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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

Mona N. Högberg[1], Maria J. I. Briones[2,3], Sonja G. Keel[1], Daniel B. Metcalfe[1], Catherine Campbell[4], Andrew J. Midwood[5], Barry Thornton[5], Vaughan Hurry[4], Sune Linder[6], Torgny Näsholm[1] and Peter Högberg[1]

[1]Department of Forest Ecology and Management, Swedish University of Agricultural Sciences (SLU), SE-901 83 Umeå, Sweden; [2]Departamento de Ecología y Biología Animal, Universidad de Vigo, 36310 Vigo, Spain; [3]Centre for Ecology and Hydrology, Lancaster Environment Centre, Lancaster LA1 4AP, UK; [4]Department of Plant Physiology and Umeå Plant Science Centre, University of Umeå, SE-901 87 Umeå, Sweden; [5]Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen AB15 8QH, UK; [6]Southern Swedish Forest Research Centre, SLU, PO Box 49, SE-230 53 Alnarp, Sweden

Author for correspondence
Mona N. Högberg
Tel: +46 90 786 8424
Email: mona.hogberg@sek.slu.se

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Summary

- The flux of carbon from tree photosynthesis through roots to ectomycorrhizal (ECM) fungi and other soil organisms is assumed to vary with season and with edaphic factors such as nitrogen availability, but these effects have not been quantified directly in the field.
- To address this deficiency, we conducted high temporal-resolution tracing of ^{13}C from canopy photosynthesis to different groups of soil organisms in a young boreal *Pinus sylvestris* forest.
- There was a 500% higher below-ground allocation of plant C in the late (August) season compared with the early season (June). Labelled C was primarily found in fungal fatty acid biomarkers (and rarely in bacterial biomarkers), and in Collembola, but not in Acari and Enchytraeidae. The production of sporocarps of ECM fungi was totally dependent on allocation of recent photosynthate in the late season. There was no short-term (2 wk) effect of additions of N to the soil, but after 1 yr, there was a 60% reduction of below-ground C allocation to soil biota.
- Thus, organisms in forest soils, and their roles in ecosystem functions, appear highly sensitive to plant physiological responses to two major aspects of global change: changes in seasonal weather patterns and N eutrophication.

Introduction

Soil organisms are almost exclusively fuelled by energy derived from plant photosynthesis. Litter from above- and below-ground plant parts feed heterotrophic organisms, chiefly bacteria and fungi, with typical time lags of months to centuries between photosynthesis and the decomposition of the organic matter. An equally large flux of C is, however, allocated below ground by the plants within h to months to sustain an autotrophic soil component consisting of plant roots, their symbiotic mycorrhizal fungi, and a rich flora of other microorganisms dependent on labile C compounds exuded by the roots (Hanson *et al.*, 2000; van Hees *et al.*, 2005; Högberg & Read, 2006). The heterotrophic pathway, particularly the early stages of decomposition of above-ground litter, is the most thoroughly studied, because inputs of above-ground litter can be easily manipulated and their degradation can be readily observed (Berg & McClaugherty, 2003). Processes further down in the soil are more difficult to follow (Wardle *et al.*, 2004). This is especially true for the activity of mycorrhizal roots, which is disrupted if the C supply from the plant canopy is severed by sampling of the soil (Söderström & Read, 1987; Högberg *et al.*, 2001).

Despite the mounting interest in the C cycle, the fate of the plant C distributed through roots to soil organisms has mainly been followed in detail in micro- or mesocosms, or in short-stature ecosystems such as grasslands (Ostle *et al.*, 2000, 2007; Leake *et al.*, 2006; Bahn *et al.*, 2009). An important advance has been the application of C isotopes (Staddon, 2004). For example, small trees have been labelled with the radioactive ^{14}C to study C allocation patterns (Horwath *et al.*, 1994; Carbone *et al.*, 2007) and patches of forest or model systems with trees have been labelled with very low tracer amounts of the stable ^{13}C in studies of the effects of elevated CO_2 concentrations (Lin *et al.*, 1999; Matamala *et al.*, 2003; Körner *et al.*, 2005). Pulse-labelling with ^{14}C enables a much higher temporal resolution than so-called FACE (free-air carbon dioxide enrichment) experiments, in which the degree of isotopic enrichment is relatively small and the ecosystems become slowly labelled with ^{13}C. Indeed, Horwath *et al.* (1994) were able to demonstrate a 250% higher below-ground ^{14}C allocation to roots and the return soil CO_2 efflux in the late as compared with the early season, which is in agreement with other results from studies of the physiology of trees in temperate and boreal climates (Hansen *et al.*, 1997; Waring & Running, 1998; Kagawa *et al.*, 2006). Recently, a few studies have demonstrated the possibility of high-tracer level pulse-labelling with ^{13}C of trees directly in forest field settings (Högberg *et al.*, 2008; Plain *et al.*, 2009). In these studies, single trees (Plain *et al.*, 2009) or a patch of a forest (Högberg *et al.*, 2008) were labelled over a period of a few hours. The latter study demonstrated significant labelling of the cytoplasm of soil microorganisms, but labelling was not high enough to study incorporation of tracer C in phospholipid fatty acids (PLFAs), biomarkers for different groups of microorganisms.

No previous study has been designed to follow *in situ* the rapid translocation of C to different groups of soil organisms via the autotrophic pathway in a forest ecosystem. We therefore lack a detailed picture of how a large amount of photosynthetically fixed C, an amount several times larger than all anthropogenic C emissions (Schimel, 1995), is distributed among groups of soil organisms and how this varies seasonally and in response to the ongoing N eutrophication (Vitousek *et al.*, 1997; Aber *et al.*, 1998) of forests. This paucity of knowledge limits our understanding of the future roles of these organisms in overall ecosystem functioning. With regard to climate change, much attention has recently been paid to potential direct effects on soil biota of higher temperatures and changes in soil moisture, but less attention has been shown to possible indirect effects on soil biota of climate-related changes in plant below-ground C allocation (Högberg & Read, 2006). For example, the production season of sporocarps of ectomycorrhizal (ECM) (Gange *et al.*, 2007) fungi in Britain became longer during the period 1950–2005, which was primarily attributed to direct effects of changes in temperature and moisture. However, changes in the seasonality of C supply from the tree hosts to the fungi could also be involved. Moreover, N eutrophication is expected to lead to a reduction in tree below-ground C allocation (Waring & Running, 1998; Mäkelä *et al.*, 2008). Indeed, several studies have reported decreases in ECM fungal sporocarp production and mycelium in response to N additions (Wallenda & Kottke, 1998; Nilsson & Wallander, 2003), and in a tree-girdling experiment the autotrophic soil respiratory component appeared twice as high in nonfertilized as compared with N-fertilized spruce forest (Olsson *et al.*, 2005), but the implied reductions in tree below-ground C allocation have not been quantified directly in the field. Hence, we do not know how important this effect may be.

Here, we report results of a large-scale ^{13}C tracer pulse-chase experiment in a boreal 14-yr-old *Pinus sylvestris* forest ecosystem, which allowed a highly resolved analysis of the C flux from trees to different groups of soil organisms. We addressed two major questions: first, how large is the difference in below-ground C allocation between the early and late seasons; and second, how much does added N affect the below-ground allocation of C?

Materials and Methods

Forest stand and site

The forest is a naturally regenerated (i.e. using the seed rain from seed trees left after most large trees were felled) boreal *Pinus sylvestris* L. forest in northern Sweden (64°09′N, 19°05′E) (Högberg *et al.*, 2008). Trees were *c.* 14 yr old in 2007. Each labelled 50 m^2 plot (n = 8) contained 62 ± 9 trees (mean ± 1 SE, mean tree height 2.5 ± 0.2 m; the taller trees were 3–5 m). There is a sparse understorey composed of dwarf shrubs of *Calluna vulgaris* L. and *Vaccinium vitis-idaea* L., and a ground layer of *Cladonia* spp. lichens. The soil is a podzol, with an organic mor layer of 2–3 cm thickness. This layer has a C : N ratio of 33 ± 1 and a pH(H_2O) of 4.5 ± 0.1 (Högberg *et al.*, 2008). The texture of the mineral soil is fine sand.

Chambers for ecosystem $^{13}CO_2$ labelling

We used two closed chambers of the same design as the one tested in a pilot study in 2006 at the same site (Högberg *et al.*, 2008). The chamber was octagonal and covered a ground surface area of 50 m^2. We labelled eight 50 m^2 plots twice (once in 2007 and again in 2008; Table 1). Four of the eight plots were fertilized with N 2 wk before ^{13}C labelling in 2007. Fertilized (N) plots, control (C) plots and unlabelled reference (K) plots were located in groups (Fig. 1). In 2007 we used 4 m tall chambers, but with taller trees in 2008 the chambers were extended to 5 m. The

Table 1 The experimental plots, their nitrogen treatments, and dates of $^{13}CO_2$-labelling

	Nitrogen added (kg ha^{-1})		Date of ^{13}C labelling	
Plot	2007	2008	2007	2008
K1	–	–	–	–
C1	–	–	11 June	4 August
N1	100	100	11 June	4 August
K2	–	–	–	–
C2	–	–	19 June	15 August
N2	100	100	19 June	15 August
K3	–	–	–	–
C3	–	–	21 August	12 August
N3	100	100	21 August	12 August
K4	–	–	–	–
C4	–	–	23 August	6 August
N4	100	100	23 August	6 August

K plots served as reference plots providing natural abundance data on ^{13}C.

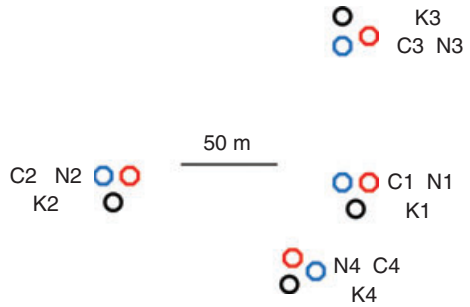

Fig. 1 Layout of the pulse-labelling experiment at Rosinedalsheden, northern Sweden. Treatments were as follows: black/K, control (unlabelled i.e. natural abundance reference); blue/C, ^{13}C-labelled control; red/N, ^{13}C-labelled N-addition plots. Plots 1 and 2 were labelled in early-season 2007, and plots 3 and 4 were labelled in late-season 2007. All plots were relabelled in late-season 2008 (cf. Table 1).

temperature inside the chambers was controlled and set to track the ambient air temperature outside (cf. Högberg *et al.*, 2008). We released 25 l of 99 atom% $^{13}CO_2$ into each chamber, which resulted in 21.9 ± 0.5 and 16.5 ± 0.3 atom% ^{13}C (compared with 1.1 atom% ^{13}C in ambient CO_2), and 531 ± 7 and 456 ± 5 ppm CO_2 (compared with *c.* 380 ppm in ambient air) at the start of labelling in 2007 and 2008, respectively. Note that these differences between years are unimportant, because we studied aspects of seasonality in 2007 and effects of N in 2008. The final CO_2 concentration was 317 ± 11 and 275 ± 5 ppm in 2007 and 2008, respectively. The CO_2 concentration inside the chambers was monitored using infrared gas analysers (IRGAs). These are conventionally set to measure $^{12}CO_2$, and were used here to follow the overall draw-down of CO_2 inside the chambers caused by photosynthesis exceeding ecosystem respiration. To obtain the accurate measurements of CO_2 concentration mentioned earlier, and the uptake of $^{13}CO_2$, we took air samples for isotope ratio analysis from inside the chamber directly after the release of $^{13}CO_2$, in the middle of the labelling period, and immediately before its end. We always labelled the two chambers in parallel (Table 1) with a time lag of *c.* 0.5 h between the start of the first and the second. Labelling periods varied between 1.5 and 3.5 h. The time of labelling was adjusted so that the amounts of ^{13}C taken up across plots were similar. In 2007, the ecosystem ^{13}C uptake, calculated as the difference between the initial and final contents of $^{13}CO_2$ in the chamber air of control plots, was 8.5 ± 0.8 g ^{13}C in June and 10.3 ± 0.5 g ^{13}C in August. In 2008, the calculated ecosystem ^{13}C uptake was 6.9 ± 0.7 g in control plots and 7.0 ± 0.4 g in N-fertilized plots. None of these two differences were statistically significant (one-way ANOVA, $P > 0.05$). The soil temperature at 5 cm depth at the time of $^{13}CO_2$ labelling was 9.1°C in June 2007, 11.5°C in August 2007, and 11.6°C in August 2008.

Nitrogen treatment

Nitrogen was added by broadcasting pellets of $Ca(NO_3)_2$ at a dose of 100 kg N ha^{-1} 2 wk before $^{13}CO_2$ labelling of the N plots in 2007 and then again in June 2008 (i.e. before the $^{13}CO_2$ labelling in August 2008; Table 1). A dose of 200 kg N ha^{-1} within 2 yr is equivalent to the highest N deposition rates in Europe, but here it served the purpose of indicating the sensitivity of the system to N additions rather than mimicking the effects of a more realistic increase in N supply, which is < 5 kg N ha^{-1} yr^{-1} in this area.

Sampling and analyses

Labelled C was traced in the soil respiratory efflux, in microbial cytoplasm, through stable isotope probing (Neufeld *et al.*, 2007) of signature PLFAs for fungi and bacteria, in fruit bodies (sporocarps) of fungi and in soil animals (Collembola, Enchytraeids and Acari). Soil respiratory efflux was sampled using cylindrical head-spaces (n = 3 per plot). Gas samples were analysed on an isotope ratio mass spectrometer (IRMS; ANCA TG system, 20-20 analyser, Europa Scientific Ltd, Crewe, UK). For analyses of microbial cytoplasm and PLFAs, samples of the organic mor-layer were taken (0.15-m-diameter corer, n = 4 per plot). Roots were carefully sorted out. The microbial cytoplasm in root-free soil was released using the chloroform fumigation-extraction method (Vance *et al.*, 1987; Högberg *et al.*, 2008) within hours after sampling. The ^{13}C in the extracts was analysed by IRMS after wet oxidation (Högberg *et al.*, 2008). PLFAs were extracted from freeze-dried soils and the δ^{13}C signatures of 35 PLFAs were determined (Paterson *et al.*, 2007). For studies of soil fauna, soil samples were

Table 2 Number of samples analysed by isotope ratio mass spectrometry

	No. of samples analysed
Soil CO_2 efflux	6750
Microbial cytoplasm	1056
Soil animals	492
Phospholipid fatty acids	480
Fungal sporocarps	206
Total	8984

Note that analyses of phospholipid fatty acids (PLFAs) in one sample yield the $\delta^{13}C$ in 35 individual fatty acids.

taken with a 15-cm-diameter core before the ^{13}C labelling in August and every second day during the first week thereafter, then after 2 wk and after a month. Soil mesofauna were extracted, using a standard dry method with Tullgren funnels for Collembola and Acari, and a modified wet funnel method (O'Connor, 1955) for Enchytraeidae. In 2007 we collected all fungal sporocarps in the plots; the fruiting season was August–September. The $\delta^{13}C$ of freeze-dried fungal sporocarps and samples of soil fauna were analysed by IRMS (isotope ratio mass spectrometer model 20-20, interfaced to an element analyser ANCA NT system module Europa Scientific, Ltd. Crewe, UK, and (Delta^{plus} Finnigan MAT, Bremen, Germany, interfaced to an element analyser CHN1110, Carlo Erba, Milan, Italy). A record of the number of samples analysed is given in Table 2.

PLFA data

Standard nomenclature is used to describe PLFAs (Tunlid & White, 1992). Fatty acids are described by the total number of C atoms:number of double bonds, followed by the position of the double bond from the methyl end of the molecule. The prefixes 'a' and 'i' refer to anteiso- and iso-branching. We analysed 35 PLFAs, of which 12 were considered to be of bacterial origin (i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7, i17:0, a17:0, cy17:0, 17:0, 18:1ω7, and cy19:0) and two were used as indicators of fungi (18:2ω6,9 and 18:1ω9) (Frostegård & Bååth, 1996). Of the latter two, 18:2ω6,9 is probably the better marker for ECM fungi, since it showed the greatest reduction after tree-girdling (Högberg *et al.*, 2007b), and the ratio of ECM to total fungal sequences correlated significantly with the ratio of 18:2ω6,9 to total fungal PLFA (Yarwood *et al.*, 2009).

Isotopic data

We report most data in ‰, using the δ notation to report deviations from the natural abundance as defined by the Vienna Peedee belemnite (V-PDB) standard:

$$\delta^{13}C(‰) = (R_{sample} - R_{standard}/R_{standard}) \times 1000, \quad \text{Eqn 1}$$

where $R = {}^{13}C/{}^{12}C$. The $\delta^{13}C$ of microbial cytoplasmic C (MiC) was calculated as follows:

$$\delta^{13}C_{MiC} = (\delta^{13}C_{fum} \times C_{fum} - \delta^{13}C_{nonfum} \times C_{nonfum})/(C_{fum} - C_{nonfum}), \quad \text{Eqn 2}$$

where fum and nonfum are extracts from chloroform-fumigated and nonfumigated soil samples, respectively. The data on ^{13}C in soil respiration (in mg m^{-2} h^{-1}), in excess of natural abundance of ^{13}C in the nonlabelled reference (K) plots, are shown in Figs 2(a) and 3(a). In these calculations we multiply the flux of C in CO_2 from the soil in the labelled plots by the atom% excess ^{13}C (calculated as the excess in relation to the atom% ^{13}C in reference plots). ^{13}C in microbial cytoplasm (mg m^{-2}), in excess of natural abundance of ^{13}C in the reference plots, is shown in Fig. 3(b), and isotopic data on a fungal biomarker (PLFA) extracted from extramatrical mycelial cell membranes in soil, expressed in ‰ in excess of natural abundance in the reference plots ($\delta^{13}C$ in C-plots minus $\delta^{13}C$ in K-plots), are given in Fig. 3(c).

The soil respiratory efflux of ^{13}C

In the pilot study (Högberg *et al.*, 2008), we found that a small quantity of labelled C was returned to the atmosphere as $^{13}CO_2$ starting immediately after the removal of the chamber. Since this flux of ^{13}C occurred well before labelled photosynthates (as monitored through sampling of sugars in the phloem) reached the soil, we speculated that this rapid return flux was abiotic. This was confirmed, using high temporal-resolution monitoring of the flux from root-free soil collars, and collars with active tree roots in plots C1 and N1 (Table 1) during the labelling in June 2007 (Subke *et al.*, 2009). Hence, we do not report soil respiratory data for the time interval before the flux of labelled C from tree photosynthesis reaches the below-ground system (Högberg *et al.*, 2008); the first data we show, apart from prelabelling data, are from the day after labelling.

Isotopic mixing-model analysis and calculation of turnover of labelled C

For calculations of the percentage contribution of ectomycorrhizal C (ECM-C) to total soil microbial cytoplasm C (MiC) we used an isotope mixing-model analysis:

$$\text{Percent ECM-C} = 100 \times ({}^{13}C_{labelMiC} - {}^{13}C_{nonlabelMiC})/({}^{13}C_{labelECMsporoc} - {}^{13}C_{nonlabelMiC}), \quad \text{Eqn 3}$$

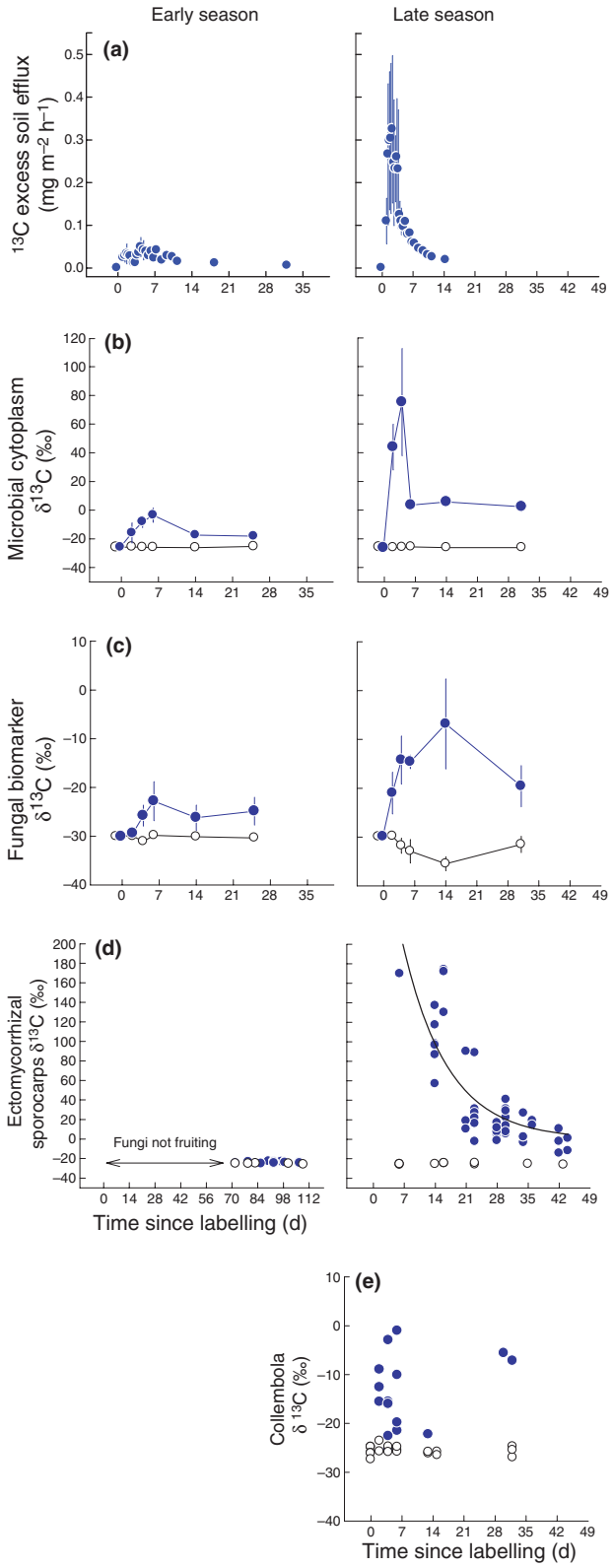

Fig. 2 Seasonal variation in tree below-ground allocation of labelled C, ^{13}C. (a) Soil respiratory efflux. (b) Soil microbial cytoplasm. Amounts of microbial cytoplasm C were 1.9 ± 0.1 and 2.1 ± 0.2 (mg C g^{-1} organic matter) (mean ± 1 SE, n = 24) in June and August, respectively. A two-way ANOVA (α = 0.05, n = 48) on ranks showed no difference in microbial cytoplasm C between months (P = 0.291) or between treatments (P = 0.668). (c) Fungal fatty acid biomarker phospholipid fatty acid (PLFA) 18:2ω6,9. (d) Ectomycorrhizal fungal sporocarps (n = 1). (e) Collembola (springtails) (n = 1). Data (mean ± 1 SE) are from the control plots labelled with ^{13}C in June or in August 2007 and from the corresponding ^{13}C natural abundance plots. Excess ^{13}C in soil respiratory efflux denotes the amount of ^{13}C in excess of that in the nonlabelled ^{13}C natural abundance (K) plots (cf. Table 1). Closed circles, $^{13}CO_2$-labelled; open circles $^{13}CO_2$-nonlabelled.

where $^{13}C_{labelMiC}$ is the δ^{13}C of labelled microbial cytoplasm at peak labelling (i.e. after 4 d), $^{13}C_{nonlabelMiC}$ is the δ^{13}C of nonlabelled microbial cytoplasm at peak labelling, and $^{13}C_{labelECMsporoc}$ is the δ^{13}C of ECM fungal sporocarps. For calculations of the turnover of labelled C, we used a simple decay function:

$$\delta^{13}C_t = \delta^{13}C_{t0}e^{\lambda t} \qquad \text{Eqn 4}$$

where t is the time (in d) after peak labelling, t_0, and λ is the decay constant.

Results and Discussion

Differences in below-ground C allocation between seasons and a detailed analysis of the C flux to soil organisms

In 2007, the seasonality in tree below-ground C allocation to soil biota was quantified. Two pairs of plots were labelled in mid-June (early summer) and another two pairs in mid-August (late summer) (Fig. 1, Table 1). As in the previous pilot study (Högberg *et al.*, 2008), the transfer of labelled C from the canopies of trees was rapid, with a time lag of 2–4 d between photosynthetic C fixation and peak soil respiratory activity involving labelled C (Fig. 2a; see also Fig. 3a). There were no short-term (2 wk) effects of the N additions on tree below-ground C allocation. In the following analysis of seasonality, we therefore focus on data from the control plots.

Very clearly, the amounts of C allocated to below-ground biota were, as predicted, based on previous studies on tree physiology (Horwath *et al.*, 1994; Hansen *et al.*, 1997; Waring & Running, 1998; Kagawa *et al.*, 2006), greater in August than in June. The amounts of labelled C found in August were 660, 450 and 410% higher, respectively, in soil respiratory efflux, microbial cytoplasm and the fungal fatty acid biomarker 18:2ω6,9 (Fig. 2). The latter is considered to be the best PLFA marker for ECM fungi (Högberg *et al.*, 2007b; Yarwood *et al.*, 2009). Labelled C was also found in the other fungal biomarker 18:1ω9, but only in one-sixth of the bacterial biomarkers (i15:0 and 16:1ω7c, indicative of Gram-positive and Gram-negative bacteria, respectively).

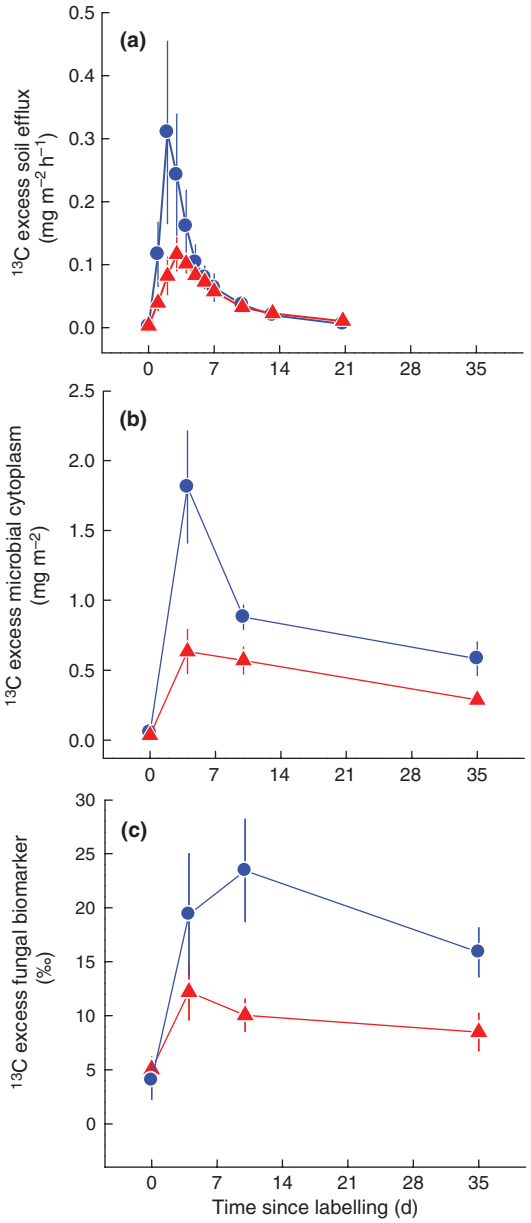

Fig. 3 Effects of added N on tree below-ground allocation of labelled C, ^{13}C. (a) Soil respiratory efflux. (b) Soil microbial cytoplasm. The microbial cytoplasm C amounts were 2.9 ± 0.2 and 2.5 ± 0.2 mg C g^{-1} organic matter (mean ± 1 SE, n = 16) in control and N-fertilized plots, respectively, and did not differ between treatments (Student's t-test, ω = 0.05, P = 0.089). (c) The fungal fatty acid biomarker phospholipid fatty acid (PLFA) 18:2ω6,9. Data (mean ± 1 SE) are from four control and four N-fertilized plots labelled in August 2008 and report values of ^{13}C in excess of those from nonlabelled ^{13}C natural abundance (K) plots (cf. Table 1). Circles, $^{13}CO_2$-labelled C plots; triangles, $^{13}CO_2$-labelled N plots.

Our figure of a 500% higher allocation below ground in the late season compares with a 250% higher allocation in late-season temperate poplar trees in the study by Horwath *et al.* (1994). This difference may reflect a real difference between our boreal pine system and their temperate poplar system, but also possibly a decrease in leaf area attributed to drought stress in the late season in the poplar system (Horwath *et al.*, 1994).

Among the 134 identified ECM sporocarps found in all plots, *Cortinarius semisanguineus* (Fr.) Gill., other *Cortinarius* sp., and *Lactarius rufus* (Scop.) Fr. comprised 87 (65%), 20 (15%) and 23 (17%), respectively. When the plots had been labelled in June, very little tracer ^{13}C was found in ECM sporocarps produced during the peak period of fruiting in September, whereas there was a rapid and massive transfer of ^{13}C to sporocarps in plots labelled in August (Fig. 2d). This demonstrates that production of sporocarps of these fungi is directly dependent on continuous C inputs (Wessels, 1993), contradicting the assumption that sporocarps are produced from C in primordia laid down early in the summer, and that their emergence is a matter of hydraulic expansion during wetter conditions in late summer and autumn. In fact, C is needed during the production of sporocarps for expansion of cell walls and to maintain high concentrations of sugars acting as osmotica causing influx of water and maintenance of turgor pressure (Wessels, 1993). The mean residence time (MRT) of the C in the pool, from which the C supplied to sporocarps was drawn during August–September, was estimated (using the equation of the regression line in Fig. 2d, $y = 385.4 \times e^{0.098t}$) to be 10.2 d ($R^2$ = 0.7, $P < 0.0001$). A few previous studies have used C isotopes to estimate the ecosystem age (time since this C was fixed through photosynthesis) of the C in sporocarps of ECM fungi in forests (Hobbie *et al.*, 2002; Steinmann *et al.*, 2004). Hobbie *et al.* (2002) used the so-called 'bomb-^{14}C' to estimate the ecosystem age of this C to 0–2 yr, while Steinmann *et al.* (2004), found that the ^{13}C labelling of sporocarps of ECM fungi was similar to that of the recent leaf C pool in the first season of their FACE experiment. The higher resolution obtained here suggests that the C used for production of these sporocarps is from tree below-ground allocation in the late season, which is corroborated by the observation that virtually no sporocarps of ECM fungi were found in August in plots in which the pine trees were girdled in June in a previous experiment (Högberg *et al.*, 2001).

We used the data on δ^{13}C in sporocarps of ECM fungi and in microbial cytoplasm to calculate (see Eqn 3) the fractional contribution of extramatrical ECM hyphae to total microbial cytoplasm C. This analysis is, of course, based on the assumption that sporocarps are produced from C derived from the cytoplasm of the mycelium of ECM fungi. Sporocarps of ECM fungi were not present on days 4 and 5 after labelling in August, which means that it is uncertain on which day $^{13}C_{labelECMsporoc}$ peaked. We used the equation of the regression line (see above) in Fig. 2(d) to estimate the mean values for days 4 (the day $^{13}C_{labelMiC}$ peaked), 5 and 6. Our estimate of the percentage ECM-C

varied from 35.2 (day 4) to 42.1 (day 6). Thus, we conclude that the ECM mycelium contributes 39 ± 1% (mean ± 1 SE) to total soil microbial biomass C in August–September. This is higher than the 32% previously estimated for this type of forest in a study employing tree-girdling as a means to stop the flow of C to the ECM soil mycelium (Högberg *et al.*, 2001; Högberg & Högberg, 2002). Tree-girdling should cause accelerated mortality of this mycelium. Hence, the estimate obtained here is probably more realistic, which highlights the importance of mycorrhizal fungi in the soil (Heinemeyer *et al.*, 2007) and of the tree below-ground C pathway.

The size of trees has hindered direct detailed study of the below-ground flux of C from photosynthesis to soil animals in a forest ecosystem. Here, the Collembola (springtails of families Podoruidea and Entomobryoidea) became labelled within days in August (Fig. 2e). We therefore suggest that they are grazers on live mycorrhizal fungal mycelium rather than detritivores feeding on dead organic matter. The rapid labelling of Collembola suggests that they turn fungal C over rapidly, as was also found in a study of temperate grassland (Johnson *et al.*, 2005). The rapid labelling also suggests that Collembola selectively graze highly active mycelium, that is, the mycorrhizal mycelium which is the strongest sink for recent photosynthate. In animals of two other groups, Enchytraeidae (here chiefly *Cognettia sphagnetorum* (Vejdovsky)) and Acari (here Oribatei mites, especially *Carabodes* sp.), there was no labelled C, which is in accordance with the current understanding of the diets of these species (Salminen & Haimi, 2001; Ostle *et al.*, 2007; Siira-Pietikäinen *et al.*, 2008). Our results differ from those obtained in a FACE experiment in temperate deciduous forest, in which many groups of soil animals were labelled but in which the labelling period was much longer (Pollierer *et al.*, 2007). Obviously, the longer the labelling period and the time elapsed after labelling, the greater the numbers of individuals and animal groups that are likely to be labelled.

Effects of N addition on below-ground allocation to soil biota

In June 2008, we added another 100 kg N ha^{-1} to the four plots fertilized in 2007. Then, in mid-August, we once again relabelled all eight plots labelled in 2007 (Table 1). Tracing this second pulse of ^{13}C, we found a large reduction in tree below-ground C allocation in response to the N treatment (Fig. 3); the labelling of the soil respiratory efflux, microbial cytoplasm and the prime ECM fungal biomarker 18:2ω6,9 were reduced by 62, 65 and 48%, respectively.

As explained earlier, a reduction was expected according to plant allocation theory (Hansen *et al.*, 1997; Waring & Running, 1998; Hermans *et al.*, 2006). There are numerous reports of lower production of sporocarps of ECM fungi in plots treated with N (see review by Wallenda & Kottke, 1998), but the direct effect on the tree below-ground C flux to groups of soil biota in response to N has never been quantified. The reduction in the biomass of soil fungi, especially of ECM fungi, is likely to have important consequences for ecosystem functions attributed to mycorrhizal symbiosis (Smith & Read, 2008). For example, loss of mycorrhizal mycelium may contribute to N loss from forests in areas with high rates of N deposition (Dise & Wright, 1995; Aber *et al.*, 1998). Indeed, in the pilot study of an unfertilized plot at this site, the ECM root tips, which were most highly labelled by ^{13}C from photosynthesis, were also the strongest sinks for $^{15}NH_4^+$ added to the soil (Högberg *et al.*, 2008). Moreover, previous studies of soil N turnover in this area have shown a high immobilization of added $^{15}NH_4^+$ in systems with a high ratio of fungi to bacteria (Högberg *et al.*, 2006), and a strong negative correlation between gross N mineralization and the ratio of fungi to bacteria (Högberg *et al.*, 2007a). The latter study included a natural soil N supply gradient, as well as a long-term N-addition experiment, in which a reduction in fungal biomass occurred in the N-addition treatments. Thus, effects of N supply on tree below-ground C allocation, such as the reduction upon N loading demonstrated here, are very likely to feed back into the N cycle.

Concluding remarks

We have shown here, directly in the field and at the scale of a forest ecosystem, how C from tree photosynthesis is transferred through roots within a few days and then rapidly distributed through the mycorrhizal fungal mycelium and up the soil food web. The below-ground flux of C varied several-fold seasonally and was greatly reduced if the ecosystem was supplied with large amounts of N. This demonstrates how a substantial fraction of soil biota is directly dependent on physiological processes in the tree canopy, and suggests that life in the soil is sensitive to plant physiological responses to aspects of global change, such as changes in seasonal weather patterns, and N eutrophication.

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