3 to 4 leaves per tree just prior labelling using a Scholander-type pressure chamber (PMS instrument, Corvallis, Oregon, USA).

BGD
8, 885–919, 2011

Photosynthetic photon flux density (PPFD; Delta T-BF2, Cambridge, UK for beech, home-made quantum sensor using a gallium arsenide photodiode for oak and DBE, Solems, Palaiseau, France for pine), air temperature (T_A; Vaisala HMP45, Helsinki, Finland) and soil temperature at 10 cm depth (T_S, home-made copper-constantan thermocouples) were recorded half-hourly at each site. Values were averaged for 24, 48 or 72 h after labelling. Soil water content (SWC) in the vicinity of each labelled tree inside the area delimited by the trench was recorded half hourly at 30 cm depth for pine with TDR probes (CS616, Campbell Scientific, Logan, UT, USA) or at 10 cm depth for oak with an impedance probe (ML2x ThetaProbes, Delta-T Device, Cambridge, UK), or measured once a week at 15 cm depth for beech using TDR probes (Trase, Soil-Moisture Equipment Corp., Santa Barbara, CA, USA).

2.2 Crown labelling

The whole crown of the tree was inserted into a 20 to 40 m^3 chamber made of 200 µm polyane film and held by two 12 m height stainless steel scaffoldings put up on both sides of the tree. Air temperature inside the crown labelling chamber was recorded, controlled and maintained at the outside air temperature. Evolution of [$^{12}CO_2$] and [$^{13}CO_2$] inside the chamber was monitored with a $^{12}CO_2/^{13}CO_2$ infrared gas analyser (S710, SICK/MAIHAK, Reute, Germany, accuracy of 5%). A total amount of 50.4 g of pure $^{13}CO_2$ (99.299 atom %, Eurisotop, Cambridge Isotope Laboratory Inc., Andover, MA, USA) was progressively injected for 1 to 5 h at a flow rate adjusted to maintain the $^{13}CO_2$ mixing ratio in the chamber between 300 and 400 µmol mol^{-1}, except in November and February for pine where higher $^{13}CO_2$ mixing ratios (800–1000 µmol mol^{-1}) were used to compensate a lower photosynthetic activity (see Dannoura et al., 2011; Plain et al., 2009 for more details). By only inserting the crown into the labelling chamber, we avoided contamination of the soil atmosphere by diffusion of $^{13}CO_2$ into the soil pores that would later have back-diffused into the atmosphere leading to an artefact in soil CO_2 efflux isotopic composition (Subke et al., 2009).

BGD
8, 885–919, 2011

2.3 ^{13}C in soil CO_2 efflux

The isotope composition of soil CO_2 efflux ($\delta^{13}C_{FS}$) was computed from [$^{12}CO_2$] and [$^{13}CO_2$] measured at the inlet and outlet of flow-through soil CO_2 efflux chambers (Marron et al., 2009; Plain et al., 2009) by tuneable diode laser absorption spectroscopy with a trace gas analyzer (TGA 100A; Campbell Scientific, Logan, UT). Two or three collars were installed for each tree at 60 cm from the trunk. Air was pumped continuously through the chamber at a flow rate ranging from 18 to 72 $dm^3 h^{-1}$. The chamber design ensured that differences in pressure between the inside and the outside of the chamber remained below 10^{-2} Pa. A manifold was used to switch between working standards and the chamber inlet and outlet lines. A mean mixing ratio was recorded over 20 s after a 30 s stabilisation. The isotope composition of each working standard that was 0.5% certified for CO_2 mixing ratios (Air Products, Paris, France for beech, Air Liquide, Paris, France for oak and DEUSTE Steininger GmbH, Mühlhausen, Germany for pine) was measured by an isotope ratio mass spectrometer (IRMS, Delta S, ThermoFinnigan, Bremen, Germany). The ranges of available mixing ratios were respectively from 293.2 to 1281.2 µmol mol^{-1} for $^{12}CO_2$ and from 3.17 to 13.75 µmol mol^{-1} for $^{13}CO_2$. The precision of the instrument at reproducing calibration tank values was 0.3 µmol mol^{-1} and 0.007 µmol mol^{-1} for $^{12}CO_2$ and $^{13}CO_2$ (Dannoura et al., 2011).

The isotopic composition of soil CO_2 effluxes (δC_{FS}, ‰) was calculated as:

$$\delta^{13}C_F = \frac{\frac{\left[^{13}CO_2\right]_{out} - \left[^{13}CO_2\right]_{in}}{\left[^{12}CO_2\right]_{out} - \left[^{12}CO_2\right]_{in}}}{R_{VPDB}} - 1 \quad (1)$$

where R_{VPDB} is the isotopic ratio of Vienna Pee Dee Belemnite (0.011179602).

BGD
8, 885–919, 2011

Total CO_2 mixing ratio ($[CO_2]$, µmol mol^{-1}) was calculated from the mixing ratios of individual isotopologues by:

$$[CO_2] = \frac{\left[^{12}CO_2\right] + \left[^{13}CO_2\right]}{(1 - f_{other})} \quad (2)$$

where f_{other} is the fraction of CO_2 containing all isotopologues other than $^{12}C^{16}O^{16}O$ and $^{13}C^{16}O^{16}O$, and assumed to be 0.00474 (Griffis et al., 2004).

2.4 ^{13}C in respiratory CO_2 of roots and soil microorganisms

Two soil cores (20 cm depth) were collected 1, 3, 6, 9 and 15 days after labelling and sliced into 3 parts (0–5, 5–10 and 10–20 cm). The soil was immediately sieved through a 2-mm mesh and living fine root fragments (diameter < 2 mm) were picked up and rinsed. Coarse roots fragments were discarded. For each core, the fine roots collected at all depths were pooled together. Oak and pine roots of the two cores were further pooled to obtain a sufficient sample size while beech roots were kept separately for each core. Each root sample was then enclosed in a 125 mL glass flask. A pump was used to circulate gas from the flask to an IRGA (EGM1, PPSystems, Hitchin, UK, LI840, Licor, Lincoln, USA or S710, SICK/MAIHAK, Reute, Germany). The CO_2 initially inside the flask was removed using a soda lime trap. After 10 min, the soda lime trap was by-passed and the increase in CO_2 mixing ratio in the flask was recorded until reaching values above 400 ppmv. Air was then sampled in a 10 mL Exetainer glass-vial (Labco limited, High Wycombe, UK) or 25-mL glass flask for IRMS analyses. Root-free soil subsamples (approx 150 g) from 0–5 and 5–10 cm depth were incubated similarly in 250 mL flask. Soil from the two cores was pooled for oak. The isotopic composition (δ) of CO_2 in sampled air was measured within a week IRMS (Delta-S, Finnigan-Mat, Thermoquest Corp., San Jose, CA, USA, for beech and pine samples or VG Optima, Fison, Villeurbanne, France, for oak samples) coupled to a gas purification device (Gas-Bench II, ThermoFinnigan, Bremen, Germany). Incubations were done

BGD
8, 885–919, 2011

on all beech and oak trees but for pine only those labelled in June. Temperature during incubation was 19–22 °C for beech and oak, and 22–24 °C for pine.

2.5 ^{13}C in root and microbial carbon

After incubation, roots were immediately plunged in liquid nitrogen, transferred into a −80 °C freezer and finally freeze-dried. Freeze-dried root samples were ground, weighed and analysed for carbon isotope composition and total carbon using an elemental analyser (NA 1500 NCS, Carlo Erba, Milan, Italy) coupled to IRMS.

After incubation, soil subsamples were stored at 4 °C for less than 4 days before processing for microbial carbon. The $\delta^{13}C$ of soil microbial carbon was determined from $\delta^{13}C$ of soluble carbon extracted from two 40 g of soil subsamples. One subsample was fumigated for 24 h with chloroform vapour, while the other was not fumigated (Vance et al., 1987). Extraction was performed using 0.05 M of K_2SO_4 for one hour under vigorous shaking. The extracts were filtered, analysed for carbon content using a total organic carbon analyser (Shimadzu TOC-VCSH, Tokyo, Japan), either freeze-dried or oven-dried at 60 °C (we observed no difference between the two methods) and then analysed for carbon isotope composition IRMS. Microbial carbon was also extracted from the deepest part of the soil cores in beech and pine (10–20 cm).

The isotope composition of soil microbial carbon ($\delta^{13}C_M$) was calculated as

$$\delta^{13}C_M = \frac{\delta^{13}C_{fum} \times C_{fum} - \delta^{13}C_{nonfum} \times C_{nonfum}}{C_{fum} - C_{nonfum}} \quad (3)$$

where C_{fum} and C_{nonfum} ($mg\,C\,kg_{soil}^{-1}$) refer to fumigated and non fumigated extracts.

Soil microbial carbon was computed as

$$C_M = \frac{(C_{fum} - C_{unfum})}{0.45}$$

where 0.45 is a correction factor used to account for the non-extractable fraction of microbial carbon (Vance et al., 1987). However, the fumigation efficiency in the sandy

BGD
8, 885–919, 2011

soils of the pine stands can be higher since the microbe protection is probably low in these unstructured soils (Achat et al., 2010).

The isotope composition of microbial carbon was also estimated in the two root exclusion cores that were collected 3 and 6 days after labelling in beech and oak, and 6 and 9 days after labelling in pine, and sliced into two parts (0–5 and 5–10 cm).

Gravimetric soil water content was determined by comparing the mass of approx. 40 g of soil before and after drying at 105 °C.

2.6 Calculations

The relative abundance of ^{13}C in any efflux or compartment (Ab) was calculated from the isotope composition as:

$$\mathrm{Ab} = \frac{^{13}C}{^{12}C + ^{13}C} = \frac{\left(\frac{\delta^{13}C}{1000} + 1\right) \times R_{\mathrm{VPDB}}}{\left[\left(\frac{\delta^{13}C}{1000} + 1\right) \times R_{\mathrm{VPDB}}\right] + 1} \quad (4)$$

Ab was corrected for the background ^{13}C content measured on unlabelled trees or on the same tree one day before labelling ($\mathrm{Ab_{UN}}$), and multiplied by X, X being either the total carbon content in a compartment (root, C_R, or microbial carbon, C_M) or the CO_2 flux to estimate the excess amount of ^{13}C (excess ^{13}C) in that efflux or compartment:

$$\text{Excess } ^{13}C = (\mathrm{Ab} - \mathrm{Ab_{UN}}) \times X \quad (5)$$

Root and microbial respiration (R_R and R_M, mg C kg^{-1} h^{-1}) were calculated from the linear slope of the increase in CO_2 mixing ratio in the incubation flasks ($d[CO_2]/dt$, mol mol^{-1} h^{-1}):

$$R = \frac{d[CO_2]}{dt} \times \frac{P \times V \times (13 \times \mathrm{Ab} + 12 \times (1 - \mathrm{Ab})) \times 10^{-3}}{8.314 \times T \times M} \quad (6)$$

BGD
8, 885–919, 2011

where P is the atmospheric pressure (Pa), V is the total volume including flask, tubes and IRGA cells (m^3), M is the dry mass of the root or of the soil (kg), T is the temperature (K), and 8.314 J mol^{-1} K^{-1} is the ideal gas constant.

Soil CO_2 effluxes (F_S, mg C m^{-2} h^{-1}) were calculated as:

$$F_S = \frac{\left([CO_2]_{out} - [CO_2]_{in}\right) \times P \times F \times (13 \times Ab + 12 \times (1 - Ab)) \times 10^{-3}}{8.314 \times T \times S} \quad (7)$$

where F is the air flow through the chamber (m^3 h^{-1}) and S is the surface of soil chamber (m^2).

The cumulative label recovered in soil CO_2 efflux (CLR_{FS}) was calculated by summing daily averages of ^{13}C in excess in the soil CO_2 efflux.

$$CLR_{FS}(d) = \sum_{0}^{d} \overline{(F_S \times (Ab - Ab_{UN}))} \times 24 \quad (8)$$

The kinetics of the label recovered in soil CO_2 efflux were described using a four-pool model that was fitted on the observed CLR_{FS} values (Fig, 1). A quantity of the label that will be respired belowground (Q) leaves the crown pool (C) at a rate following a first-order kinetics and arrives after a time lag (L) into an active belowground pool (B1) where it can be transferred and back transferred to an inactive pool (B2) at rates following also a first-order kinetics. Finally, the label leaves the substrate pool in a similar way and accumulates in the atmosphere (A). The rates of change in the different pools are:

BGD
8, 885–919, 2011

$$\begin{cases} \frac{dQ_C(t)}{t} = -k_{CB1} \times Q_C(t) \\ \text{if } t < L \\ \frac{dQ_{B1}(t)}{dt} = \frac{dQ_{B2}(t)}{dt} = \frac{dQ_A(t)}{dt} = 0 \\ \text{if } t \geq L \\ \frac{dQ_{B1}(t)}{dt} = +k_{CB1}Q_C(t-L) + k_{B2B1}Q_{B2}(t) - k_{B1B2}Q_{B1}(t) - k_{B1A}Q_{B1}(t) \\ \frac{dQ_{B2}(t)}{dt} = k_{B1B2}Q_{B1}(t) - k_{B2B1}Q_{B2}(t) \\ \frac{dQ_A(t)}{dt} = +k_{B1A}Q_{B1}(t) \end{cases} \quad (9)$$

where k_{CB1}, k_{B1B2}, k_{B2B1} and k_{B1A} are the rate constant of transfer between the crown to the B1 pool, between B1 and B2 pools (transfer and back transfer), and between B1 and the atmosphere (respiration).

The cumulative label recovered in soil CO_2 efflux at any time t is therefore

$$CLR_{FS}(t) = \int_0^t \frac{dQ_A(t)}{dt} \quad (10)$$

and the asymptotic value, $CLR_{FS}(\infty)$ is equal to $Q_C(0)$. $CLR_{FS}(\infty)$ multiplied by the trenched surface represents the amount of carbon allocated to belowground respiration.

The B1 pool is a metabolically active pool that contributes to soil CO_2 efflux while the B2 pool is metabolically inactive, and does not contribute to soil CO_2 efflux. We also unsuccessfully tested a two-metabolically-active-pool model that would have represented roots and microorganisms. We are aware that our model is oversimplified and that it does not describe the complexity of the fate of carbon belowground. We used it for estimating seasonal variation in the amount of carbon allocated to soil CO_2 efflux and the half residence time of soil respired ^{13}C into the plant-soil system ($t_{1/2}$), defined as the time need to reach 50% of $CLR_{FS}(\infty)$.

BGD
8, 885–919, 2011

The time lag was not estimated from Eq. (9) but determined by fitting a quadratic function to the relationships between excess ^{13}C in F_S and the time after labelling (Fig. 1) using non linear least-squares regression (PROC NLIN of SAS software, Marquardt-Levenberg method, SAS Institute Inc., Cary, NC, USA):

$$\begin{cases} \text{excess}^{13}C = 0 & \text{if } t < L \\ \text{excess}^{13}C = a\,(t-L) + b\,(t-L)^2 & \text{if } t \geq L \end{cases} \quad (11)$$

The model predicted satisfactorily CLR_{FS} values at 7, 14 and 20 days after labelling across species and seasons ($R^2 = 0.99$, $p < 0.001$).

2.7 Statistical analyses

Data were analysed separately for each species using SAS software (SAS Institute Inc., Cary, NC, USA). Two-way ANOVA (tree and sampling date, nested within tree) were used for excess ^{13}C in F_S, C_R and R_R and three-way ANOVA (tree, depth and sampling date nested within tree and depth, including interaction between tree and depth when the two cores were not pooled) were used for ^{13}C in excess in C_M and R_M. A four-way ANOVA was used to compare ^{13}C in excess in C_M of normal cores and root exclusion cores (tree, depth, core type nested within tree and depth and sampling date nested within tree, depth and core type, including interactions between tree and depth when the two cores were not pooled). The residual errors of each model are shown as vertical bars in the figures. Linear correlations between the different parameters and environmental variables were tested with a two-tailed test for significance.

BGD
8, 885–919, 2011

3 Results

3.1 Excess ^{13}C in soil CO_2 efflux

Soil CO_2 efflux (F_S) varied within a similar range in the three species according to seasons, with highest values in July for beech and oak and lowest values in February for pine (Table 1). A significant excess ^{13}C in F_S was rapidly observed in beech and oak (Fig. 2) after a time lag (L, Table 1) of less than 0.5 to 1.5 days. In oak, L was negatively correlated to cumulative PPFD on the day of labelling ($R = -0.86$, $p < 0.05$). Low cumulated PPFD were observed in September. L was higher in pine than in the two broadleaved species during the growing season, including October labelling (1.6 to 2.5 days) and increased markedly in November and February, up to 4.4 days, and this increase was clearly related to the drop in temperature. In pine, L was indeed negatively correlated to both air and soil temperature, with the highest correlation between L and soil temperature averaged for 4 days since the start of the labelling ($R = -0.84$, $p < 0.01$).

In pine, the lowest $CLR_{FS}(\infty)$ values were observed for trees labelled in November and February, reflecting lower F_S values in these seasons (Table 1). $CLR_{FS}(\infty)$ was indeed positively related to both air and soil temperature, with the highest R between $CLR_{FS}(\infty)$ and air temperature averaged for 24 h since the start of the labelling ($R = 0.84$, $p < 0.01$). The marked influence of temperature on the shape of the time courses of excess ^{13}C in soil CO_2 efflux (Fig. 2) may explain the negative correlation between $CLR_{FS}(\infty)$ and L in this species ($R = -0.92$, $p < 0.01$), which was not observed for the two broadleaved species. In beech and oak, $CLR_{FS}(\infty)$ was not related to any of the measured environmental factors. Despite the lack of relationships with climatic factors, there were clear seasonal differences in $CLR_{FS}(\infty)$ in beech with the higher $CLR_{FS}(\infty)$ observed in July (Table 1). Despite lower soil water content under rainfall exclusion roofs, this had no marked influence on $CLR_{FS}(\infty)$ in beech.

Half residence time of soil respired ^{13}C in the tree-soil system averaged 6.9 and 6.5 days in beech and oak respectively with no clear seasonal trend. It increased markedly

BGD
8, 885–919, 2011

in pine in November and February compared to June, August and October (Table 1), with the highest correlation between $t_{1/2}$ and air temperature averaged for 72 h since the start of the labelling ($R = -0.77$, $p < 0.05$). In oak and in pine, $t_{1/2}$ and L are correlated ($R = 0.87$, $p < 0.05$ and $R = 0.84$, $p < 0.01$, respectively) but not in beech.

3.2 Excess ^{13}C in root and microbial respiration and root and microbial carbon

Fine root biomass (0–20 cm depth) was higher in beech (255 g DM m^{-2}, values for each tree are in Table 1) than in oak (96 g DM m^{-2}) and pine (50 g DM m^{-2}), while specific root respiration was similar for beech and oak (87 mg C kg^{-1} DM h^{-1}) and much higher in pine (411 mg C kg^{-1} DM h^{-1}, measured only in June 2009). Microbial carbon (0–10 cm depth) was higher in oak (68 g C m^{-2}) than in beech (39 g C m^{-2}) and pine (22 g C m^{-2}). Microbial respiration was higher in oak (5.1 mg C kg_{soil}^{-1} h^{-1} on average) than in beech (3.6 mg C kg_{soil}^{-1} h^{-1}), with lower values in August especially for beech trees under rainfall exclusion roofs, and lower in pine (2.2 mg C kg_{soil}^{-1} h^{-1}, measured only in June 2009).

An excess amount of ^{13}C was already found one day after labelling in the root and microbial compartments (both carbon and respiration) in oak and beech. It occurred later in pine, i.e. three days after labelling during the growth season (June to October) and six days after labelling in November and February excess ^{13}C in the microbial compartment was higher in the top soil (0–5 cm) than at deeper soil depths for all species.

The patterns of excess ^{13}C in microbial (Fig. 3) and root (Fig. 4) respiration and in microbial (Fig. 5) and root biomass (Fig. 6) were on the whole consistent with TDLAS data (Fig. 2). The mean value of excess ^{13}C in F_S calculated over the chase period was well related with mean excess ^{13}C in R_R in beech ($R = 0.86$, $p < 0.01$), better than with excess ^{13}C in R_M ($R = 0.77$, $p < 0.05$). A similar correlation was observed between excess ^{13}C in F_S and excess ^{13}C in C_R ($R = 0.91$, $p < 0.01$) while no correlation was observed between excess ^{13}C in F_S and excess ^{13}C in C_M. In oak, excess ^{13}C in F_S was

BGD
8, 885–919, 2011

only related to excess ^{13}C in R_R ($R = 0.85$, $p < 0.05$) while no correlation was observed between excess ^{13}C in F_S and excess ^{13}C in R_M, C_M or C_R. In pine, we found a weak correlation between excess ^{13}C in F_S and excess ^{13}C in C_R ($R = 0.72$, $p < 0.001$).

Excess ^{13}C in C_M and R_M was significantly lower in root exclusion cores compared to normal cores for beech, especially in July while $CLR_{FS}(\infty)$ is maximal (Fig. 7). A similar but not significant difference between normal and root exclusion cores was observed for oak and pine.

4 Discussion

4.1 Transfer time of carbon belowground

Time lags estimated for beech and oak ranged from 0.5 to 1.3 d and excess ^{13}C peaked 2 to 3 days after labelling, a value similar to those observed on 3 m tall poplars (Horwath et al., 1994; Mikan et al., 2000). The ^{13}C peak was observed after one day in 0.5 m tall beech seedlings (Ruehr et al., 2009) and after 0.5 d in silver birch seedlings (Pumpanen et al., 2009). Considering the path length from tree leaves to the soil in our experiment, the transfer times we report are shorter. This might be due to a better time resolution of measurements based on tunable diode laser absorption spectrometry compared to those based on off-site isotope ratio mass spectrometry.

No other labelling experiments on tall trees were reported so far in natural conditions in the field. The transfer time of photosynthates to ecosystem respiration has been indirectly estimated by tracing fluctuation in natural abundance of ^{13}C related to climate-induced variations in discrimination during photosynthesis. Time lags of three to five days observed on 8–9 m tall Douglas fir (Bowling et al., 2002; McDowell et al., 2004) and in a loblolly pine plantation and a mixed hardwood forest (Mortazavi et al., 2005), which is higher than those observed in this study during the active growing season (1.6–2.7 days for pine).

BGD
8, 885–919, 2011

In contrast with beech and oak for which recovery of ^{13}C was observed in soil CO_2 efflux after a time lag of less than 1 day on average during the growing season, the time lags for pine ranged between 1.6 to 2.7 days from June to October, and more than four days in the resting season (November and February). The tracer peaked after 4 to 6 days in the first period, a range that is in agreement with those reported for 2-m tall Scots pines (Högberg et al., 2008) and 4-m tall black spruce (Carbone et al., 2007), after considering the difference in tree size. Similar differences were reported after ^{14}C labelling of small potted seedlings in the laboratory between coniferous species (Norway spruce and Scots pine) and silver birch (Pumpanen et al., 2009). The markedly different transfer time of ^{13}C belowground between the two broadleaved species and the pine highlights differences in the velocity of photosynthate transport *via* the phloem sap between angiosperm and gymnosperm that were related to differences in phloem anatomy (Dannoura et al., 2011; Wingate et al., 2010). The doubling of time lag and peak time between summer and winter time, as well as the delayed peak time (more than 10 days in the resting season) were consistent with an effect of temperature on phloem loading, especially on retrieval after leaching (Peuke et al., 2006), on the viscosity of phloem sap (Hölttä et al., 2009) or on the carbohydrate sink strength (Wingate et al., 2010) that may affect the velocity of phloem sap (Dannoura et al., 2011).

The soil tortuosity and moisture might have influenced the transfer time of CO_2 from roots to the soil surface (Mencuccini and Hölttä, 2010; Stoy et al., 2007). However, this putative effect would have led to an underestimation of the contrast found between broadleaved and coniferous species since the pine trees of our study were on a sandy soil with higher porosity than the soils of the two broadleaved trees. In addition, the close coupling between soil surface efflux and incubation of either root or root-free soil indicates that additional time lags related to transport are negligible.

The rainfall exclusion experiment conducted on two beech trees in August did not reveal any change in transfer time in contrast to what was observed on potted beech seedlings (Ruehr et al., 2009). Despite marked differences in soil water content (0–15 cm depth), there was however no difference in predawn leaf water potential

BGD
8, 885–919, 2011

(−0.4 MPa) that would have affected the rate of phloem transport (Hölttä et al., 2009).

4.2 Amount of carbon allocated to soil CO_2 efflux

The compartment model (Eq. 9) used to estimate the amount of ^{13}C allocated to soil CO_2 efflux accounted for labelled substrates that are available for root and microbial respiration during the chase period. It does not account for any subsequent mobilisation of stored labelled carbon nor for decomposition of labelled roots, mycelia and microorganisms.

Our experiment highlighted the species specific pattern in the seasonality of the carbon allocation to soil respiration. In pine, a four-times lesser amount of ^{13}C was recovered in soil CO_2 efflux after labelling done in November and February compared to those done in June, August and October, indicating that carbon is differently retained in other plant compartments depending on the season Inhibition of root growth and rhizosphere activity by low soil temperature in late autumn and winter may explain the seasonality of carbon allocation belowground (Kagawa et al., 2006). During the resting season in pine, the amount of ^{13}C allocated to soil CO_2 efflux accounted for less than 1–4% of amount of $^{13}CO_2$ taken up by the crown estimated as the difference between the amount of ^{13}C injected into the labelling chamber and the residual amount of $^{13}CO_2$ in the chamber at the end of the labelling. It raised to 7–10% during the active growing season, with no strong difference between June, August and October labelling, which contrasts with recent observations on Scots pine (Högberg et al., 2010).

In beech, the relative amount of ^{13}C allocated to belowground respiration varied markedly throughout of the leafy season with a maximum of 18–21% of ^{13}C recovered in soil CO_2 efflux in July against only 3% in May and 1–6% in August. This was also evident from both fine root and microbial respiration, and from both fine root and microbial biomass. This finding agrees well with the high rhizosphere respiration found in early July as inferred from a trenched plot experiment in the same beech forest (Epron et al., 2001). Low allocation of ^{13}C to soil CO_2 efflux in May might be related to the sink

BGD
8, 885–919, 2011

competition with active above-ground growth that occurs essentially in the few weeks between early May and the end of June. This timing is obvious from leaf phenology observations and recordings of tree diameter growth of this forest stand (Granier et al., 2008). At the end of the growing season until leaf fall, the main carbon sink may be the build-up of storage (Barbaroux et al., 2003) that diverts potentially most carbohydrates resources away from belowground metabolism. In oak, there was low variation in the amount of ^{13}C allocated to soil CO_2 efflux among seasons (7–11%), which was contrasting with the pattern in beech. This may suggest that stored carbon acts as a buffer between CO_2 uptake and C allocation belowground in this species. Indeed, oak is known to have higher non structural carbohydrate concentration in stem and coarse roots than beech, with also more pronounced seasonal variations in these non structural carbohydrates (Barbaroux et al., 2003).

Despite these differences in the carbon allocation patterns among the three species, we found no difference in residence time of ^{13}C allocated to belowground respiration that averaged 6–7 days during the active growing season. This value is longer than the 4–5 days reported for shrub species (Carbone and Trumbore, 2007), and much more than the 35 h value reported for small Scots pines (Högberg et al., 2008) but it still represents a fast turnover. In pine, the residence time of ^{13}C allocated to belowground respiration was much higher in November and February, confirming that carbon is retained in other plant compartments during the resting season.

4.3 C fluxes through root and microbial compartments

In all species, excess ^{13}C in F_S was more related to excess ^{13}C in the root compartment (respiration and biomass) than in the microbial compartment. Root respiration that was measured on excised fine roots retrieved from soil cores might have been affected by wounding (Marsden et al., 2008). Flushes in microbial respiration can occur after sieving, due to aeration and increased substrate for respiration (Achat et al., 2010). Both potentially overestimate and lead to high variability in measured fine root and microbial respiration in the short term, making comparisons with soil CO_2 efflux

BGD
8, 885–919, 2011

difficult. However, root and microbial respiration seem to be well scaled and observations were repeatable through seasons and between labelled trees. Higher specific fine root respiration in pine compared to beech and oak could not be explained by a higher specific root length since gymnosperm species are known to have thicker fine roots than angiosperm species (Ostonen et al., 2007; Steele et al., 1997). Indeed, reported ranges of specific root lengths for our species in non-fertilized stands were 5.7–31.5 for beech (Ostonen et al., 2007), 7.2–29.1 for oak (Bakker, 1999) and 4–12 $m\,g^{-1}$ for pine (Bakker et al., 2006). Maritime pine is a fast growing species in contrast to beech and oak, and one fast growing pine species was found to have a higher specific root respiration rate than other slow-growing Pinaceae and Fagaceae species (Comas et al., 2002). Of course, root sorting in not trivial, and we are aware that our root samples could have included different proportion of low-active old roots or even-dead roots depending on the species.

The tracer was recovered at the same time in the fine root and the microbial compartments and peaked after 3 to 6 days, indicating a fast transfer of carbon between roots and microorganisms. Large variability among collected soil samples confounds spatial with temporal variations of excess ^{13}C in soil compartments, precluding attempts to partition excess ^{13}C between roots and microbial biomass and to estimate the residence time of carbon within each compartment. Microbial biomass is composed of a large number of different microorganisms and includes the extraradical mycelium of mycorrhizal fungi. A rapid transfer of photosynthate to ectomycorrhiza has indeed been reported (Esperschütz et al., 2009; Högberg et al., 2010; Leake et al., 2001) and was recently observed from truffle sporocarps after pulse-labelling of chestnut trees (B. Zeller, unpublished data) . The fact that the excess ^{13}C in microbial biomass was less in the root exclusion cores than in normal cores indicates that root exudation also contributes to the fast transfer of photosynthates to soil micro-organisms, among which gram negative bacteria that were found to use recent plant derived carbon (Kramer and Gleixner, 2008). However, the excess ^{13}C in non fumigated soil extracts that would have reflected the dissolved organic carbon released from roots remained very low

BGD
8, 885–919, 2011

expect in few oak samples (data not shown). This might be caused by a rapid absorption of the released carbon by the bacteria and/or by an important dilution into a large pool of soluble carbon (Esperschütz et al., 2009). Spatial variability of ^{13}C distribution after pulse-labelling is indeed expected at a millimetre scale (Grieve et al., 2006). We did not attempt to separate bulk soil from root adhering soil where more excess ^{13}C is expected in the vicinity of the roots both in non fumigated soil extracts and in microbial biomass because of root exudation and rhizodeposition (Esperschütz et al., 2009) and the rhizospheric soil might not be well represented in our fumigated soil extracts.

5 Conclusions

Fine temporal resolution of laser absorption spectrometry-based measurements of the isotope composition of soil CO_2 efflux after in situ pulse labelling of trees is a promising tool for studying carbon transfer belowground. Our study has highlighted important seasonal variation in carbon transfer and allocation belowground. Beech appeared to rely more on recent carbon uptake while allocation/mobilisation of stored carbon may damp seasonal variations of carbon allocation belowground in oak. Despite a lower velocity of carbon transfer belowground in pine compared to the two angiosperm species during the active growing season, a similar amount of carbon is allocated to soil CO_2 efflux, with a strong seasonal variability. This study highlighted the species specific seasonal pattern of the carbon allocation to fulfil energy requirements of metabolic processes occurring belowground. This study also indicated a fast transfer of photosynthate to the microbial biomass including mycorrhizal mycelia and micro-organisms feeding on root exudates. However, further analyses will be needed to better describe the fate of carbon in the complex soil microbial community, a prerequisite for improving our understanding of soil carbon dynamics in forest ecosystems and their contribution to the global carbon cycle.

BGD
8, 885–919, 2011

Acknowledgement. Financial support was provided by the French National Research Agency (ANR) through the CATS Project (ANR-07-BLAN-0109). The CATS labelling experimentation was a team work and the authors would like to thank all persons taking part in it. Authors also acknowledge the "Métabolisme-Métabolome" platform of the IFR87 and the "functional ecology" platform of the IFR 110 for IRMS analyses.

References

Achat, D. L., Morel, C., Bakker, M. R., Augusto, L., Pellerin, S., Gallet-Budynek, A., and Gonzalez, M.: Assessing turnover of microbial biomass phosphorus: combination of an isotopic dilution method with a mass balance model, Soil Biol. Biochem., 42, 2231–2240, 2010.

Bahn, M., Reichstein, M., Davidson, E. A., Grünzweig, J., Jung, M., Carbone, M. S., Epron, D., Misson, L., Nouvellon, Y., Roupsard, O., Savage, K., Trumbore, S. E., Gimeno, C., Curiel Yuste, J., Tang, J., Vargas, R., and Janssens, I. A.: Soil respiration at mean annual temperature predicts annual total across vegetation types and biomes, Biogeosciences, 7, 2147–2157, doi:10.5194/bg-7-2147-2010, 2010.

Bakker, M. R.: The effects of liming and gypsum applications on a sessile oak (*Quercus petraea* (M.) Liebl.) stand at La Croix-Scaille (French Ardennes) II. Fine root dynamics, Plant Soil, 206, 109–121, 1999.

Bakker, M. R., Augusto, L., and Achat, D.: Fine root distribution of trees and understory in mature stands of maritime pine (*Pinus pinaster*) on dry and humid sites, Plant Soil, 286, 37–51, 2006.

Barbaroux, C., Bréda, N., and Dufrêne, E.: Distribution of above-ground and below-ground carbohydrate reserves in adult trees of two contrasting broad-leaved species (*Quercus petraea* and *Fagus sylvatica*), New Phytol., 157, 605–615, 2003.

Bowling, D. R., McDowell, N. G., Bond, B. J., Law, B. E., and Ehleringer, J. R.: ^{13}C content of ecosystem respiration is linked to precipitation and vapor pressure deficit, Oecologia, 131, 113–124, 2002.

Bowling, D. R., Pataki, D. E., and Randerson, J. T.: Carbon isotopes in terrestrial ecosystem pools and CO_2 fluxes, New Phytol., 178, 24–40, 2008.

Carbone, M. S. and Trumbore, S. E.: Contribution of new photosynthetic assimilates to

BGD

8, 885–919, 2011

respiration by perennial grasses and shrubs: residence times and allocation patterns, New Phytol., 176, 124–135, 2007.
Carbone, M. S., Czimczik, C. I., McDuffee, K. E., and Trumbore, S. E.: Allocation and residence time of photosynthetic products in a boreal forest using a low-level ^{14}C pulse-chase labeling technique, Glob. Change Biol., 13, 466–477, 2007.
Comas, L. H., Bouma, T. J., and Eissenstat, D. M.: Linking root traits to potential growth rate in six temperate tree species, Oecologia, 132, 34–43, 2002.
Craine, J. M., Wedin, D. A., and Chapin III, F. S.: Predominance of ecophysiological controls on soil CO_2 flux in a Minnesota grassland, Plant Soil, 207, 77–86, 1999.
Dannoura, M., Maillard, P., Fresneau, C., Plain, C., Berveiller, D., Gérant, D., Chipeaux, C., Bosc, A., Ngao, J., Damesin, C., Loustau, D., and Epron, D.: In situ assessment of the velocity of carbon transfer by tracing ^{13}C in trunk CO_2 efflux after pulse labelling: variations among tree species and season, New Phytol., doi:10.1111/j.1469-8137.2010.03599.x, in press, 2011.
Ekblad, A., Boström, B., Holm, A., and Comstedt, D.: Forest soil respiration rate and $\delta^{13}C$ is regulated by recent above ground weather conditions, Oecologia, 143, 136–142, 2005.
Epron, D.: Chapter 8: Separating autotrophic and heterotrophic components of soil respiration: lessons learned from trenching and related root exclusion experiments, in: Soil Carbon Flux Measurements. An Integrated Methodology, edited by: Kutsch, W., Bahn, M., and Heinemeyer, A., Cambridge University Press, Cambridge, 562–602, 2009.
Epron, D., Farque, L., Lucot, E., and Badot, P. M.: Soil CO_2 efflux in a beech forest: dependence on soil temperature and soil water content, Ann. For. Sci., 56, 221–226, 1999.
Epron, D., Le Dantec, V., Dufrêne, E., and Granier, A.: Seasonal dynamics of soil carbon dioxide efflux and simulated rhizosphere respiration in a beech forest, Tree Physiol., 21, 145–152, 2001.
Esperschütz, J., Buegger, F., Winkler, J. B., Munch, J. C., Schloter, M., and Gattinger, A.: Microbial response to exudates in the rhizosphere of young beech trees (*Fagus sylvatica* L.) after dormancy, Soil Biol. Biochem., 41, 1976–1985, 2009.
Gaumont-Guay, D., Black, T. A., Barr, A. G., Jassal, R. S., and Nesic, Z.: Biophysical controls on rhizospheric and heterotrophic components of soil respiration in a boreal black spruce stand, Tree Physiol., 28, 161–171, 2008.
Giardina, C. P. and Ryan, M. G.: Total belowground carbon allocation in a fast-growing *Eucalyptus* plantation estimated using a carbon balance approach, Ecosystems, 5, 487–499,

BGD
8, 885–919, 2011

2002.
Granier, A., Bréda, N., Longdoz, B., Gross, P., and Ngao, J.: Ten years of fluxes and stand growth in a young beech forest at Hesse, North-eastern France, Ann. For. Sci., 65, 2008.
Grieve, I. C., Davidson, D. A., Ostle, N. J., Bruneau, P. M. C., and Fallick, A. E.: Spatial heterogeneity in the relocation of added ^{13}C within the structure of an upland grassland soil, Soil Biol. Biochem., 38, 229–234, 2006.
Griffis, T. J., Baker, J. M., Sargent, S. D., Tanner, B. D., and Zhang, J.: Measuring field-scale isotopic CO_2 fluxes with tunable diode laser absorption spectroscopy and micrometeorological techniques, Agric. For. Meteorol., 124, 15–29, 2004.
Högberg, M. N., Briones, M. J. I., Keel, S. G., Metcalfe, D. B., Campbell, C., Midwood, A. J., Thornton, B., Hurry, V., Linder, S., Näsholm, T., and Högberg, P.: Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest, New Phytol., 187, 485–493, 2010.
Högberg, P. and Read, D. J.: Towards a more plant physiological perspective on soil ecology, Trends Ecol. Evol., 21, 549–554, 2006.
Högberg, P., Högberg, M. N., Gottlicher, S. G., Betson, N. R., Keel, S. G., Metcalfe, D. B., Campbell, C., Schindlbacher, A., Hurry, V., Lundmark, T., Linder, S., and Nasholm, T.: High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil microorganisms, New Phytol., 177, 220–228, 2008.
Hölttä, T., Mencuccini, M., and Nikinmaa, E.: Linking phloem function to structure: analysis with a coupled xylem-phloem transport model, J. Theor. Biol., 259, 325–337, 2009.
Horwath, W. R., Pregitzer, K. S., and Paul, E. A.: ^{14}C Allocation in tree-soil systems, Tree Physiol., 14, 1163–1176, 1994.
Janssens, I. A., Lankreijer, H., Metteucci, G., Kowalski, A. S., Buchmann, N., Epron, D., Pilegaard, K., Kutsch, W., Longdoz, B., Grünwald, T., Montagnani, L., Dore, S., Rebmann, C., Moors, E. J., Grelle, A., Rannik, Ü., Morgenstern, K., Oltchev, S., Clement, R., Gudmundsson, J., Minerbi, S., Berbigier, P., Ibrom, A., Moncrieff, J., Aubinet, M., Bernhofer, C., Jensen, N. O., Vesala, T., Granier, A., Schulze, E.-D., Lindroth, A., Dolman, A. J., Jarvis, P. G., Ceulemans, R., and Valentini, R.: Productivity overshadows temperature in determining soil and ecosystem respiration across European forests, Glob. Change Biol., 7, 269–278, 2001.
Kagawa, A., Sugimoto, A., and Maximov, T. C.: Seasonal course of translocation, storage and remobilization of ^{13}C pulse-labeled photoassimilate in naturally growing *Larix gmelinii* saplings, New Phytol., 171, 793–804, 2006.

BGD
8, 885–919, 2011

Kramer, C. and Gleixner, G.: Soil organic matter in soil depth profiles: distinct carbon preferences of microbial groups during carbon transformation, Soil Biol. Biochem., 40, 425–433, 2008.

Leake, J. R., Donnelly, D. P., Saunders, E. M., Boddy, L., and Read, D. J.: Rates and quantities of carbon flux to ectomycorrhizal mycelium following ^{14}C pulse labeling of *Pinus sylvestris* seedlings: effects of litter patches and interaction with a wood-decomposer fungus, Tree Physiol., 21, 71–82, 2001.

Litton, C. M. and Giardina, C. P.: Below-ground carbon flux and partitioning: global patterns and response to temperature, Funct. Ecol., 22, 941–954, 2008.

Litton, C. M., Raich, J. W., and Ryan, M. G.: Carbon allocation in forest ecosystems, Glob. Change Biol., 13, 2089–2109, 2007.

Marron, N., Plain, C., Longdoz, B., and Epron, D.: Seasonal and daily time course of the ^{13}C composition in soil CO_2 efflux recorded with a tunable diode laser spectrophotometer (TDLS), Plant Soil, 318, 137–151, 2009.

Marsden, C., Nouvellon, Y., and Epron, D.: Relating coarse root respiration to root diameter in clonal *Eucalyptus* stands in the Republic of the Congo, Tree Physiol., 28, 1245–1254, 2008.

McDowell, N. G., Bowling, D. R., Schauer, A., Irvine, J., Bond, B. J., Law, B. E., and Ehleringer, J. R.: Associations between carbon isotope ratios of ecosystem respiration, water availability and canopy conductance, Glob. Change Biol., 10, 1767–1784, 2004.

Mencuccini, M. and Hölttä, T.: The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked, New Phytol., 185, 189–203, 2010.

Mikan, C. J., Zak, D. R., Kubiske, M. E., and Pregitzer, K. S.: Combined effects of atmospheric CO_2 and N availability on the belowground carbon and nitrogen dynamics of aspen mesocosms, Oecologia, 124, 432–445, 2000.

Mortazavi, B., Chanton, J. P., Prater, J. L., Oishi, A. C., Oren, R., and Katul, G.: Temporal variability in ^{13}C of respired CO_2 in a pine and a hardwood forest subject to similar climatic conditions, Oecologia, 142, 57–69, 2005.

Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M. R., Lõhmus, K., Majdi, H., Metcalfe, D., Olsthoorn, A. F. M., Pronk, A., Vanguelova, E., Weih, M., and Brunner, I.: Specific root length as an indicator of environmental change, Plant Biosyst., 141, 426–442, 2007.

Peuke, A. D., Windt, C., and Van As, H.: Effects of cold-girdling on flows in the transport phloem in *Ricinus communis*: is mass flow inhibited?, Plant Cell Environ., 29, 15–25, 2006.

BGD
8, 885–919, 2011

Plain, C., Gérant, 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, 1433–1447, 2009.

Pumpanen, J., Heinonsalo, J., Rasilo, T., Hurme, K.-R., and Ilvesniemi, H.: Carbon balance and allocation of assimilated CO_2 in Scots pine, Norway spruce, and silver birch seedlings determined with gas exchange measurements and ^{14}C pulse labelling, Trees-Struct. Funct., 23, 611–621, 2009.

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, 2009.

Ryan, M. G. and Law, B. E.: Interpreting, measuring, and modelling soil respiration, Biogeochemistry, 73, 3–27, 2005.

Steele, S. J., Gower, S. T., Vogel, J. G., and Norman, J. M.: Root mass, net primary production and turnover in aspen, jack pine and black spruce forests in Saskatchewan and Manitoba, Canada., Tree Physiol., 17, 577–587, 1997.

Stoy, P. C., Palmroth, S., Oishi, A. C., Siqueira, M. B. S., Juang, J.-Y., Novick, K. A., Ward, E. J., Katul, G. G., and Oren, R.: Are ecosystem carbon inputs and outputs coupled at short time scales? A case study from adjacent pine and hardwood forests using impulse-response analysis, Plant Cell Environ., 30, 700–710, 2007.

Subke, J.-A., Inglima, I., and Cotrufo, M. F.: Trends and methodological impacts in soil CO_2 efflux partitioning: a metaanalytical review, Glob. Change Biol., 12, 921–943, 2006.

Subke, J.-A., Vallack, H. W., Tord, M., 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, 2009.

Vance, E. D., Brookes, P. C., and Jenkinson, D. S.: An extraction method for measuring soil microbial biomass C, Soil Biol. Biochem., 19, 703–707, 1987.

Wingate, L., Ogée, J., Burlett, R., Bosc, A., Devaux, M., Grace, J., Loustau, D., and Gessler, A.: Photosynthetic carbon isotope discrimination and its relationship to the carbon isotope signals of stem, soil and ecosystem respiration, New Phytol., 188, 576–589, 2010.

BGD
8, 885–919, 2011

Table 1. Diameter at 1.3 m height (DBH, cm), cumulative photosynthetic photon flux density (PPFD, mol m^{-2} d^{-1}) over 24 h following the beginning of labelling, air temperature (T_A, °C) averaged over 24 h following the beginning of labelling, volumetric soil water content (SWC, m^3 m^{-3}) on the day of labelling. Soil CO_2 efflux (F_S, mg C m^{-2} h^{-1}), root mass per unit area (M_R, g DM m^{-2}), specific root respiration (R_R, mg C kg_{DM}^{-1} h^{-1}), microbial carbon per unit area (C_M, g C m^{-2}) and microbial respiration (R_M, mg C kg_{soil}^{-1} h^{-1}) were averaged over the entire chase period (20 days). Time lag in ^{13}C recovery in soil CO_2 efflux (L, day), asymptotic value of cumulative label recovered in soil CO_2 efflux ($CLR_{FS}(\infty)$ in mg ^{13}C) and half residence time ($t_{1/2}$, day) were estimated by fitting cumulative label recovered in soil CO_2 efflux to Eqs. (9)–(11).

Date of labelling	DBH	PPFD	T_A	SWC	F_S	M_R	R_R	C_M	R_M	L	$CLR_{FS}(\infty)$	$t_{1/2}$
Beech												
19 May 09	5.0	48.1	16.4	0.37	206	205	65	39	4.5	1.13	426	6.63
27 May 09	7.3	40.1	13.1	0.36	105	297	93	33	4.4	0.92	371	9.13
6 Jul 09	7.8	43.8	17.5	0.32	214	343	104	44	4.3	0.67	2534	6.21
10 Jul 09	7.3	46.2	14.8	0.31	231	282	122	48	5.4	0.50	2128	4.92
17 Aug 09	4.9	43.6	21.2	0.39	79	186	67	35	2.7	1.33	93	4.83
19 Aug 09	6.6	50.7	23.7	0.36	99	246	80	41	3.0	0.67	599	5.96
23 Aug 09	6.2	51.7	18.3	0.23	122	192	72	32	1.9	1.33	694	13.13
24 Aug 09	6.0	40.2	23.4	0.13	75	292	82	41	2.6	0.75	468	4.17
Oak												
19 May 09	8.9	40.3	14.6	0.30	227	103	79	58	6.2	0.63	828	6.25
27 May 09	8.1	26.9	15.2	0.29	133	99	66	61	6.6	0.79	914	6.13
30 Jun 09	9.6	56.5	24.3	0.24	296	95	153	57	8.1	0.58	1064	5.96
8 Jul 09	8.6	21.4	14.3	0.23	294	97	95	68	4.5	0.75	867	6.13
31 Aug 09	8.9	14.8	22.5	0.20	163	95	56	62	2.0	1.08	729	7.00
9 Sep 09	8.1	18.4	20.8	0.21	140	90	77	98	3.2	1.04	1362	7.67
Pine												
12 Jun 09	12.0	63.6	21.1	0.10	177	39	449	13	2.3	1.75	1380	4.88
17 Jun 09	14.7	76.4	19.6	0.16	246	51	374	18	2.13	1.63	1152	6.04
3 Aug 09	15.0	50.3	19.8	0.08	174	57		16		2.46	960	7.54
4 Aug 09	11.3	62.8	23.4	0.09	169	53		15		1.96	1332	6.83
27 Oct 09	12.1	18.0	13.3	0.05	102			24		2.71	948	6.21
17 Nov 09	14.1	26.9	16.6	0.12	64			32		4.04	474	15.25
18 Feb 10	14.0	15.9	10.6	0.18	23			23		4.17	96	11.00
23 Feb 10	13.8	78.1	11.6	0.17	37			31		4.38	564	23.63

BGD
8, 885–919, 2011

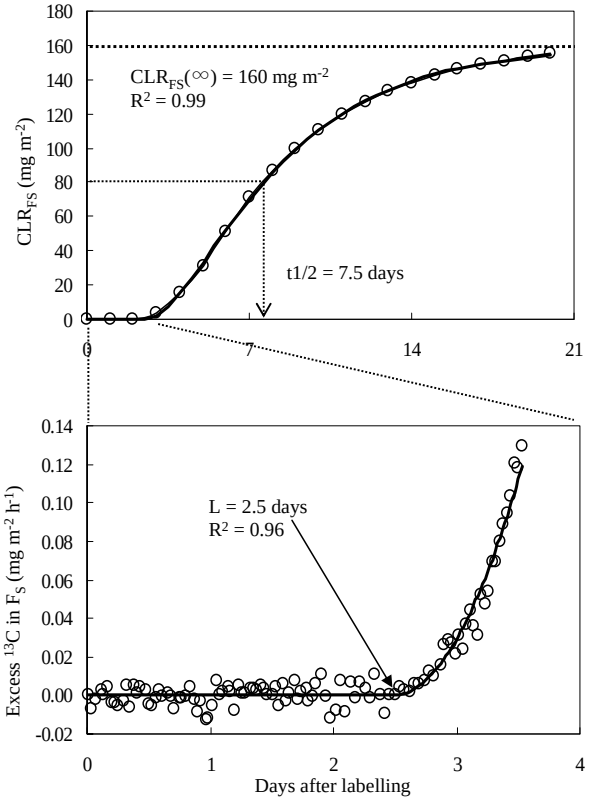

Fig. 1. Kinetics of cumulative excess ^{13}C in soil CO_2 efflux (CLR_{FS}, top panel) and kinetics of excess ^{13}C in soil CO_2 efflux (excess ^{13}C in F_S). Open circles are measured data and the curves represent the best fits of Eqs. (9) and (11), respectively. The example shown is for pine labelled on 3 August 2009. Asymptotic value of CLR_{FS} ($CLR_{FS}(\infty)$), half residence time of soil respired ^{13}C ($t_{1/2}$) and time lag (L) between the start of the labelling and the first appearance of ^{13}C in soil CO_2 efflux are depicted on the graphs.

BGD
8, 885–919, 2011

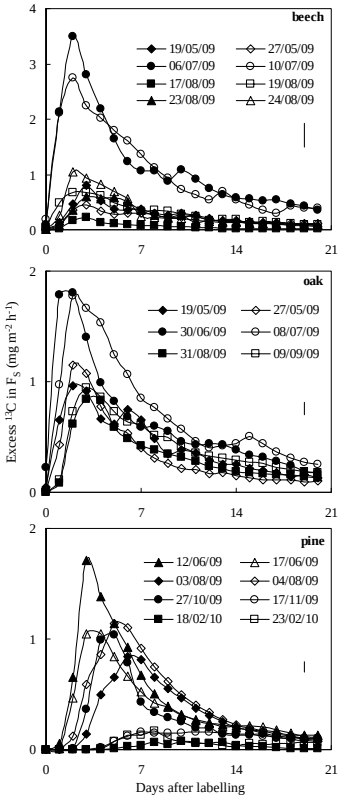

Fig. 2. Time courses of 24 h average of excess ^{13}C in soil CO_2 efflux (F_S) after whole crown pulse labelling of beeches (top), oaks (middle) and pines (bottom). Each tree is identified by its date of labelling. There are significant differences between trees ($F_{7,126} = 41.0$, $F_{5,63} = 63.9$ and $F_{7,126} = 114.2$ for beech, oak and pine, respectively, $p < 0.001$) and between sampling dates ($F_{160,126} = 2.75$, $F_{120,63} = 14.3$ and $F_{160,126} = 15.8$, respectively, $p < 0.001$). The verticals bars represent the root mean error.

BGD
8, 885–919, 2011

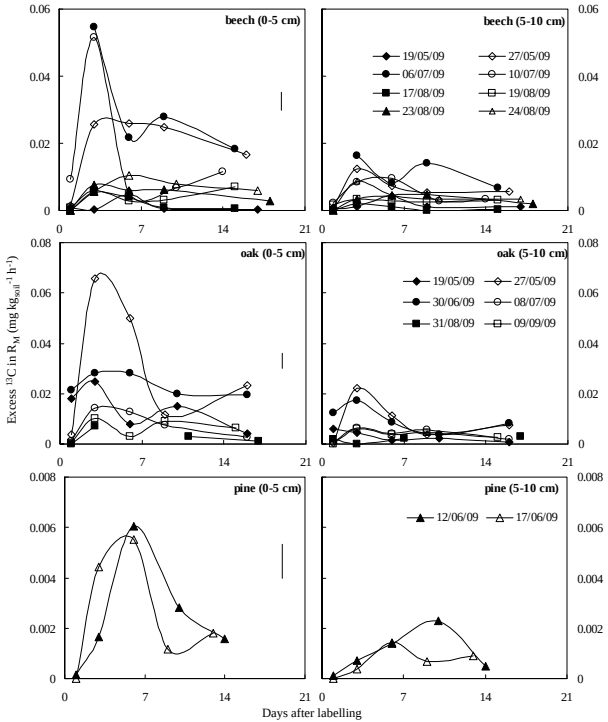

Fig. 3. Time courses of excess ^{13}C in microbial respiration (R_M) after whole crown pulse labelling of beeches (top), oaks (middle) and pines (bottom) at 0–5 cm depth (left) and 5–10 cm depth (right). Each tree is identified by its date of labelling. There are significant differences between trees for beech ($F_{7,78} = 23.1$, $p < 0.001$) and oak ($F_{5,23} = 13.3$, $p < 0.001$), between depths ($F_{1,78} = 44.9$, $F_{1,23} = 31.7$ and $F_{1,20} = 15.8$ for beech, oak and pine, respectively, $p < 0.001$) and between sampling dates ($F_{64,78} = 4.6$, $F_{23,23} = 3.5$ and $F_{16,20} = 3.2$ for beech, oak and pine, respectively, $p < 0.001$). The verticals bars represent the root mean error. Data are presented on two different panels according to depth but depth was included in the ANOVA.

BGD
8, 885–919, 2011

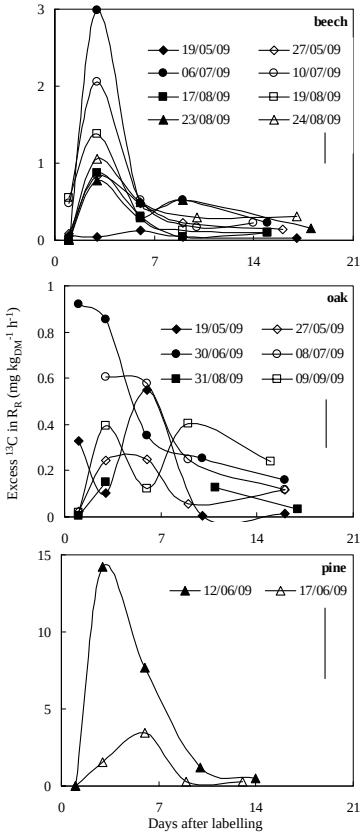

Fig. 4. Time courses of excess ^{13}C in root respiration (R_R) after whole crown pulse labelling of beeches (top), oaks (middle) and pines (bottom). Each tree is identified by its date of labelling. There are significant differences between trees for beech ($F_{7,42} = 4.1$, $p < 0.01$) and oak ($F_{5,15} = 3.0$, $p < 0.05$) and between sampling dates for beech ($F_{32,42} = 4.7$, $p < 0.001$). The verticals bars represent the root mean error.

BGD
8, 885–919, 2011

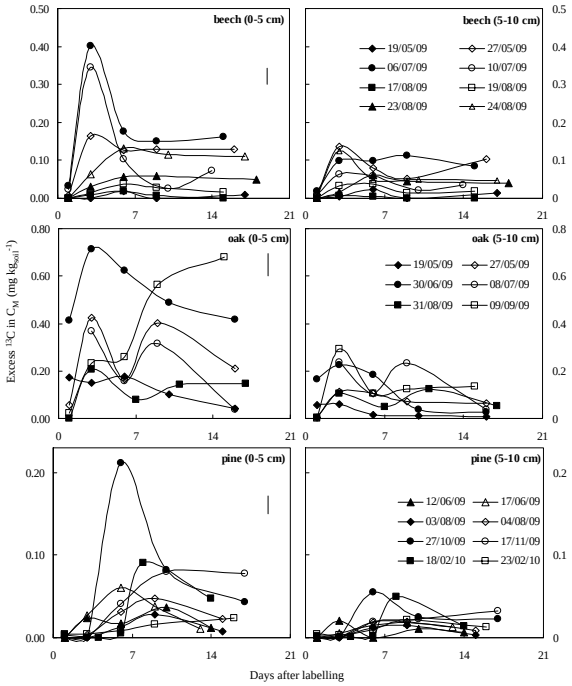

Fig. 5. Time courses of excess ^{13}C in microbial carbon (C_M) after whole crown pulse labelling of beeches (top), oaks (middle) and pines (bottom) at 0–5 cm depth (left) and 5–10 cm depth (right). Data for 10–20 cm depth in beech and pine are not shown but they are included in the statistical analyses. Each tree is identified by its date of labelling. There are significant differences between trees ($F_{7,120} = 22.0$, $F_{5,23} = 10.1$ and $F_{7,120} = 3.8$, for beech, oak and pine, respectively, $p < 0.001$), between depth ($F_{2,120} = 22.0$, $F_{1,23} = 47.1$ and $F_{2,120} = 14.5$ for beech, oak and pine, respectively, $p < 0.001$) and between sampling dates ($F_{96,120} = 2.5$ for beech, $p < 0.001$, $F_{23,23} = 2.4$ for oak, $p < 0.05$ and $F_{96,120} = 3.0$ for pine, $p < 0.001$). The verticals bars represent the root mean error. Data are presented on two different panels according to depth but depth was included in the ANOVA.

BGD
8, 885–919, 2011

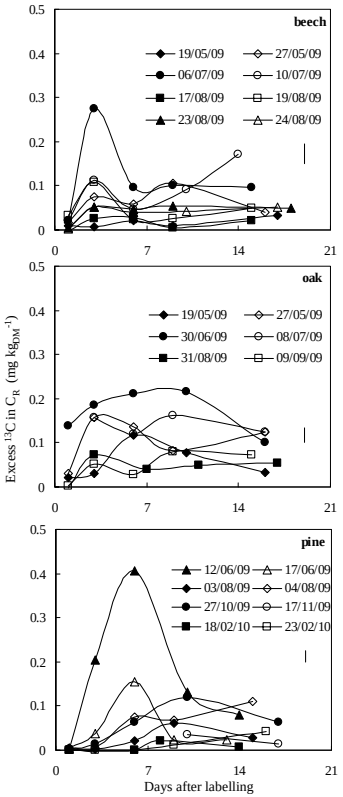

Fig. 6. Time courses of excess ^{13}C in root carbon (C_R) after whole crown pulse labelling of beeches (top), oaks (middle) and pines (bottom). Each tree is identified by its date of labelling. There are significant differences between trees ($F_{7,42} = 5.9$, $F_{5,9} = 10.7$ and $F_{7,18} = 24.1$, for beech, oak and pine, respectively, $p < 0.001$) and between sampling dates ($F_{32,42} = 2.0$ and $F_{9,15} = 2.69$ for beech and oak, respectively, $p < 0.05$, $F_{31,18} = 7.4$ for pine, $p < 0.001$). The verticals bars represent the root mean error.

BGD
8, 885–919, 2011

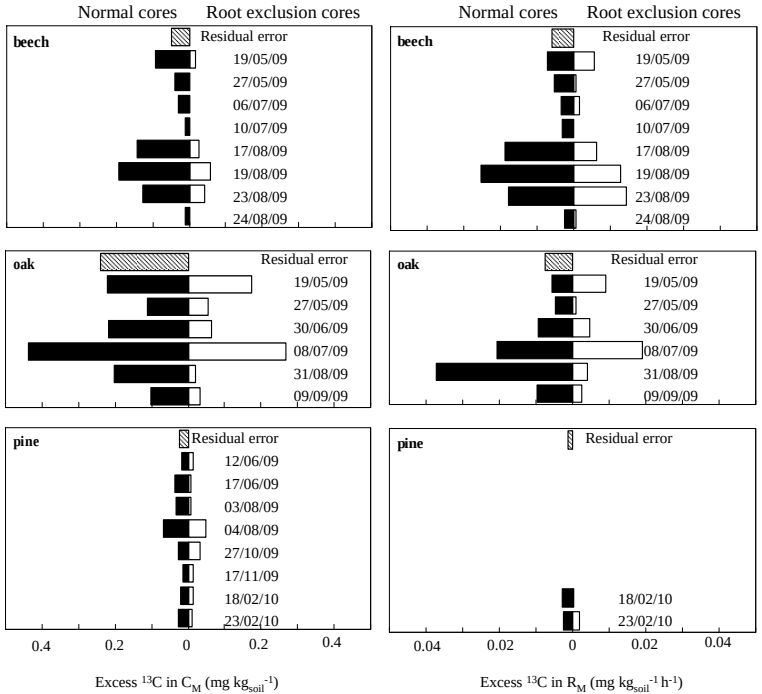

Fig. 7. Excess ^{13}C in microbial carbon (C_M, left) or excess ^{13}C in microbial respiration (R_M, right) between normal cores and root exclusion cores. Data are pooled by date of sampling and depth for presentation but the statistical analyses were done on non-pooled data. Each tree is identified by its date of labelling. For C_M, there are significant differences for beech and pine between trees ($F_{7,64} = 7.1$ and $F_{7,64} = 8.2$, $p < 0.001$), between depth for beech and oak ($F_{1,64} = 4.5$ and $F_{1,62} = 3.9$, respectively, $p < 0.05$) and between sampling dates for beech and pine ($F_{32,64} = 2.0$ and $F_{32,62} = 2.2$, $p < 0.05$). For R_M, there are significant differences between trees and between sampling dates for beech ($F_{7,63} = 10.9$ and $F_{32,63} = 3.7$, $p < 0.001$), between depths for all species ($F_{1,63} = 14.9$, $F_{1,16} = 18.2$ and $F_{1,24} = 13.5$ for beech, oak and pine, respectively, $p < 0.001$). The dashed bars represent the root mean error.

BGD
8, 885–919, 2011