

High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil microorganisms

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Summary

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- Half of the biological activity in forest soils is supported by recent tree photosynthate, but no study has traced in detail this flux of carbon from the canopy to soil microorganisms in the field.
- Using $^{13}\text{CO}_2$, we pulse-labelled over 1.5 h a 50-m² patch of 4-m-tall boreal *Pinus sylvestris* forest in a 200-m³ chamber.
- Tracer levels peaked after 24 h in soluble carbohydrates in the phloem at a height of 0.3 m, after 2–4 d in soil respiratory efflux, after 4–7 d in ectomycorrhizal roots, and after 2–4 d in soil microbial cytoplasm. Carbon in the active pool in needles, in soluble carbohydrates in phloem and in soil respiratory efflux had half-lives of 22, 17 and 35 h, respectively. Carbon in soil microbial cytoplasm had a half-life of 280 h, while the carbon in ectomycorrhizal root tips turned over much more slowly. Simultaneous labelling of the soil with $^{15}\text{NH}_4^+$ showed that the ectomycorrhizal roots, which were the strongest sinks for photosynthate, were also the most active sinks for soil nitrogen.
- These observations highlight the close temporal coupling between tree canopy photosynthesis and a significant fraction of soil activity in forests.

Key words: carbon, forests, mycorrhiza, photosynthesis, plant allocation, Scots pine, soil microorganisms.

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Introduction

Soils have been described as a final frontier in ecosystem science and as a hidden world (Copley, 2000; Sugden *et al.*, 2004) because their exploration has been hindered by their opacity, extreme diversity of microscopic organisms (Torsvik & Ovreas, 2002; Gans *et al.*, 2005) and complex food webs (Wardle *et al.*, 2004). Plant carbon (C) reaches the soil and its organisms as above-ground litter, or via the plant below-ground

allocation pathway, which supports root production and hence below-ground litter production, but also provides sugars and other labile C compounds to mycorrhizal fungi and other root- or mycorrhiza-associated microorganisms (Smith & Read, 1997; van Hees *et al.*, 2005).

Half of the biological activity in soil is fuelled by C that was fixed through photosynthesis a few hours (grasslands) or a few days (forests) ago, while the other half is fuelled by C, in dead organic matter supplied as litter, that was fixed months to

years ago, which compounds problems of studying organisms in soils (Högberg & Read, 2006). For example, the flux of recent photosynthate is distributed through roots and mycorrhizal fungal hyphae which act as pipelines for recently fixed C through a matrix of older C in organic matter, but which are severed when soil samples are extracted. The problems of studying soils are aggravated in systems where plants are large, as in forests, in which case laboratory mesocosms may not realistically mimic the complexity and dynamics found in the field. Hence, we lack a detailed understanding of several critical aspects of forest soil C dynamics, including processes that globally represent a C release to the atmosphere several times larger than that from burning of fossil fuels (Schimel, 1995).

A considerable uncertainty relates to the quantitative distribution of tree below-ground allocation. A few decades ago it was widely held that most of the C allocated below-ground in forests supported the growth of fine roots, as the biomass of the finest roots was supposed to turn over one or several times per year (Vogt *et al.*, 1986; Waring & Running, 1998; Gill & Jackson, 2000). If this is true, mycorrhizal fungi and other soil biota should receive relatively little C by the plant below-ground C allocation pathway. However, recent tree-girdling experiments (Högberg *et al.*, 2001, 2002), and free air carbon dioxide enrichment (FACE) experiments, which provide a means of labelling photosynthate with the stable isotope ^{13}C (Matamala *et al.*, 2003; Körner *et al.*, 2005; Keel *et al.*, 2006), have, along with bomb- ^{14}C estimates of root age (Gaudinski *et al.*, 2000, 2001), indicated that fine roots of trees turn over much more slowly than previously assumed. This, in turn, indicates that more of the below-ground C flux is made available to mycorrhizal fungi and other soil biota closely associated with roots (Högberg & Read, 2006). Indeed, a recent FACE experiment in a plantation of *Populus* spp. trees indicated that mycorrhizal fungal C provides the major C input to soil organic matter (Godbold *et al.*, 2006), and data from a laboratory mesocosm study of a (herbaceous) *Plantago* species suggested that C in arbuscular mycorrhizal fungal mycelium had a residence time of < 1 wk (Staddon *et al.*, 2003).

The problems discussed above can only be resolved if we can obtain a more detailed picture in the field of the dynamics of the C flux from the tree canopy into the below-ground system. Developments in stable isotope and molecular techniques have enabled stable isotope probing of functional groups of soil organisms through labelling of plant photosynthate in short-stature vegetation such as that found in grasslands (e.g. Treonis *et al.*, 2004; Rangel-Castro *et al.*, 2005), but not yet in a forest setting. Studies in forests have been few and have mostly been in the context of FACE experiments (Andrews *et al.*, 1999; Matamala *et al.*, 2003; Steinmann *et al.*, 2004; Körner *et al.*, 2005; Keel *et al.*, 2006), which use an elevated atmospheric CO_2 concentration ($[\text{CO}_2]$), and create a relatively small deviation in ^{13}C abundance, which becomes significant only in the longer term. Hence, it is difficult to follow the C dynamics in detail. Recently, Carbone *et al.* (2007)

used a short pulse-labelling with $^{14}\text{CO}_2$ in a boreal forest to obtain a greater resolution. They argued that $^{13}\text{CO}_2$ could not be used for the same purpose unless long labelling periods or very high $^{13}\text{CO}_2$ concentrations were used, which would be associated with very high financial costs. Here, we report the first highly resolved temporal record of the forest below-ground flux of C in a natural setting based on tracing a short pulse of ^{13}C from tree canopy photosynthesis through roots to the soil microbial biomass and back to the atmosphere via the soil respiratory efflux.

Materials and Methods

Site

The site at Rosinedalsheden (64°09'N, 19°05'E, at 145 m above sea level) is located on a plain of fine sand c. 50 km north-west of Umeå in northern Sweden. The soil profile is a podzol, with an organic mor-layer of 2–3 cm thickness, and with a carbon:nitrogen (C:N) ratio of 33 ± 1 (mean ± 1 SE) and a pH (soil:solution volume ratio = 1 : 3) of 4.5 ± 0.1 . The stand is a young naturally regenerated *Pinus sylvestris* L. forest. Trees (in the 50-m² labelled area) were 2.42 ± 0.08 m tall ($n = 87$, excluding 17 individuals < 1.3 m), with a diameter at breast height (d.b.h., at 1.3 m) of 1.97 ± 0.13 cm, and were 14.2 ± 0.3 yr old. The 10 largest were 3.86 ± 0.13 m tall, had a diameter of 4.19 ± 0.51 cm, and were 16.9 ± 1.0 yr old. There was a sparse understorey of the dwarf shrubs *Calluna vulgaris* L. and *Vaccinium vitis-idaea* L. and a ground-layer of *Cladonia* spp. lichens. A reference plot was established 15 m away from the labelled plot to provide data on the natural abundance of ^{13}C of the soil CO_2 efflux and of ^{13}C and ^{15}N of ecosystem components. This 50-m² plot was on the same type of soil and contained 98 small trees and seedlings, of which the 10 largest were 4.82 ± 0.26 m tall, had a diameter of 6.27 ± 0.59 cm and were 19.3 ± 0.7 yr old.

^{13}C and ^{15}N labelling

On the 23 August 2006, a 200-m³ chamber covering an octagon-shaped surface area of 50 m² and with a height of 4 m was erected over a patch of the forest. The frame was put in place first, and the plastic cover was left folded on the top rim of the frame until shortly before the labelling event. Then (at 14:30 h) the plastic cover was quickly unfolded and sealed to the ground with a barrier of fine sand. The chamber air was cooled to the temperature of the outside air ($\pm 1^\circ\text{C}$). Chamber air was re-circulated through the cooling battery at a rate of 400 m³ min⁻¹, which ensured good temperature control and thorough mixing of the air. The $[\text{CO}_2]$ (measured with an infrared gas analyser; Li-Cor 6400; Li-Cor, Lincoln, NE, USA) inside the chamber was 360 ppm (i.e. just below ambient atmospheric concentrations) a few minutes after the chamber was sealed, but declined (Fig. 1), as a result of a predominance

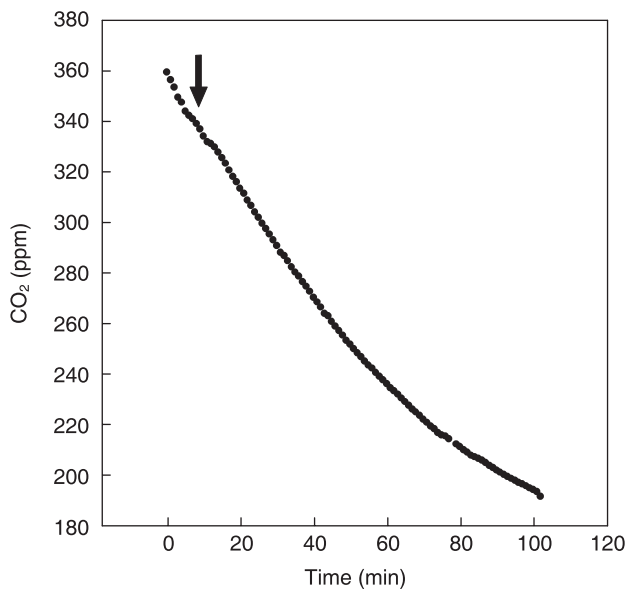


Fig. 1 Change in CO₂ concentration ([CO₂]) in chamber air during the ¹³CO₂-labelling period. The decline in [CO₂] reflects the predomination of photosynthesis over ecosystem respiration. Arrow, 5 l of ¹³CO₂ was released after 9 min.

of photosynthesis over respiration, to 190 ppm after 102 min (when the plastic cover was removed). Nine minutes after the chamber was sealed, a quantity of *c.* 5 l of CO₂ with ≥ 95 atom% ¹³C (Spectra Gases, Alpha, NJ, USA) was released into the air stream through the cooling battery. Directly after this release, the atom% ¹³C of CO₂ in the chamber was 5.74 ± 0.04 , but declined, during the labelling period of 93 min, to 3.74 ± 0.01 atom% ¹³C (atmospheric CO₂ has a ¹³C natural abundance of *c.* 1.11 atom%) in parallel with the decline in [CO₂] as a consequence of respiratory release of unlabelled CO₂ from soil and plants into the chamber air, and also because of isotopic exchange with unlabelled CO₂ in soil air and water. No attempt was made to hinder the activity of roots from unlabelled trees in the central 10-m² area of the labelled plot, in which all of the soil sampling was conducted. To assess the potential influence of unlabelled roots belonging to trees outside the 50-m² ¹³CO₂-labelled area, the soil in the central 10 m² was labelled with ¹⁵NH₄Cl. Fifteen ml of a solution with 9.9 atom% ¹⁵N was injected at 160 points regularly distributed within this circular 10-m² area to give a final addition of 0.2 g N m⁻². Determination of ¹⁵N in needles from trees inside and outside the labelled area was later used to estimate the influence of roots from trees not labelled with ¹³C. The ¹⁵N labelling of the soil also enabled studies of ¹⁵N uptake into tree roots.

Sampling

Samples for estimates of soil respiration and its C isotope composition were taken from four 0.046-m² circular plots in

the central 10 m² of the labelled area and from four plots in the reference area, using cylindrical head-spaces (Högberg & Ekblad, 1996). At sampling, a lid was put onto the cylinder, and five consecutive samples were taken at time intervals of 2 min from each cylinder, after which the cylinder lid was taken off. The first sampling was performed on the day before ¹³CO₂ labelling, and then every 4 h for the first 72 h after labelling. After this initial period, the sampling was less frequent and was terminated 30 d after labelling. Samples of phloem from the pine trees were taken at 1.3 and 0.3 m above the ground at the same time as the sampling of the soil respiratory efflux, and the soluble fraction of the phloem was isolated as described by Betson *et al.* (2007). The organic mor-layer of the soil was sampled with a 10-cm-diameter corer, on the day before labelling, and 1, 2, 4, 7, 13 and 33 d after labelling. Soil samples were brought immediately to the laboratory, where roots were extracted. Ectomycorrhizal fine-root tips were then sampled under a dissecting microscope and put directly into tin capsules for freeze-drying and isotope ratio analysis. The remaining soil sample was passed through a 2-mm sieve, and used for determination of microbial cytoplasm C and N following the chloroform fumigation-extraction method (Vance *et al.*, 1987; Högberg & Högberg, 2002), but using a 10-fold lower concentration of the salt extractant (Merckx & Van der Linden, 1988). Extracts from fumigated and nonfumigated soil were also used for determination of the $\delta^{13}\text{C}$ of microbial cytoplasm. The chloroform fumigation-extraction methodology measures mainly the microbial cytoplasm C in the soil and correction factors are generally used to convert the data into full estimates of microbial biomass (Jenkinson *et al.*, 2004 and references therein). Whereas the cytoplasmic portion of microbes is often defined as the chloroform-labile microbial C, we prefer to use the term 'microbial cytoplasm' (MCYT).

Current-year needles from the uppermost whorl were collected from three trees in the labelled area and three in the reference area immediately after the end of ¹³CO₂ labelling, and then six more times until 30 d after the labelling. Four days after the labelling, needles from all 104 trees and seedlings inside the labelled area and from 25 trees outside it were sampled to assess the distribution of tree root activity (¹⁵N uptake) inside and around the central 10 m² of the ¹³C-labelled plot. Very little ¹⁵N tracer was found after 4 d in needles of trees in the central 10-m² ¹⁵N-labelled plot. A second sampling was therefore conducted in March 2007, when the site was still covered by winter snow. Samples of needles formed in 2006 were taken from 20 trees inside the central 10-m² chamber plot, and from one tree in each of four directions (north, east, south and west) at 3, 4, 6, 8 and 10 m from the plot centre. In autumn 2006, samples of tree biomass were obtained from 23 individual trees in the stand, encompassing the range of diameters at breast height found in the chamber plot. It was found that a linear equation could be used to describe the relationship between foliar biomass per tree and

diameter at breast height (foliar biomass (in kg) = 0.0426 + 0.0035 × d.b.h. (in mm); $R^2 = 0.96$). Using the data on the 87 trees that reached > 1.3 m, and this equation, the foliar biomass in the chamber plot was estimated.

Analyses

Gas samples were admitted to a module focusing the CO_2 peak before being admitted to an isotope ratio mass spectrometer (IRMS; Högberg & Ekblad, 1996). The soil respiration rate was calculated as the linear increase in $[\text{CO}_2]$ in each head-space; series of samples with an $R^2 < 0.80$ were discarded. Keeling plots (linear regressions analysis of $\delta^{13}\text{C}$ vs $1/[\text{CO}_2]$; Keeling, 1958) were used to estimate the $\delta^{13}\text{C}$ of soil respired CO_2 , unless there was no change ($< 1\%$) in $\delta^{13}\text{C}$ despite a clear increase in $[\text{CO}_2]$, which occurs at $\delta^{13}\text{C}$ values of soil respiration close to that of ambient air, in which case the average of the five samples was used as the estimate. Keeling plots with an $R^2 < 0.80$ were discarded. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of needle and ectomycorrhizal fine-root samples were analysed on an elemental analyser (EA) coupled to an IRMS (Ohlsson & Wallmark, 1999). The ^{13}C abundance in soil extracts and in microbial cytoplasm extracted after chloroform fumigation were analysed in CO_2 produced by wet oxidation using dichromate (Allison, 1960). Total C in soil extracts was determined by combustion followed by analysis on an infrared gas analyser (TOC-5000; Shimadzu Corporation, Kyoto, Japan). Results of isotopic analyses are reported in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ (‰):

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000 \quad \text{Eqn 1}$$

(R , $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$.) The standards used were 5‰ CO_2 (for samples of soil CO_2 efflux) in N_2 with a $\delta^{13}\text{C}$ of 5‰ relative to the Vienna Pee Dee Belemnite (V-PDB) standard or wheat flour (for samples of plant material) calibrated against atmospheric N_2 and V-PDB, respectively. In the case of extracts of soil and microbial cytoplasm, standard curves based on analysis of sugars at ^{13}C abundances of -25, -11, 94 and 535‰ were used. The $\delta^{13}\text{C}$ of MCYT was calculated as follows:

$$\delta^{13}\text{C}_{\text{MCYT}} = [\delta^{13}\text{C}_{\text{FUM}} \times C_{\text{FUM}} - \delta^{13}\text{C}_{\text{NONFUM}} \times C_{\text{NONFUM}}] / [C_{\text{FUM}} - C_{\text{NONFUM}}] \quad \text{Eqn 2}$$

(FUM and NONFUM, extracts from chloroform-fumigated and nonfumigated soil samples, respectively.) Declines in $\delta^{13}\text{C}$ of a particular C pool after peak labelling were fitted to a first-order exponential decay function:

$$N_t = N_0 \times e^{-\lambda t} \quad \text{Eqn 3}$$

N_0 and N_t are the $\delta^{13}\text{C}$ values at peak labelling and at time t , respectively, and λ is the decay constant, which was used in the subsequent calculation of the half-life, $t_{1/2}$, of the C in the respective C pool or C flux:

$$t_{1/2} = \ln(2)/\lambda \quad \text{Eqn 4}$$

The half-life, $t_{1/2}$, was then used to calculate the mean residence time, τ :

$$\tau = t_{1/2}/\ln(2) \quad \text{Eqn 5}$$

We estimated the rate of photosynthesis and uptake of ^{13}C in two ways. First, we assumed that CO_2 was removed from the chamber air through plant photosynthesis, but added to it through plant and soil respiration. We used the initial (during the 8 min before $^{13}\text{CO}_2$ was added) rate of change in chamber $[\text{CO}_2]$ plus an estimated ecosystem respiration. The latter was based on the mean rate of soil CO_2 efflux on the day before and the day after the labelling period and the assumption that soil respiration accounted for 70% of ecosystem respiration (Janssens *et al.*, 2001). The rate of photosynthesis and the average atom% ^{13}C in the chamber air were then used to estimate uptake of ^{13}C . Secondly, we used the difference in the $\delta^{13}\text{C}$ of current needles between the chamber and the reference plots immediately after the labelling, and the foliar biomass in the chamber, to estimate the rate of uptake of ^{13}C and of total C through photosynthesis. Current (Curr) and 1-yr-old (Curr + 1) needles are more active than older needles, and to take this into account, we assumed that in August Curr, Curr + 1, Curr + 2 and Curr + 3 needles contributed 30, 30, 30 and 10%, respectively, of the foliar biomass (Flower-Ellis & Persson, 1980), and that the rate of photosynthesis by Curr + 1 needles equalled that of Curr needles (the age-class of needles sampled here), while the rate was 25 and 50% lower in Curr + 2 and Curr + 3 needles, respectively (Linder & Troeng, 1980; Linder & Lohammar, 1981).

Results and Discussion

Carbon dioxide was quickly taken up by photosynthesis in the chamber (Fig. 1). The initial rate of draw-down of $[\text{CO}_2]$ was $-2.75 \pm 0.14 \text{ ppm min}^{-1}$, or $0.29 \text{ g C min}^{-1}$. With a soil respiration rate of $0.04 \text{ g C min}^{-1}$ and an estimated ecosystem respiration rate of $0.06 \text{ g C min}^{-1}$, photosynthesis was thus estimated at $0.35 \text{ g C min}^{-1}$ (or 0.43 g C m^{-2} (ground surface area) h^{-1}). Given an average atom% ^{13}C of CO_2 of 4.74 in the chamber, this gives an uptake of *c.* 1.5 g of ^{13}C . Using the second estimate, the one based on estimated foliar biomass and $\delta^{13}\text{C}$ in current needles immediately after labelling, the uptake of ^{13}C was estimated at 1.7 g, and the rate of photosynthesis was $0.58 \text{ g C m}^{-2} \text{ h}^{-1}$. The foliar biomass in the chamber plot was estimated at 10.25 kg (dry weight (DW)) and the foliar C at *c.* 5 kg, while the ^{13}C enrichment of current needles was estimated to be 18‰ above ambient (see below). Both estimates of uptake of ^{13}C given above (i.e. 1.5 g and 1.7 g) include the natural background of the isotope, *c.* 1.11 atom%, representing 0.4–0.6 g of C. Thus, when corrected for this, the real uptake of tracer ^{13}C becomes

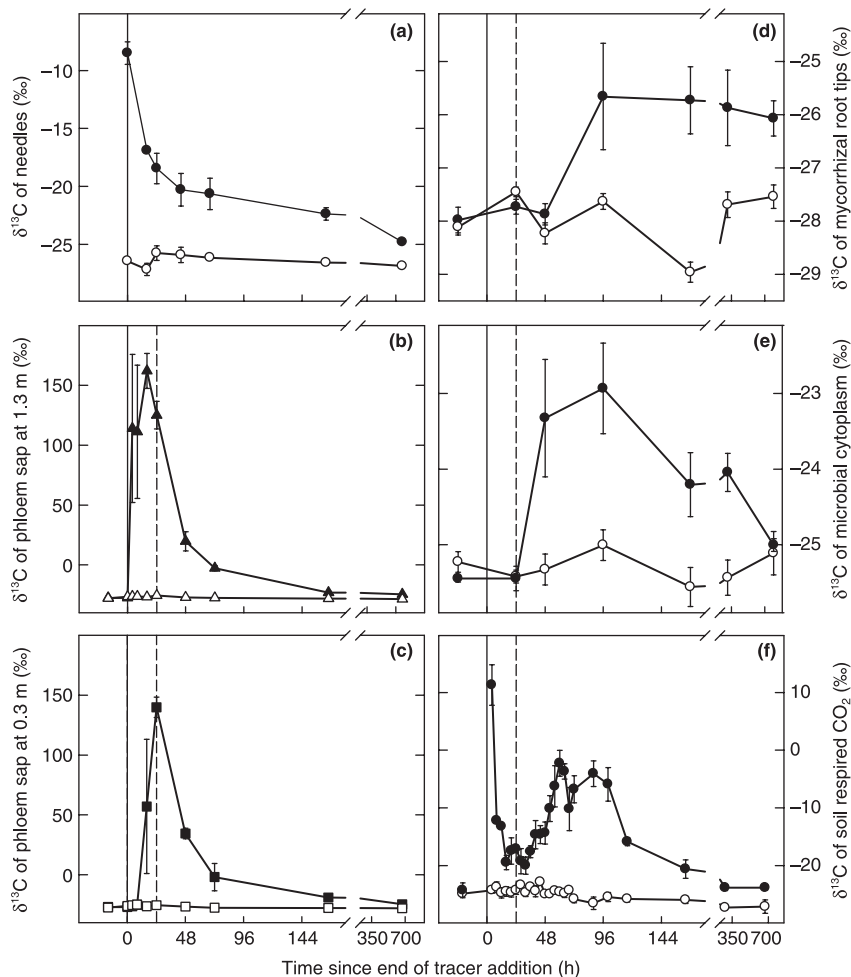


Fig. 2 Progression of ^{13}C label through ecosystem carbon pools and fluxes in a boreal Scots pine (*Pinus sylvestris*) forest labelled through photosynthetic incorporation of ^{13}C by trees exposed to $^{13}\text{CO}_2$. (a) Current needles; (b) soluble carbohydrates (sugars) in tree phloem sap at 1.3 m above the ground; (c) soluble carbohydrates (sugars) in tree phloem sap at 0.3 m above the ground; (d) ectomycorrhizal fine-root tips; (e) microbial cytoplasmic C; (f) soil CO_2 efflux. Closed symbols, labelled plot; open symbols, unlabelled reference plot. The solid vertical line shows the 93-min labelling event, while the broken line indicates the time of peak labelling of the soluble carbohydrates in the phloem sap at a height of 0.3 m. Data are means ± 1 SE.

c. 1.1 g in both cases. Because one of the estimates, the one based on tree foliar biomass, ascribes all photosynthesis to the trees, while the other does not, the photosynthesis by lichens and ericaceous dwarf shrubs should be minor. The uptake of 1.1 g compares with the 2.9 g of ^{13}C (according to specifications given by the supplier) released into the chamber, and means that almost 40% of the tracer was taken up. Around 0.6 g of ^{13}C , or 20%, remained in the chamber head-space air at the end of the labelling period. Nonbiological release of ^{13}C with the soil respiratory efflux directly after labelling suggests that tracer ^{13}C exchanged with C in soil CO_2 and in carbonic acid in soil water (see further below), which is a likely explanation of some of the 40% unaccounted for.

Immediately after the end of the labelling period, the $\delta^{13}\text{C}$ of the current needles was elevated by 18‰ above that of the control (Fig. 2a), but the needle $\delta^{13}\text{C}$ fell very quickly in the first 45 h after labelling and then more slowly. This probably reflects direct export of labelled sucrose (concomitant with production of new unlabelled photosynthate), followed by a phase of export of sucrose derived from labelled C that was stored for a while. The labelled C exported from needles

during the first phase (0–45 h) had a half-life of 22 h (Table 1).

Before labelling there were no differences between the chamber plot and the reference plot in ^{13}C natural abundance of soluble carbohydrates in the tree stem phloem, ectomycorrhizal fine roots, microbial cytoplasmic C and soil respiratory efflux (Fig. 2). Elevated $\delta^{13}\text{C}$ of soluble carbohydrates (sugars) in the phloem at a height of 1.3 m was already observed 4 h after the labelling event, but peaked after 16 h (Fig. 2b). Further down the stems, at a height of 0.3 m, elevated $\delta^{13}\text{C}$ was first observed 16 h after labelling, and peaked 8 h later, that is, 24 h after labelling (Fig. 2c). This means that there was an 8-h difference between the peaks in $\delta^{13}\text{C}$ at heights of 1.3 and 0.3 m, which translates to a transport velocity of 0.1 m h^{-1} . This is below previous estimates of velocities of phloem transport of $0.2\text{--}2 \text{ m h}^{-1}$ (Ekblad & Höglberg, 2001; Nobel, 2005). Moreover, the phloem became highly enriched in ^{13}C , with abundances of 190 and 165‰ above ambient at heights of 1.3 and 0.3 m, respectively (Fig. 2b,c). The close agreement between these two figures suggests that there is little exchange with carbohydrates outside the phloem during the downward

Table 1 Half-lives and mean residence times (MRTs) of carbon pools and fluxes in a young boreal Scots pine (*Pinus sylvestris*) forest labelled through photosynthetic incorporation of $^{13}\text{C}\text{O}_2$

Carbon pool or flux	Half-life (h)	MRT (h)	R^2	P
Rapidly turning over needle C pool*	22	32	0.96	0.015
Phloem at tree height of 0.3 m	17	24	0.99	0.003
Labile C in ectomycorrhizal root tips†	65	94	1.00	–
Microbial (cytoplasmic) C‡	277	400	0.87	0.013
Soil CO_2 efflux	35	50	0.87	0.005

Adjusted R^2 and probability (P) refer to the first-order exponential decay functions used for calculations of half-lives and mean residence times.

*Representing the decline in labelling during the first 45 h after labelling.

†Representing the decline in labelling over 624 h after peak ^{13}C labelling; the data fit the decay function perfectly, but the number of sampling times (three) does not allow a statistical analysis.

‡Representing the decline in labelling over 744 h after peak ^{13}C -labelling of this pool.

transport. In fact, some of the difference may be caused by an influx into the phloem of C fixed after the labelling period by needles on branches between heights of 1.3 and 0.3 m. The decline in the $\delta^{13}\text{C}$ of the phloem was rapid, with a half-life of 17 h at a height of 0.3 m (Table 1).

The soil respiratory efflux appeared already to be highly labelled 4 h after the chamber was removed (i.e. at the first sampling after the labelling period), but the $\delta^{13}\text{C}$ of this flux decreased very quickly, and was already almost as low as reference values 16 h after the labelling event (Fig. 2f). However, this was clearly before the pulse of labelled tree photosynthate reached the below-ground system, as labelling of phloem sap C at a height of 0.3 m was first observed after 16 h and peaked at 24 h after labelling (Fig. 2c). We attribute the initial 16-h decline in $\delta^{13}\text{C}$ in the soil respiratory efflux to isotopic exchange, because during the labelling period C in CO_2 in soil air and in carbonic acid in soil water must have equilibrated isotopically, at least partly, with the CO_2 in the chamber air (which was on average 3200‰ ^{13}C). Upon removal of the chamber, another phase of equilibration, this time with an atmosphere with $\delta^{13}\text{C}$ at natural abundance (c. –8‰ ^{13}C), would lead to a decline, as observed (Fig. 2f). Furthermore, the rate of this decline was far faster than any of the biological components or processes studied (Fig. 2, Table 1). Fixation of labelled soil air through carboxylation by phosphoenolpyruvate (PEP) carboxylase in roots and other non-Rubisco carboxylations in roots or microorganisms are possible; such C could later contribute to soil respiration, but its contribution should be minor, especially since no ^{13}C label was found in ectomycorrhizal fine roots or in microbial cytoplasm 24 h after the labelling (Fig. 2d,e).

The phloem flux-driven pulse of label ^{13}C in the soil respiratory efflux started 24 h after labelling, and showed a broad peak at c. 20‰ above ambient between 48 and 96 h after labelling (Fig. 2f). A 2-d time lag between photosynthesis and soil respiration was also observed in ^{14}C -based studies of similarly sized trees by Howarth *et al.* (1994) and Carbone *et al.* (2007). The subsequent decline after the peak in $\delta^{13}\text{C}$ of the soil respiratory efflux in our study was relatively fast, with

a half-life of 35 h (Fig. 2f, Table 1). This is half the rate of the decline in $\delta^{13}\text{C}$ observed in the phloem (Table 1), and is consistent with the notion of the rapidly turning over 'autotrophic' C flux (with a half-life of 17 h for the labelled phloem flux) contributing about half of soil respiration (Högberg *et al.*, 2001; Högberg & Read, 2006), and the remainder being a heterotrophic component with a much lower rate of turnover. Furthermore, such a mixing ratio of the autotrophic and heterotrophic components of soil activity would suggest that the autotrophic component was labelled at c. 40‰ above ambient, which is 25% of the labelling of soluble carbohydrates in the phloem. This in turn indicates that the labelled phloem flux mixed with a large pool of labile C in the below-ground system, a suggestion also corroborated by the relatively broad ^{13}C peak of the soil respiratory efflux (Fig. 2f). The broadness of this peak may also relate to the fact that some of the ^{13}C -labelled CO_2 could have been contributed by roots and microbes deeper down in the soil profile, having a longer pathway through the soil before its release at the surface (Stoy *et al.*, 2007); however, in a dry soil, like the one studied here, diffusion of CO_2 is orders of magnitude faster than through a wet soil.

The ^{13}C label was first observed in ectomycorrhizal root tips after 4 d; however, these were not sampled between 2 and 4 d after labelling (Fig. 2d). The level of labelling was relatively low, c. 2–3‰ above the reference. Accordingly, variations in the reference interfered somewhat with the analysis of the flux of labelled C through this pool. We subtracted the average $\delta^{13}\text{C}$ of the reference to remove the background variability (Fig. 3a). The data obtained could be taken as evidence of an initial faster turnover of a more labile C pool (Table 1), as was the case with needles. In fact, as ectomycorrhizas are dual organs, composed of plant and fungal partners, one should consider the possibility that both partners contain rapidly and slowly turning over pools of C in future studies. Our observations, based on three samplings performed within 1 month, cannot be used to calculate accurately the half-life of C in roots (Table 1). It will take a longer period of observation

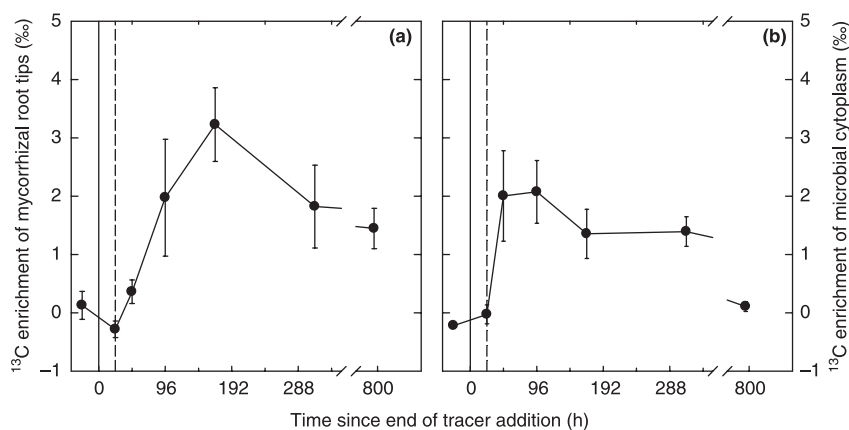


Fig. 3 Progression of $\delta^{13}\text{C}$ in ectomycorrhizal root tips (a) and microbial cytoplasmic carbon (b) after labelling of canopy photosynthate by exposing *Pinus sylvestris* trees to $^{13}\text{CO}_2$. The data shown are deviations from mean values of samples from the unlabelled reference plot. The solid vertical line shows the 93-min labelling event, while the broken line indicates the time of peak labelling of the soluble carbohydrates in the phloem sap at a height of 0.3 m.

and a higher tracer ^{13}C level to determine whether the biomass of ectomycorrhizal tree roots turns over many times a year or whether the rate of turnover is considerably slower, as suggested by, for example, observations in FACE experiments (Matamala *et al.*, 2003; Körner *et al.*, 2005).

Furthermore, the population of ectomycorrhizal fine roots sampled was highly heterogeneous in terms of variability in ^{13}C labelling (Figs 2d, 3a, 5), despite the fact that all the mycorrhizal roots sampled appeared to be young when examined visually under the dissecting microscope. Thus, the population of ectomycorrhizal fine roots appears to be a mix of highly active roots, which are strong sinks for photosynthate, and less active roots. This dichotomy may explain why ^{13}C labelling was more clearly observed earlier in microbial cytoplasm than in the fine roots; in the former, the peak in $\delta^{13}\text{C}$ occurred just 2 d after the labelling (Figs 2e, 3b). It should also be noted that this is shortly before the peak in ^{13}C in the soil CO_2 efflux (Fig. 2f). Thus, we speculate that a small fraction of the population of ectomycorrhizal fine roots is exceptionally active in transferring C to the extramatrical mycorrhizal mycelium and other root-associated microbes.

As found in roots, the increase above the reference in the $\delta^{13}\text{C}$ of the microbial cytoplasm was small, at the most 2‰. However, if the ectomycorrhizal component of this C is a highly labelled fraction (being the interface between plant roots and soil) and accounts for one-third of the total microbial cytoplasmic C, as suggested by the decrease after tree-girdling of another Scots pine forest in this area (Högberg & Högberg, 2002), then the extramatrical mycelium should be labelled at *c.* 6‰ (above the reference). The estimated half-life for C in the microbial cytoplasm of 277 h (Table 1), and the fact that the $\delta^{13}\text{C}$ was not elevated above that of the control after 33 d (Figs 2e and 3b), suggest that the microbial cytoplasm C turns over within less than 1 month. This compares with observations of turnover times of roughly 1 month for microbial cytoplasm N from another Scots pine forest in the same area (Högberg *et al.*, 2006).

The soil was labelled with $^{15}\text{NH}_4^+$ to enable assessment of the potential impact of root activity of trees from outside the

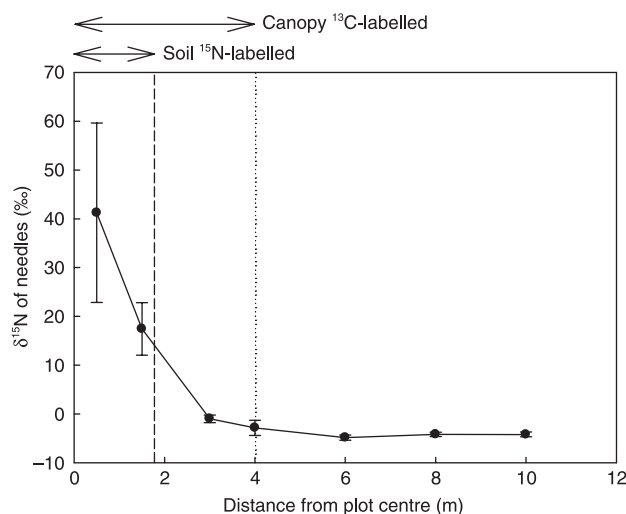


Fig. 4 Abundance of ^{15}N of current needles of Scots pine (*Pinus sylvestris*) in March 2007 after labelling the soil in August 2006 with $^{15}\text{NH}_4^+$. The area labelled with $^{13}\text{CO}_2$ is indicated for comparison; note that all soil and root sampling was performed inside the ^{15}N -labelled area in the chamber plot.

labelled area. Four days later we could not observe any elevation in ^{15}N in trees outside the 50-m² area labelled with ^{13}C , and there was very little evidence of elevated ^{15}N just outside the central ^{15}N -labelled 10 m². This pattern was clearer months later, in spring 2007 (Fig. 4), and indicates that root activity inside the central 10-m² area, for example respiration, was mainly contributed by trees inside the labelled plot. Other recent ^{15}N root uptake studies in nearby coniferous forests, including one Scots pine forest, suggest that most roots of trees in these forests do not extend > 4–5 m from the stems (S. G. Göttlicher, unpublished results). Hence, there should be little influence of roots from trees not labelled with $^{13}\text{CO}_2$ inside the central 10-m² area of the chamber plot.

The simultaneous ^{15}N labelling also provided an opportunity to analyse interactions between the C and N cycles. For example, despite the fact that the ^{15}N injections provided

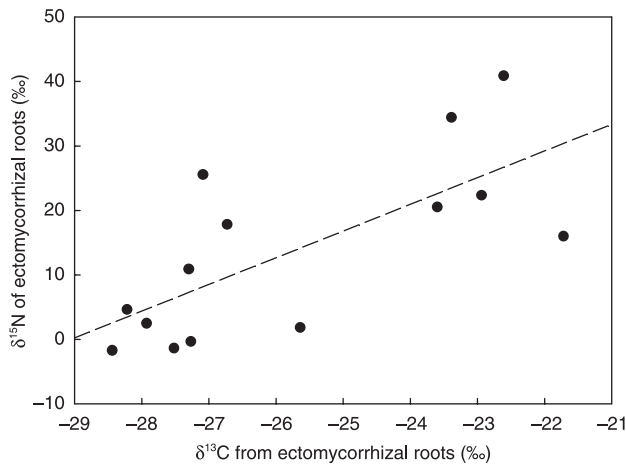


Fig. 5 Labelling of ectomycorrhizal fine tree roots with ^{15}N supplied via the soil as $^{15}\text{NH}_4^+$ vs labelling by ^{13}C supplied via canopy photosynthesis 7 d after $^{13}\text{CO}_2$ labelling.

a very heterogeneous labelling of the soil, there was a weak correlation ($P < 0.05$; data not shown) between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in ectomycorrhizal fine roots in samples collected between 4 and 33 d after labelling. At the sampling 7 d after labelling, the day on which the difference in $\delta^{13}\text{C}$ between ectomycorrhizal fine roots from the labelled plot and the control plot was largest (Figs 2d, 3a), there was a stronger correlation ($r^2 = 0.46$, $P < 0.01$) between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in ectomycorrhizal fine roots (Fig. 5). This correlation lends support to the assumption that the roots, which are the strongest sinks for plant photosynthate, are also the strongest sink for soil N, and further emphasizes the heterogeneity within the population of ectomycorrhizal fine roots. Reasons for this heterogeneity could be variations in fungal symbiont species, age of the mycorrhiza and its position within the root system.

Carbone *et al.* (2007) suggested that ^{14}C labelling should be superior to ^{13}C labelling, in particular because ^{13}C labelling would require longer labelling periods or very high $[\text{CO}_2]$, and hence impose risks of exposure of the system studied to artificial conditions. We demonstrated that a comparatively short (93 min) period of labelling was sufficient to produce traceable quantities of ^{13}C in the same components as those studied by Carbone *et al.* (2007), and also in microbial cytoplasm. It is evident that release of a greater quantity of $^{13}\text{CO}_2$, which should produce the higher $\delta^{13}\text{C}$ needed for precise estimates of turnover of root C, and traceable quantities of ^{13}C in fatty acid biomarkers for different functional groups of soil biota, is possible and would not increase the costs of a study of this type prohibitively. The temporal resolution reported by us (Fig. 2) is unique, as is the size of the ecosystem labelled.

Our previous studies in boreal forests using tree-girdling (Högberg *et al.*, 2001) or variations in the natural abundance of ^{13}C in photosynthate (Ekblad & Högberg, 2001) have

suggested a time lag of a few days between canopy photosynthesis and respiratory activity by the 'autotrophic' soil component, which is strongly supported by the data presented here. Those studies also demonstrated that half or more of the soil activity is driven by recent photosynthate. Taken together, these results suggest a close temporal coupling between a very significant fraction of soil activity and tree canopy photosynthesis in these forests.

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