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Mycorrhizal hyphal turnover as a dominant process for carbon input into soil organic matter

Douglas L. Godbold[1,9], Marcel R. Hoosbeek[2], Martin Lukac[1], M. Francesca Cotrufo[3], Ivan A. Janssens[4], Reinhart Ceulemans[4], Andrea Polle[5], Eef J. Velthorst[2], Giuseppe Scarascia-Mugnozza[6], Paolo De Angelis[6], Franco Miglietta[7] & Alessandro Peressotti[8]

[1]*School of Agricultural and Forest Sciences, University of Wales, LL57 2UW, Bangor, Gwynedd, UK.* [2]*Laboratory of Soil Science and Geology, Department of Environmental Sciences, Wageningen University, P.O. Box 37, 6700AA, Wageningen, The Netherlands.* [3]*Dipartimento di Scienze Ambientali, Seconda Università degli Studi di Napoli, Via Vivaldi 43, I-80110, Caserta, Italy.* [4]*Department of Biology, University of Antwerpen (UA), Universiteitsplein 1, B-2610, Wilrijk, Belgium.* [5]*Institut für Forstbotanik, Universität Göttingen, Büsgenweg 2, 37077, Göttingen, Germany.* [6]*Di.S.A.F.Ri., Università degli Studi della Tuscia, Via S. Camillo De Lellis, I-01100, Viterbo, Italy.* [7]*Insitute of Biometeorology, IBIMET-CNR, P.le delle Cascine 18, I-50144, Firenze, Italy.* [8]*Dipartimento Produzione Vegetale eTechnologie Ambientali, Università di Udine 208, Via delle Scienze, 33100, Udine, Italy.* [9]*Corresponding author**

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Abstract

The atmospheric concentration of CO_2 is predicted to reach double current levels by 2075. Detritus from aboveground and belowground plant parts constitutes the primary source of C for soil organic matter (SOM), and accumulation of SOM in forests may provide a significant mechanism to mitigate increasing atmospheric CO_2 concentrations. In a poplar (three species) plantation exposed to ambient (380 ppm) and elevated (580 ppm) atmospheric CO_2 concentrations using a Free Air Carbon Dioxide Enrichment (FACE) system, the relative importance of leaf litter decomposition, fine root and fungal turnover for C incorporation into SOM was investigated. A technique using cores of soil in which a C_4 crop has been grown ($\delta^{13}C$ −18.1‰) inserted into the plantation and detritus from C_3 trees ($\delta^{13}C$ −27 to −30‰) was used to distinguish between old (native soil) and new (tree derived) soil C. In-growth cores using a fine mesh (39 μm) to prevent in-growth of roots, but allow in-growth of fungal hyphae were used to assess contribution of fine roots and the mycorrhizal external mycelium to soil C during a period of three growing seasons (1999–2001). Across all species and treatments, the mycorrhizal external mycelium was the dominant pathway (62%) through which carbon entered the SOM pool, exceeding the input via leaf litter and fine root turnover. The input via the mycorrhizal external mycelium was not influenced by elevated CO_2, but elevated atmospheric CO_2 enhanced soil C inputs via fine root turnover. The turnover of the mycorrhizal external mycelium may be a fundamental mechanism for the transfer of root-derived C to SOM.

Introduction

Over 75% of the carbon (C) in terrestrial ecosystems is stored in forests, with more than half of this C in soil organic matter (SOM) (Schlesinger, 1997). Soil organic matter has the potential to sequester the largest amount of C for the longest period of time (Schlesinger, 1997; Del Galdo et al., 2003), and is a key component if changes in land management are to mitigate the current rise in atmospheric carbon dioxide concentration.

* FAX No: +44-1248-382459.
E-mail: d.l.godbold@bangor.ac.uk

Detritus from aboveground and belowground plant parts constitutes the primary source of C for SOM (Vogt et al., 1986). Belowground inputs of biomass are considerable, and biomass inputs to soil from fine root turnover are on average 30–40% of aboveground litter inputs (Vogt et al., 1986; Godbold et al., 2003). Most of the C input in plant detritus is rapidly respired by the soil microbial biomass and only recalcitrant compounds are eventually stored as SOM (Aber et al., 1990). Although mycorrhizal fungal symbionts are now known to be a large biomass pool (Fogel and Hunt, 1983; Wallander et al., 2001), the turnover of mycorrhizal fungal symbionts as a potential contributor to SOM formation has largely been neglected in studies of SOM formation. In early work, Fogel (1980) suggested that in a Douglas fir ecosystem mycorrhizae accounted for 50% of the throughput of biomass to the soil. This estimate was based on the biomass of mycorrhizal root tips. However, mycorrhizal fungi use an extensive hyphal mycelium to explore the soil and acquire nutrients. Mineral nutrient acquisition is mediated by mycorrhizae in the majority of plant species. Estimation of the biomass of the extramatrical mycelium of ectomycorrhizae is difficult, especially as only recently have methods been developed to distinguish between hyphae from ectomycorrhizal and saprotrophic fungi in forest soils (Nilsson and Wallander, 2003). In a Douglas fir forest, the total hyphal biomass (mycorrhizal and saprotrophic) in the soil was estimated to be ca. 700 g m^{-2} (Fogel and Hunt, 1983). In a previous estimate these authors calculated the ectomycorrhizal hyphal biomass to be 660 g m^{-2}, a soil biomass pool which exceeded the biomass of fine roots (Fogel and Hunt, 1979). In more recent work, using sand filled mesh bags inserted into the soil in a pure Norway spruce forest and a mixed oak–spruce forest, biomass of the ectomycorrhizal external mycelium was estimated at 59 and 42 g m^{-2} respectively (Wallander et al., 2001). Total ectomycorhizal biomass (external mycelium and hyphal mantles) was estimated to be 480 and 580 g m^{-2} respectively for the pure Norway spruce forest and a mixed oak–spruce forest. In a girdling experiment, 50% of soil respiration was shown to originate from current photosynthate (Högberg et al., 2001). In this girdling experiment, soil microbial respiration was rapidly reduced by up to 56%, of which 41% was due to the loss of ectomycorrhizal mycelium, suggesting that C in extramatrical mycelium and associated bacteria form a carbon pool with a fast turnover rate (Högberg and Högberg, 2002). Based on an observational study, the turnover of arbuscular mycorrhizal (AM) hyphae is indeed assumed to be rapid, with a lifespan of only 5–7 days (Friese and Allen, 1991). These fast turnover rates have recently been supported by another study using CO_2 depleted in ^{14}C and accelerated mass spectrometry (Staddon et al., 2003). These authors (Staddon et al., 2003; Fitter et al., 2004) suggested that turnover of arbuscular hyphae in this system was only 5–6 days. However Leake et al. (2004) have suggested that Staddon et al. (2003) did not take into account that much of the C allocated to hyphae is used in respiration and only ca. one third is used in biomass production, and thus turnover time may be longer than 30 days. There are no estimates of ectomycorrhizal hyphal turnover and moreover, the extramatrical mycelium of ectomycorrhizae will be composed of more than one type of hyphae (Agerer, 2001), which may have different lifespans. Indeed the rhizomorphs, cord-like fungal structures thicker than mycelium, of expanding ectomycorrhizal fronts have been observed to live for several months (Coutts and Nicoll, 1990). However, finer foraging hyphae may have a considerably shorter lifespan. Thus, the large biomass and high turnover rate of mycorrhizal hyphae make them a potentially large source of soil C.

The atmospheric concentration of CO_2 is predicted to reach double current levels by 2075. Elevated atmospheric CO_2 has been shown to increase rates of photosynthesis and increase productivity (Ceulemans et al., 1999), and in most cases elevated atmospheric CO_2 increases aboveground biomass. However, an increase in belowground biomass greater than the increase in aboveground biomass is often found (Norby et al., 2002; Lukac et al., 2003). To investigate the effects of elevated atmospheric CO_2 on whole ecosystems, Free Air Carbon Dioxide (CO_2) Enrichment (FACE) systems have been developed (Hendrey et al., 1999; Miglietta et al., 2001). FACE systems are almost unanimously considered to be the best systems to expose ecosystems to elevated atmospheric

CO_2 concentrations with minimal alteration of the ambient environment (Miglietta et al., 2001). In the EUROFACE system, pure CO_2 is released at high velocity into the atmosphere through a very large number of small gas jets. Such FACE systems allow estimation of C accumulation in forest soils over a number of years (Loya et al., 2003; Hoosbeek et al., 2004). To increase the applicability of our system to natural forest ecosystems, where poplar and aspen are major components of boreal forests, we used three species of poplar with contrasting ecological and physiological characteristics, and exposed the trees from the outset to elevated CO_2, thus avoiding an unrealistic stepwise change in CO_2 concentration.

Changes in soil C content are hard to detect because the soil C pool is usually very large compared to the annual C input, and the soil C turnover time is long compared to the duration of most experiments (Hungate et al., 1996). Furthermore, the usually high spatial variability in soil C content tends to blur effects of C sequestration. The natural abundance of ^{13}C has been used in a number of studies to estimate changes in soil C stores (Balesdent et al., 1988; Del Galdo et al., 2003; Binkley et al., 2004). The ^{13}C signature in SOM reflects the natural abundance of ^{13}C in plant materials growing on the soils. In plant/soil systems where the ^{13}C signature of the input materials differs to that of the native SOM, the natural abundance of ^{13}C can be used to quantify the input of new organic matter (Balesdent et al., 1988). Commonly, these studies grow C_3 plants ($\delta^{13}C$ ca. $-28‰$) on soils with organic matter derived from C_4 plants ($\delta^{13}C$ ca. $-12‰$). Using this technique, Del Galdo et al. (2003) could show that afforestation of former arable land increased the total amount of soil C by 23 and 6% at 0–10 and 10–30 cm soil depth respectively. In contrast Binkley et al. (2004) showed that the new C entering the system from the C_3 eucalyptus was balanced by loss of the older C_4 soil carbon.

In FACE systems, the CO_2 source used to elevate atmospheric CO_2 is commonly derived from fossil fuel and is thus depleted in ^{13}C (^{13}C ca. -40 to $-50‰$) compared to ambient CO_2 (^{13}C $-8‰$) (Schlesinger and Lichter, 2001), and this can be used to trace C movement through soil pools. However, this does not allow a comparison between ambient and elevated CO_2 plots. To overcome this problem, Hoosbeek et al. (2004) used cores of C_4 soils inserted into the native C_3 soil to measure changes in new soil and old C under ambient and elevated CO_2 (FACE). Total soil C contents increased under control and FACE, respectively, by 12 and 3%, i.e. 484 and 107 g C m^{-2}, while 704 and 926 g C $m^{-2}$of new carbon was sequestered under control and FACE during the experiment. It was concluded that FACE increased the loss of old soil C and simultaneously increased new soil C.

The results reported in the work presented here were obtained in a high density poplar forest in central Italy. Using cores of soil in which a C_4 crop has been grown ($\delta^{13}C$ $-18.1‰$) inserted into the plantation and C_3 trees ($\delta^{13}C$ -27 to $-30‰$) we were able to distinguish between old (native soil) and new (tree derived) soil C. Ingrowth cores using a fine mesh to prevent in-growth of roots, but allow in-growth of fungal hyphae, were used to assess contribution of roots and mycorrhizal external mycelium to soil C during a period of three growing seasons.

Material and methods

Site description and plantation lay-out

The EuroFACE experimental facility is located in central Italy near Viterbo (42° 37′04″N, 11° 80′87″E, alt.150 m) on a former agricultural field. Within the 9 ha plantation, three control plots were left under natural conditions, while in the remaining three plots an elevated CO_2 treatment (550 ppm) was provided using the FACE technique. Average concentrations ($\pm$SD) of CO_2 were measured at 544 ppm ($\pm$48), 532 ppm ($\pm$83) and 554 ppm ($\pm$95) during the first (1999), second (2000) and third (2001) growing seasons in FACE plots. The CO_2 concentration was measured at 1 min intervals, and was within 20% of the target value 89, 72 and 65% of the time for the first, second and third years. A detailed description of the FACE installation and the performance of the system is given by Miglietta et al. (2001). Each circular plot (22 m diameter) was divided into six segments, and two segments each planted with trees of a single *Populus* genotype. The genotypes utilised were as follows: *P. alba* L. (genotype

2AS11), *P. nigra* L. (Jean Pourtet) and *P. x euramericana* Dode (Guinier) (*P. deltoides* Bart. ex Marsh. x *P. nigra* L., I-214). Further information on genotype properties is detailed in Calfapietra et al. (2001).

The soil at the sites is classified as a Pachic Xerumbrept (Hoosbeek et al., 2004). The soil has a loamy consistency, and a pH between 4.8 and 5.5. All the plots except one FACE plot (plot 5) had a C content of 1.1–1.2%. Plot 5 had a slightly lower C content of 0.7% C. All plots had an N content of 0.1%. A full description of the soils is given in Hoosbeek et al. (2004).

The whole of the plantation was drip irrigated at a rate of 6–10 mm of water per day during the growing season, starting approximately at the beginning of April until the beginning of November (Calfapietra et al., 2001). The amount of water applied increased from spring to summer, and from the first growing season to the third growing season, in order to match transpiration. Irrigation was important especially at the height of the summer in order to avoid water stress caused by high temperatures and windy conditions. We did not find any evidence of drip irrigation affecting the spatial distribution of roots.

Leaf litter production and decomposition

Leaf litter production

During the second and the third growing season (years 2000 and 2001) litter production was monitored in control and FACE plots of the plantation using leaf litter traps. Traps consisted of plastic baskets (0.13 m^2) placed on the ground in each segment either under one tree or between four adjacent trees. Three replicated traps were placed in each segment. Litter fall was collected twice a month during the entire growing season, and litter derived from the three replicated traps per segment pooled together (Cotrufo et al., 2005). After sampling, litter was dried in an oven at 80 °C for 48 h, and dry weight recorded. The C concentration of leaf litter was determined on litter fall in the year 2000. Litter produced in the months of October and November was pooled by segment and three sub-samples were ground to a fine powder, dried in an oven at 70 °C and analysed independently in an elemental analyser Carlo Erba NA 1500 (Carlo Erba Strumentazione, Milan, Italy).

Field decomposition experiment

Litter decomposition was studied as described in Cotrufo et al. (2005) using the litterbag technique with bags made of a PVC coated fibreglass net, 2 mm mesh size. A known amount of air-dried litter (4 g) was enclosed in each bag (20 cm^2) together with a plastic label for later identification. Litter sub-samples were dried in an oven (70 °C) for correction of dry weight. For the purpose of this study, the experiment was designed in order to assess the decay rates of leaf litter generated and incubated in FACE (F) CO_2 plots as compared to those of litter generated and incubated in control (C) rings. Thus two litter types were generated for each poplar clone, and they were: CC: litter produced in control plots and incubated in control plots; FF: litter produced in FACE plots and incubated in FACE plots. On 21/01/2001, litterbags were laid within the litter layer. At 2 month intervals until 28/09/2001, four replicate bags per plot were retrieved from the field and brought to the laboratory, where remaining litter was dried in an oven at 70 °C and mass loss determined as percentage of original weight. Dry litter from individual bags was then milled and analysed for C in an elemental analyser (Carlo Erba NA1500).

From this study, decay rates by species and treatment were obtained by fitting the mass loss curves with a singular exponential decay model (*mass remaining* (%) $= A + (100 - A)e^{(-kt)}$; where A is the asymptotic decay value, k is the decay constant and t the time) using Origin *version 6* (OriginLab, Maine, USA). For the duration of this study, leaf litter C input to soil was calculated by multiplying the cumulative leaf litter C production by the percentage asymptotic value obtained for the same litter (Cotrufo et al., 2005).

Root necromass

Annual root necromass production was determined as the difference between annual fine root production in in-growth cores and standing biomass. Standing root biomass was sampled using an 8 cm diameter corer to a depth of 40 cm. In-growth cores 40 cm deep and 4 cm in diameter were used to estimate fine root production using a modification of the in-growth-coring method

(Lukac and Godbold, 2001). Cores were wrapped in 2 mm mesh and care was taken to compact the soil to the original bulk density of undisturbed soil. Samples were taken in November 1999, March, July and November 2000, and March, May July, September and November 2001. Each *Populus* species had five replicate cores per plot, three cores were taken from one segment and two from the other, and alternated between sample dates. The values for each plot were pooled to give one value per species per plot, thus each plot was a replicate. The in-growth cores were harvested 2.5–4 months after insertion into the soil. All roots were removed from the soil, washed, dried and weighed. Root turnover was determined as a ratio between annual production and maximum standing crop (Dahlman and Kucera, 1965; Gill and Jackson, 2000).

Measurement of mycorrhizal fungal colonisation

To estimate colonisation by AM fungi, two samples of fine roots were collected from the depth of 0–20 cm at randomly chosen locations within the sampling range of each segment within each plot. Samples were taken in November 1999, March, July and November 2000, and March, May July, September and November 2001. Root colonisation by AM fungi was then estimated according to a modified version of a protocol described by McGonigle et al. (1990). In order to measure ectomycorrhizal fungal (EM) colonisation of root tips, three fine root samples per *Populus* species per plot were collected, on the same sampling dates as above. After collection, the roots were carefully shaken free of soil particles and placed into sealed vials containing moist cotton and kept at 4 °C until analysis. One distinct ectomycorrhizal morphotype was responsible for over 90% of the root tips colonised for all *Populus* species. RAPD examination of DNA extracted from the EM hyphae using a modified protocol of Doyle and Doyle (1987) confirmed that only one EM fungal species colonised roots of all three *Populus* species under both FACE and control treatments. A comparison with DNA from a fruiting bodies collected within experimental plots identified this EM species as *Laccaria laccata*.

^{13}C abundance in plant and fungal materials

The ^{13}C abundance versus the Pee Dee Belemnite was measured in leaves, coarse and fine roots and fungal fruiting bodies. The materials were air-dried and milled and analysed using a Carlo Erba EA 1108 (Carlo Erba Strumentazione, Milan, Italy) CHN analyser coupled to a continuous flow measurement mass spectrometer Finnigan Delta Plus (TermoQuest Italia, Milan, Italy). The statistics programme SYSTAT 7.0 (Systat, California, USA) was used for a two-way analysis of variance for treatment and species effects on the ^{13}C signature of plant materials.

New soil C and the input of hyphal and root C.

The CO_2 gas used for fumigation had a $\delta^{13}C$ of $-6‰$ vs. the Pee Dee Belemnite standard and could therefore not be used as an isotopic signal. However, this allowed us to use the same mixing model in control and FACE treatments, since the $\delta^{13}C$ signature was the same in FACE and in control. To estimate the fraction of new soil carbon, the C_3/C_4 stable isotope method was utilised. The soil of the plantation was previously used for wheat cultivation and had an average $\delta^{13}C$ of $-24.7‰$. The C_4 soil used in the in-growth cores was taken from Udine (north eastern Italy) and had been under continuous corn production for at least 45 years. This soil resembled the average characteristics of the soil at the plantation, including its nutrient status, but had an average $\delta^{13}C$ value $-18.1‰$. The C content of the soil was 0.9% and the N content 0.1%, and was thus not significantly different to the soil of the surrounding plots. Five root and hyphal in-growth cores per species per plot were used to estimate the C input of these structures. The root in-growth cores of 4 cm diameter and 40 cm length were inserted flush with the soil surface. One series was covered with a 2 mm mesh that allowed fine roots and mycorrhizal hyphae to grow in. The other series was covered with a 39 μm mesh which prohibited *Populus* roots growing in to the core, but allows penetration by fungal hyphae. The 39 μm mesh is sufficiently large to allow penetration of both arbuscular and ectomycorrhizal hyphae (Agerer, 1987–1996; Friese and Allen, 1991). The ^{13}C signature of ectomycorrhizal fruiting bodies has been shown to be the same as that of the external

mycelium (Wallander et al., 2001). Using measured fungal $\delta^{13}C$ signature of *L. laccata* fruiting bodies collected at the plots, new soil C originating from mycorrhizal external mycelium was determined using the simple mixing model shown in Equation (1). At the end of the incubation period (from June 1999 until November 2001), two sub-samples of each core at 10 and 30 cm depth were taken, mixed and kept at 4 °C until analysis. The hyphal in-growth cores were checked for roots, and any cores with signs of root in-growth were discarded. ^{13}C abundance in the soil, expressed as $\delta^{13}C$ (‰), was determined after conversion of total C to CO_2, purified by CuO and Ag, in a VG/SIRA 9 Mass Spectrometer (VG Instruments, Manchester, UK). The mean value of the replicate cores were used to calculate the fraction of new C during the incubation period (*f*) using a mixing model (Balesdent et al., 1988),

$$f = (\delta^{13}C_{\text{incubated soil}} - \delta^{13}C_{\text{initial C4 soil}}) / (\delta^{13}C_{\text{poplar}} - \delta^{13}C_{\text{initial C4 soil}}). \quad (1)$$

First, the amount of new C originating from fungal biomass was established from fungi only cores. This new C was then subtracted from the total C measured in root plus mycorrhiza cores and the amount of new C originating from roots was then calculated. Measured $\delta^{13}C$ values were used both for mycorrhizae and roots. The $\delta^{13}C$ signature of the C_4 soil did not change significantly in stored soils. The effect of decomposition of C_4 derived material on the $\delta^{13}C$ signature was estimated using a sensitivity model (Phillips and Gregg, 2001); no significant influence was shown. Total soil carbon was determined by flash combustion in an elemental analyzer (EA 1108) (Van Lagen, 1996). The SPSS 10.1 (SPSS Inc., Chicago, USA) general linear model was used to calculate univariate analysis of variance and to evaluate treatment effects on the levels of new soil C.

Results and discussion

FACE conditions did not significantly influence the $\delta^{13}C$ signature of leaves and coarse roots (Table 1). However, for fine roots a significant treatment ($P < 0.01$) effect was shown, with the $\delta^{13}C$ signature was lower under FACE. The $\delta^{13}C$ signature of mycorrhizal tissue, determined from fruiting bodies of *L. laccata* was −27.6% (SD ±0.6‰), over 2‰ higher than the leaf and root materials.

During the 2-year period that C_4 in-growth cores were in the soil, the $\delta^{13}C$ signature of the soil in the cores decreased by over 3‰ relative to the initial value of −18.1‰ (Table 2). Using the $\delta^{13}C$ signatures of the soil from the cores, and from fine roots and mycorrhizal materials, the C input from root mortality and fungal mycelium was estimated (Table 3). The soil C input via mycorrhizal fine roots was calculated using two different methods. (a) Annual root mortality was determined as the difference between annual fine root production measured with in-growth cores, and seasonal changes in standing biomass measured by repeated auger coring (Lukac et al., 2003). The in-growth cores used a 2 mm mesh that allowed in-growth of roots and mycorrhizal hyphae. (b) Soil C input via mycorrhizal fine roots was also estimated from the change in ^{13}C in the SOM. The C input from mycorrhizal external mycelium was estimated using a series of C_4 in-growth cores with 39 μm mesh which prevented in-growth of *Populus* roots, but allowed penetration by mycorrhizal hyphae.

For all *Populus* species, the values obtained using standing root biomass showed a clear influence of CO_2 enrichment (Table 3). An increase in

Table 1. Mean $\delta^{13}C$ signatures (‰, ±SE, $n = 3$) of leaves, coarse and fine roots of three species of *Populus* grown for 3 years under ambient or elevated CO_2 (550 ppm)

	P. alba		*P. nigra*		*P. x euramericana*	
	Ambient	FACE	Ambient	FACE	Ambient	FACE
Leaves	−29.7 ± 0.3	−29.6 ± 0.3	−28.8 ± 0.3	−29.0 ± 0.3	−29.6 ± 0.2	−30.0 ± 0.2
Fine roots (< 2 mm)	−29.5 ± 0.1	−29.9 ± 0.1	−29.6 ± 0.1	−30.2 ± 0.2	−29.5 ± 0.2	−30.1 ± 0.2
Coarse roots (> 5 mm)	−29.1 ± 0.4	−30.0 ± 0.5	−29.2 ± 0.4	−29.4 ± 0.2	−29.5 ± 0.1	−30.1 ± 0.4

Table 2. Mean $\delta^{13}C$ signature (‰, ±SE, $n=3$) of soil from root exclusion and root in-growth cores of three species of *Populus* grown for 2 years under ambient or elevated CO_2 (550 ppm)

	P. alba Ambient	FACE	*P. nigra* Ambient	FACE	*P. x euramericana* Ambient	FACE
Roots excluded	−21.4 ± 0.1	−21.0 ± 0.2	−21.3 ± 0.1	−21.3 ± 0.2	−21.4 ± 0.3	−21.0 ± 0.4
Roots present	−21.6 ± 0.2	−21.7 ± 0.5	−21.4 ± 0.2	−21.4 ± 0.1	−21.4 ± 0.2	−21.5 ± 0.2

The initial $\delta^{13}C$ signature of the soil in the cores was −18.1‰.

Table 3. Soil C inputs from root litter and the mycorrhizal external mycelium in three species of *Populus* grown under ambient or elevated CO_2 (550 ppm)

C soil fluxes (g C m^{-2})	*P. alba* Ambient	FACE	*P. nigra* Ambient	FACE	*P. x euramericana* Ambient	FACE
C input fine roots (in-growth cores)[a]	104 ± 33	210 ± 14	126 ± 13	274 ± 22	157 ± 26	335 ± 13
C input fine roots (^{13}C)[b]	840 ± 40	566 ± 117	845 ± 104	805 ± 28	832 ± 83	801 ± 27
C input mycorrhizal external mycelium (^{13}C)	1228 ± 40	954 ± 80	1364 ± 36	1311 ± 110	1412 ± 113	1267 ± 132
New soil C (^{13}C)	2068 ± 72	1520 ± 187	2209 ± 85	2116 ± 113	2244 ± 120	2068 ± 130

(Mean ± SE, $n=3$).
The data shown are the cumulative values for the period June 1999 until November 2001. C input from fine roots estimated using standing biomass and turnover rate[a] and the isotopic method[b].

the C input from root mortality seen under elevated atmospheric CO_2 in the in-growth core data is not reflected in the isotopic measurements. In the values obtained from $\delta^{13}C$, no significant differences between the ambient and FACE treatments could be shown. Hoosbeek et al. (2004) using the same C_3/C_4 technique could show an greater accumulation of new soil C under FACE compared to ambient in the second growing season (March–November 2001), but not during the first growing season (March–November 2000). However, the values obtained from measurements of standing root biomass and fine root turnover are lower than results of $\delta^{13}C$ analysis. This difference may be due to an underestimation of the rate of fine root turnover caused by fine root dieback within in-growth cores prior to their extraction from the soil profile (Lukac et al., 2003; Fitter et al., 2004), and in part to isotopic fractionation during decomposition (Balesdent and Mariotti, 1996). Stabile isotopes are unevenly distributed in plant materials, and lignin is depleted by 3–5‰ compared to total organic matter. Assuming lignin is the major plant material remaining in the SOM, depleting the $\delta^{13}C$ signature of the input material by 5‰ would decrease the size of the new soil C components by ca. 30%.

The estimated C input to the soil from the mycorrhizal fungal external mycelium greatly exceeded that of fine roots, and accounted on average for 62% of new soil C in all treatments. No clear effect of elevated CO_2 was seen in the C input via the mycorrhizal fungal external mycelium ($P=0.101$), and subsequently no significant effect of elevated CO_2 was found on total new soil C in any of the species ($P=0.056$). We did not determine the relative amounts of mycorrhizal or saprotrophic fungal hyphae in the cores, or the species of mycorrhizal hyphae in the cores. However, mycorrhizal fungal colonisation of the poplar was over 90% from *L. laccata* (Lukac et al., 2003), and fruiting bodies of this species were used to determine the $\delta^{13}C$ signature of the fungal material. Using in-growth bags filled with silica sand, Wallander et al. (2001) showed that these bags were primarily colonised by ectomycorrhizal hyphae, and not those of saprotrophic species, although in the in-growth cores used in the present work, the higher C content of the soil increases the possibility of saprotrophic fungi being present. Redistribution of soil C by saprotrophic fungi is also known to occur. Frey et al. (2003) demonstrated a considerable transfer of C in exchange for N between a wheat straw and a mineral soil layer in microcosms. In the

microcosms there was a great difference in C and N content between the two soil layers. In contrast, at the EuroFACE site the fungal in-growth cores had the same C and N contents as the surrounding soil of the plots, suggesting that unidirectional transport of C into the cores by saprotrophic fungi is unlikely. We suggest that it is reasonable to assume that C input to the fungal in-growth cores is primarily from mycorrhizal hyphae.

Due to slow decomposition rates of leaves measured at the EuroFACE site (Cotrufo et al., 2005) and the design of in-growth cores which did not permit leaf litter deposition within the core, leaf litter derived C was not included in our isotopic calculation of sources of new soil C. However, soil C input via leaf litter decomposition can be calculated from leaf litterfall and litter decay rates determined by *in situ* litterbag incubations (Cotrufo et al., 2005). During the studied period, the cumulative input of C from leaf litter averaged across species and treatment was 505 g C m^{-2}. If we assume that all litter which does not undergo decomposition on the forest floor (i.e. the fraction corresponding to the asymptotic value of decay curves) eventually enters the soil, the calculated C input to soil from aboveground litter ranged between 359 and 441 g C m^{-2} for all species. It is important to emphasise that with this approach we quantified the maximum C input from leaf litter, and given all the limitations of the litter bag method, we believe this to be an overestimation. However, this value is still less than half the putative input via the mycorrhizal external mycelium, even if isotopic fractionation during decomposition is taken into account. This however does not negate the importance of the organic layer as an important reservoir of C. But our results show that in our system the dominant input into the soil organic pool was via the mycorrhizal external mycelium. The major biomass pool of the leaf litter contributed only a small amount to C input to SOM, primarily because most of the C remained stored in the leaf litter layer during the three years of measurement, and of the C loss during decomposition, most is respired to the atmosphere and only a minor fraction enters the more stable soil C pools. Moreover, even during later stages, when part of the C stored in litter layer will enter the soil C pool, it is not expected to contribute significantly.

In this study, *Populus* roots were colonised with both ectomycorrhizal and AM fungi (Lukac et al., 2003). Among the *Populus* species, differences in the degree of mycorrhizal fungal colonisation and response to elevated CO_2 were found. Ectomycorrhizal and AM fungi both contain relatively recalcitrant compounds, chitin and glomalin respectively, which are specific to fungi. Glomalin has been suggested to contribute strongly to SOM (Treseder and Allen, 2000), and have a residence time in soils of 6–42 years (Rillig et al., 2001). Similarly, chitin contents of ectomycorrhizal hyphae have been estimated at between 5 and 12% and up to 60% of dry weight (Muzzarelli, 1977; Ekblad et al., 1998). Published estimates of ectomycorrhizal external mycelium biomass in forest soils range between 16 and 80 g m^{-2} in spruce forests (Nilsson and Wallander, 2003) and 660 g m^{-2} in Douglas fir stands (Fogel and Hunt, 1979). The new soil C at the EuroFACE site would be accounted for by an assumed hyphal biomass of ca 110 g m^{-2} and a lifespan of 9 days, i.e., a slightly longer lifespan than those measured experimentally for AM hyphae (Friese and Allen, 1991, Staddon et al., 2003). While it is unlikely that all types of hyphae have the same lifespan, this average lifespan we suggest is reasonable based on current knowledge. These results emphasise the importance of high rates of biomass turnover for SOM production. In contrast, turnover rates of leaves are about once per year and those of fine roots about three times per year (Lukac et al., 2003).

Little effect of elevated CO_2 was seen on the soil C input via putative turnover of the mycorrhizal external mycelium. This is in contrast to the effects of elevated CO_2 on ectomycorrhizal fungi shown in pot experiments (Godbold et al., 1997). In a review of the effects of elevated CO_2 on mycorrhizal biomass it was concluded that elevated CO_2 predominately increases or has no effect on mycorrhizal hyphal biomass (Treseder and Allen, 2000). The increase in mycorrhizal hyphal biomass was often associated with a shift in mycorrhizal species assemblage, an effect that was not seen at EuroFACE due to the low species diversity (Lukac et al., 2003). Since responses to elevated CO_2 have been shown to be species-specific (Kubiske and Godbold, 2001), different species of mycorrhizal fungi may still be responsive to elevated atmospheric CO_2 concentrations.

Recently, Fitter et al. (2004) have suggested that the response of AM fungi to elevated CO_2 is dominated by the response of the host plant. An increase in mycorrhizal hyphal biomass and turnover would increase C sequestration in terrestrial ecosystems. Although the results of our experiment were obtained in a fast growing poplar forest, the results are relevant for forest ecosystems in general, where mycorrhizal biomass is a large constituent of the belowground biomass. Additionally, our supposition that these results are applicable to other forest ecosystems is supported by the ratio of aboveground to belowground biomass inputs being similar to coniferous forests (Vogt et al., 1986), and rates of root turnover (Lukac et al., 2003) being similar to those of aspen in boreal forests (Steele et al., 1997). Although the absolute rates of C sequestration in forest ecosystems may differ, the importance of mycorrhizal external mycelium in C input to soils is evident. As mycorrhizae are ubiquitous in all terrestrial ecosystems, the turnover of the mycorrhizal external mycelium may be a fundamental mechanism for the transfer of root-derived C to SOM and warrants further investigation.

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