

Patterns of rhizosphere carbon flux in sugar maple (*Acer saccharum*) and yellow birch (*Betula allegheniensis*) saplings

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Abstract

Despite its importance in the terrestrial C cycle rhizosphere carbon flux (RCF) has rarely been measured for intact root–soil systems. We measured RCF for 8-year-old saplings of sugar maple (*Acer saccharum*) and yellow birch (*Betula allegheniensis*) collected from the Hubbard Brook Experimental Forest (HBEF), NH and transplanted into pots with native soil horizons intact. Five saplings of each species were pulse labeled with $^{13}\text{CO}_2$ at ambient CO_2 concentrations for 4–6 h, and the ^{13}C label was chased through rhizosphere and bulk soil pools in organic and mineral horizons for 7 days. We hypothesized yellow birch roots would supply more labile C to the rhizosphere than sugar maple roots based on the presumed greater C requirements of ectomycorrhizal roots. We observed appearance of the label in rhizosphere soil of both species within the first 24 h, and a striking difference between species in the timing of ^{13}C release to soil. In sugar maple, peak concentration of the label appeared 1 day after labeling and declined over time whereas in birch the label increased in concentration over the 7-day chase period. The sum of root and rhizomicrobial respiration in the pots was 19% and 26% of total soil respiration in sugar maple and yellow birch, respectively. Our estimate of the total amount of RCF released by roots was 6.9–7.1% of assimilated C in sugar maple and 11.2–13.0% of assimilated C in yellow birch. These fluxes extrapolate to 55–57 and 90–104 $\text{g C m}^{-2} \text{yr}^{-1}$ from sugar maple and yellow birch roots, respectively. These results suggest RCF from both arbuscular mycorrhizal and ectomycorrhizal roots represents a substantial flux of C to soil in northern hardwood forests with important implications for soil microbial activity, nutrient availability and C storage.

Keywords: mycorrhizae, pulse labeling, rhizodeposition, root exudation, trees

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Introduction

The flux of recent photoassimilate from trees to soil is one of the least understood and most poorly quantified processes in the terrestrial carbon (C) cycle (Grayston *et al.*, 1996; Cheng, 1999; Kuzyakov & Domanski, 2000). Rhizosphere C flux (RCF) results from the sloughing of cells, and the release of low molecular weight exudates and organic secretions from fine roots and mycorrhizal hyphae to soil. RCF is extremely difficult to quantify because the released C occurs in a narrow zone around roots (Norton *et al.*, 1990; Rygielwicz & Andersen, 1994) and is rapidly consumed by rhizosphere microflora

because of its high quality as a microbial substrate (Leyval & Berthelin, 1993). Estimates of RCF in trees range from <1% to 12% of assimilated C (Grayston *et al.*, 1996), although this wide range of estimates may result as much from the methodologies employed as from actual differences between tree species and forest ecosystems (Meharg, 1994).

Despite uncertainty in the magnitude of RCF, this process is regarded as a potentially responsive component to global change because it is sensitive to elevated CO_2 and temperature (Grayston *et al.*, 1996), and intimately linked to soil microbial activity, organic matter decomposition and nutrient release (Cheng, 1999; Hütsch *et al.*, 2002; Paterson, 2003). Moreover, changes in RCF may result in feedbacks to ecosystem C storage (Hu *et al.*, 1999). Numerous studies suggest RCF

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is likely to be stimulated under elevated CO₂ across a wide range of plant taxa (Norby *et al.*, 1987; Zak *et al.*, 1993; Rouhier *et al.*, 1996; Paterson *et al.*, 1997; Hungate *et al.*, 1999; Pendall *et al.*, 2004). Increases in RCF may limit C storage in soil if microbial breakdown of soil organic matter (SOM) is enhanced through priming effects (Kuzyakov, 2002). However, increases in RCF may also result in greater ecosystem C storage if microbial activity in the rhizosphere increases nutrient availability to plants, and results in greater net C assimilation rates and C storage in plant biomass (Hu *et al.*, 1999).

In northern hardwood forests of the northeastern US, acidic deposition and climate change are influencing the distribution and abundance of many tree species (Iverson & Prasad, 2002; Bailey *et al.*, 2004). Because RCF may differ several-fold within and between plant taxa (Kuzyakov & Domanski, 2000), shifts in species composition could have profound implications for C storage and nutrient availability. Sugar maple (*Acer saccharum*) and yellow birch (*Betula allegheniensis*) are regionally important, coexisting tree species in the northern hardwood forest that differ in the quality and quantity of root exudates released to soil. Birch roots exude a greater diversity of carbohydrates, amino acids and organic acids than maple roots, and nearly three times more C than maple roots (Smith, 1976). Such differences could influence C storage and nutrient dynamics in forests such as the Hubbard Brook Experimental Forest (HBEF) if declining sugar maple are replaced by yellow birch.

Most fine roots in northern hardwood forests are mycorrhizal, and the influence of mycorrhizal colonization on RCF in tree roots has only recently been examined (Norton *et al.*, 1990; Rygielwicz & Andersen, 1994; Rouhier & Read, 1998). The two broad classes of mycorrhizae in forests, ectomycorrhizae (EM) and arbuscular mycorrhizae (AM), exhibit striking differences in morphology, C sink strength and spatial extent of hyphae (Whipps, 1990). Hence, it is likely that differences in mycorrhizal association will influence RCF, microbial activity and C storage. Yellow birch roots are colonized by EM fungi whose extramatrical hyphae decompose slowly because of their high chitin content (Langley & Hungate, 2003). In contrast, sugar maple roots are colonized by AM fungi and may release the glycoprotein glomalin which promotes soil aggregation and retards organic matter decomposition (Wright & Upadhyaya, 1998). Whether differences in mycorrhizal association influence RCF is presently unknown, but may have important implications for C dynamics in northern hardwood forests where EM tree species are predicted to increase in abundance under several climate change scenarios (Iverson & Prasad, 2002).

Most studies of RCF in trees have been conducted with seedlings growing in nonnative soil (Norton *et al.*, 1990; Rygielwicz & Andersen, 1994; Rouhier *et al.*, 1996; Kelting *et al.*, 1998; Dyckmans *et al.*, 2000), and none has considered how tree species with different mycorrhizal associations influence RCF (Jones *et al.*, 2004). Empirically derived estimates of RCF in forests are rare, as most estimates of this flux are calculated by modeling approaches (Luo *et al.*, 2001) or by difference in ecosystem mass balances for C (Fahey *et al.*, 2005). The goals of this study were to compare patterns of RCF from sugar maple and yellow birch saplings, and to use these data to provide estimates of annual RCF for the HBEF.

Materials and methods

In the summer of 1998, five saplings of yellow birch and sugar maple were harvested from HBEF and transplanted into large pots (0.35 m diameter; 0.20 m depth) with native root-soil systems intact. The soils at HBEF are primarily Typic Haplorthods derived from unsorted glacial till, and are acidic (pH = ~ 4). Potted saplings were kept under a dense canopy (<20% of ambient sunlight) at Pleasant View Farm in W. Thornton, NH prior to the pulse labeling. All saplings were watered and weeded regularly. At the time of the labeling, none of the saplings showed visible signs of insect or pathogen damage, and no saplings had intensive root growth around the perimeter of the pots (i.e. the roots were not 'pot-bound'). Sapling heights averaged 1.25 m and sapling diameter averaged 1.3 cm.

Pulse labeling

Short-term ¹³CO₂ pulse labeling of five saplings of sugar maple and yellow birch was carried out in July 2002. All saplings were labeled individually for 4–6 h in a large cuvette (850 L) constructed by fitting a custom-designed, CO₂-impermeable Tedlar bag (Plastic Film Enterprises, Royal Oak, MI, USA) over a rectangular frame (2 m in height; 1 m × 0.5 m at the base) made from 2.5 cm diameter copper pipe. The entire frame was placed over each sapling, and then tightly secured to a plexiglass board (1.1 m × 0.51 m) on the rim of each pot. The plexiglass board was fit around the plant stem by cutting a narrow slice out of the board. We sealed off the cuvette from the soil atmosphere by filling the space around the plant stem with molding clay (Mortite rope caulk). This air-tight seal prevented ¹³CO₂ transport from the cuvette into the soil atmosphere, and ensured that all ¹³C in soil resulted from photoassimilated C released from roots.

A semi-closed circulation system was used to control and monitor the flow of gas through the cuvette. Air circulation into and out of the cuvette was controlled by connecting a vacuum pump to two 1 cm diameter ports on opposite sides of the cuvette (embedded in the Tedlar bag) with tygon tubing. Two large oscillating fans were placed at the base of the plant to mix air in the cuvette during labeling. A small subsample of cuvette air was re-directed to a LI-6200 infrared gas analyzer (LI-COR Inc. Lincoln, NE, USA) to continuously measure the CO₂ concentration in the cuvette. In addition, CO₂ in the cuvette was extracted continuously during the labeling period through rubber septa embedded in the Tedlar bag, and measured with gas chromatography (Schmadzu Scientific Instruments Inc., Columbia, MD, USA). The latter procedure was necessary because the LI-6200 requires a correction factor for measuring ¹³CO₂ (Svejcar *et al.*, 1990).

Ambient CO₂ was scrubbed from the entire system by routing the circulating air through a 2.5 Liter cylinder of soda lime. Once CO₂ concentrations in the chamber dropped to <50 ppm, the soda lime was removed from the air circulation stream. The 99 atom% ¹³CO₂ (Isotech, Miamisburg, OH, USA) was added to the line of circulating air in short pulses until the CO₂ in the cuvette stabilized ~450 ppm. Addition of ¹³CO₂ was carefully controlled with a fine metering valve and monitored with a flow meter. We allowed CO₂ to circulate through the system until the cuvette concentration decreased to 375 ppm because of uptake by photosynthesizing leaves. We then repeated the process by adding ¹³CO₂ to increase the CO₂ concentration to ~450 ppm. Pulsing of CO₂ lasted for 4–6 h. Temperature increases in the cuvette were minimized by blocking direct sunlight with shade cloth which reduced quantum flux by ~40% but did not lower light levels below the saturation point for either species (~500 μmol m⁻² s⁻¹; Beaudet *et al.*, 2000). Temperature, relative humidity, light and CO₂ were monitored inside and outside of the cuvette every 3 min for the duration of the labeling period. Three saplings without leaves (removed just prior to labeling) were exposed to identical conditions to serve as controls.

Chasing the label

Immediately after pulse labeling, saplings were removed from the cuvette and placed in the shade (less than 20% ambient light). At days 1, 4 and 7 after labeling, foliar subsamples were collected with a hole-puncher (0.5 cm diameter) from 25 different leaves per sapling. The amount of leaf area removed represented less than 0.1% of the total leaf area of each sapling. Roots and soil were subsampled by removing three to

four soil cores (2.5 cm diameter) on days 1, 4 and 7 after labeling. The amount of soil removed from each pot ranged from 1.7% to 3.5% of the total soil mass, and there were no significant differences between species in the percentage of soil removed. Soils and fine roots (<1 mm) were separated by horizons (organic and mineral) and soils were separated into two fractions (rhizosphere and bulk soil). Rhizosphere soil was operationally defined as any soil adhering to live fine roots after gentle shaking (Wollum, 1994) and was carefully separated from fine roots using forceps. Bulk soil was defined as all soil remaining in the core after removal of rhizosphere soil. Fine root fragments and root hairs were carefully picked from soil samples using ultra-fine forceps for ~60 min per sample.

Subsamples of rhizosphere and bulk soil were analyzed for microbially respired ¹³CO₂ (labile ¹³C; 10-day aerobic incubation), microbial biomass ¹³C (MB-¹³C; CHCl₃ fumigation-incubation), and SOM-¹³C (combustion at 1200 °C). We did not correct isotope values for carbonates in SOM (Harris *et al.*, 2001) because they are insignificant in soils at HBEF (Bailey & Hornbeck, 1992). CO₂ released from incubating soil was trapped in 10 mL of 0.1 M NaOH and CO₂-C was determined by single end-point titration with 0.1 M HCl following addition of 400 μL of 1 M BaCl₂. To collect the BaCO₃ precipitate for ¹³C analysis, the precipitate was washed with degassed DI, centrifuged at 2200 rpm for 5 min, and the supernatant decanted. This washing procedure was repeated three times and any remaining solution was evaporated in a drying oven at 65 °C for 24 h.

During the 7-day chase period, total soil respiration (TSR) was measured in mid-afternoon each day with a LI-6200 infrared gas analyzer fitted with a LI-COR 6000-09 soil respiration chamber. The isotopic signature of the CO₂ evolved from soil during the 7-day chase period was measured by trapping CO₂ in 0.1 M NaOH traps placed inside closed soil chambers. Base traps were removed every 6–10 h and titrated with 0.1 M HCl. BaCO₃ precipitates from titrated samples were analyzed for ¹³C as described above. Eight days after labeling, all pots were harvested to determine plant tissue biomass (e.g. foliage, branch and stem and roots) and soil mass. All soil and tissue samples were oven-dried at 60 °C prior to weighing and combustion for isotope analysis. Isotopes were measured on a Finnegan isotope ratio mass spectrometer (ANCA-MS) at the Cornell-Boyce Thompson Stable Isotope Laboratory in Ithaca, NY. To ensure complete recovery of ¹³C during combustion of BaCO₃ precipitates (Harris *et al.*, 1997), vanadium pentoxide (V₂O₅) was added as a catalyst, and combustion columns were replaced every 75–100 samples.

Mycorrhizal colonization of fine roots was determined on a subset of the pots using standard methods (Brundrett *et al.*, 1994). Arbuscular mycorrhizal roots of sugar maple were washed, cleared in a 10% KOH solution in a boiling water bath, bleached in hydrogen peroxide, and stained with ink-glycerol-vinegar solution overnight. Roots were de-stained in glycerol-vinegar solution for several days before mounting on slides and scoring for mycorrhizal structures (vesicles, hyphal coils and arbuscules). A total of 100 random intersections per sample were scored under a compound microscope using the gridline intersect method. Ectomycorrhizal yellow birch roots were washed gently, and counted under a dissecting scope using the gridline intersect method. For roots of both species, colonization was calculated as a percentage of the total root length.

Calculations

All $\delta^{13}\text{C}$ values (relative to a Pee Dee Belemnite standard) were converted to atom percent excess ^{13}C ($\mu\text{g } ^{13}\text{C/g}$ tissue or soil) by subtracting natural abundance $\delta^{13}\text{C}$ values measured for control plants and soils. We estimated the amount of $^{13}\text{CO}_2$ assimilated by each plant during the labeling as the difference between the amount of $^{13}\text{CO}_2$ pulsed into the cuvette and the $^{13}\text{CO}_2$ remaining in the cuvette at the end of the labeling period. $^{13}\text{CO}_2$ remaining in the cuvette was estimated by simultaneously measuring CO_2 with a gas chromatograph and the LI-6200, and using the following equation based on a correction factor for the LI-6200 developed by Svejcar *et al.*, (1990):

$$(x + y) - (y + z) = A - B, \quad (1)$$

where x is the $^{13}\text{CO}_2$ concentration in excess of ambient, y is the $^{12}\text{CO}_2$ concentration, $z = (x + 60.9)/3.09$ (based on Svejcar *et al.*, 1990) which is the $^{13}\text{CO}_2$ concentration in excess of ambient as measured by the LI-6200, A is the CO_2 concentration as measured by gas chromatography and B is the CO_2 concentration as measured by the LI-6200. Solving for x (after pulse labeling) the expression can be rewritten as:

$$x = (((A - B) \times 3.09) + 60.9)/2.09. \quad (2)$$

MB- ^{13}C was calculated by multiplying the soil MB times the atom percent excess ^{13}C (hereafter referred to as excess ^{13}C) in CO_2 released from fumigated soil. MB-C was calculated after correcting with a control and using a k -value of 0.41 (Horwath *et al.*, 1996). The pots were not watered during the chase period and the soils were not rewet prior to incubation in order to minimize disturbance to microbial cells. As a result, we calculated MB- ^{13}C for each sampling date by multiplying the

excess ^{13}C from each fumigated sample by the mean MB-C measured within each soil fraction during the chase period. Thus, our calculations assume no change in the size of the soil MB over the 7-day chase period. Pools for plant tissues and soils were calculated by multiplying the excess ^{13}C by the oven-dry mass of each tissue or soil fraction.

Respiratory losses of CO_2 from soil (TSR) were estimated in order to constrain losses of CO_2 from microbially respired rhizodeposits. Respiratory losses over the chase period were estimated by regression, using the relationship between soil temperature and CO_2 flux from soil where: $\text{TSR} = 0.1079 \times \text{soil temperature}^{1.2913}$ ($r^2 = 0.63$; $n = 30$; $P < 0.0001$). There were no significant differences between species in the slopes ($P = 0.39$) or intercepts ($P = 0.43$) of the regression equation, and thus data from both species were included in the model. This relationship allowed us to use frequent measurements of soil temperature (every 6–10 h; 20–25 measurements per pot) to predict TSR throughout the 168 h chase period. Prediction intervals (95%) were calculated around estimated CO_2 values to provide a lower and upper estimate of the TSR from each pot.

Respiratory losses from fine roots (RR) over the entire chase period were calculated two ways. First, we estimated RR based on the relationship between root respiration (RR) and soil temperature reported in Burton *et al.* (2002) for sugar maple ($\text{RR} = -0.152 \times \exp^{0.1 \times \text{soil temp.}}$) and yellow birch ($\text{RR} = -0.056 \times \exp^{0.08 \times \text{soil temp.}}$). Because RR is sensitive to temperature changes, we used frequent measurements of soil temperature to predict RR throughout the 168 h chase period. Total losses from RR over the entire chase period were calculated by multiplying the mean RR for each pot by the fine root biomass in each pot. In the second approach, we calculated RR over the entire chase period based on measurements of CO_2 flux from recently excised fine roots from a subset of the pots. Roots were washed, blotted dry, and incubated in Mason jars for 3–4 h at 22 °C (Kelting *et al.*, 1998). Short-term respiration rates were then multiplied by the fine root biomass in each pot. Values of RR calculated by these two methods were virtually identical ($r^2 = 0.97$; $y = 1.012 \times + 0.004$; $P < 0.001$), and we used the temperature-weighted values in our calculations. Because all pots were kept in deep shade during the chase period, we assumed that any diurnal variation in the relationship between soil temperature and RR was relatively minor (Kuzakov & Cheng, 2004).

The total amount of $^{13}\text{CO}_2$ released by TSR (TSR- ^{13}C) and RR (RR- ^{13}C) was calculated by multiplying the CO_2 lost as TSR and RR by the excess $^{13}\text{CO}_2$ in the base traps from the closed soil chambers and incubations of

excised fine roots. For six pots in which excised roots were not incubated, we used the relationship between $\delta^{13}\text{C}$ in fine root tissue and $\delta^{13}\text{CO}_2$ released from RR where: $\delta^{13}\text{CO}_2$ from RR = $1.2737 \times \delta^{13}\text{C}$ in fine root tissue + 548.17 ($r^2 = 0.96$; $P < 0.0001$). Rhizomicrobial respiration of labeled rhizodeposits (RMR- ^{13}C) was calculated as the difference between TSR- ^{13}C and RR- ^{13}C ; this flux provides an estimate of the amount of ^{13}C released by soil microbes utilizing the C in labeled exudates, sloughed cells, etc.

RCF was estimated in two ways. The minimum RCF (RCF_{min}) was calculated as the maximum ^{13}C recovered in the soil C pool (SOM- ^{13}C) over the 7-day chase period, as a percentage of the total ^{13}C assimilated by each individual plant. This provides the most conservative estimate of RCF because it does not account for any respiratory losses of $^{13}\text{CO}_2$ from microbial consumption of labeled rhizodeposits. RCF_{max} was calculated as the sum of RCF_{min} and the RMR- ^{13}C estimate for the time interval preceding the highest observed measurement of SOM- ^{13}C . For example, in sugar maple the maximum value of SOM- ^{13}C was detected on day 1 (24 h after initiation of the chase period). Thus, the estimated amount of RMR- ^{13}C over the first 24 h was added to the maximum SOM- ^{13}C in sugar maple to calculate RCF_{max}. In yellow birch, the maximum value of SOM- ^{13}C was detected on day 7, and thus RMR- ^{13}C was added to the maximum SOM- ^{13}C to calculate RCF_{max}. Annual estimates of RCF_{min} and RCF_{max} were calculated by assuming that the percentage of net assimilated C lost to soil as RCF in the pots was the same as for trees at the HBEF throughout the growing season, and using an average net C assimilation value from the HBEF of $800 \text{ gC m}^{-2} \text{ yr}^{-1}$ (Fahey *et al.*, 2005).

Statistical analysis

Analysis of variance (ANOVA) was used for a multi-factor split-plot model to repeatedly measure excess ^{13}C in tissue and soil fractions over time. Fixed effects were species, tissue or soil fraction, and day. A

Bonferroni adjustment was applied to the model to account for unwanted comparisons. We used *t*-tests to examine species differences in tissue biomass and respiratory losses from belowground pools. *t*-tests were also used to examine differences in labeling conditions for the two species. In all tests differences were considered significant where $P < 0.05$. All statistics were run with SAS v.9.1 (SAS Institute Inc, 2003, Cary NC, USA).

Results

Plant biomass and labeling conditions

Overall, sugar maple saplings were slightly larger than yellow birch saplings, and these differences were significant for foliage and coarse roots in the organic horizon (Table 1). The higher foliar biomass in sugar maple was offset by the greater net C assimilation rates in birch leaves, and thus there were no species differences in the total amount of $^{13}\text{CO}_2$ assimilated (Table 2). Greater variation in yellow birch than sugar maple for $^{13}\text{CO}_2$ assimilated and net C assimilation rate resulted from a single sapling which had values 59% and 50% greater than the mean, respectively. Differences in coarse root biomass between species were likely influenced by the difficulty of separating roots growing at the organic/mineral interface, and of precisely defining the boundary between the above-ground stem and the woody root at the base of the saplings.

Because the saplings were labeled individually an effort was made to minimize variation in labeling conditions, especially for light, temperature and CO_2 – all of which influence RCF (Grayston *et al.*, 1996; Paterson *et al.*, 1997). Overall, there were no significant differences in environmental conditions in the cuvette during the pulse labeling period (Table 2). Mean temperature in the cuvette ranged from 24 to 34 °C during labeling and closely tracked ambient temperature. The cuvette temperature was on average 4 °C higher than ambient. Although environmental variables

Table 1 Aboveground and belowground dry weight of sugar maple and yellow birch saplings separated by soil horizon (organic and mineral) and root diameter ($n = 5$)

Species (<i>n</i> = 5)	Grams per sapling						Total biomass
	Foliage	Branch and stem	Organic roots		Mineral roots		
			> 1 mm	< 1 mm	> 1 mm	< 1 mm	
Sugar maple	31.4* (1.1)	85.4 (5.2)	37.5* (3.6)	21.8 (4.1)	3.8 (0.8)	18.5 (3.0)	198.5 (9.0)
Yellow birch	24.6 (1.3)	85.5 (8.3)	22.2 (3.1)	14.5 (2.6)	3.0 (0.5)	15.7 (1.5)	165.4 (12.7)

*Significant differences between tree species for each tissue where $P < 0.05$.

Table 2 Cuvette conditions during pulse-labeling

Species (<i>n</i> = 5)	<i>T</i> (°C)	RH (%)	QF (μmol/m ² -s)	¹³ CO ₂ added (mg C)	¹³ CO ₂ assim. (mg C)	¹³ CO ₂ assim. (%)	Net C assim. rate (μmol/m ² -s)
Sugar maple	29.7 (1.7)	81.1 (1.5)	1000 (126)	533 (17)	423.0 (34.6)	75.9 (4.3)	3.80 (0.35)
Yellow birch	27.8 (1.8)	84.2 (1.3)	1018 (159)	577 (53)	447.1 (70.3)	72.8 (5.0)	6.34* (0.84)

Means (and standard errors) are for temperature (*T*), relative humidity (RH), quantum flux (QF), ¹³CO₂ added to the cuvette, estimated ¹³CO₂ assimilated (assim.), and net C assimilated as a percentage of ¹³CO₂ added.

*Significant differences between tree species for each variable where *P* < 0.05.

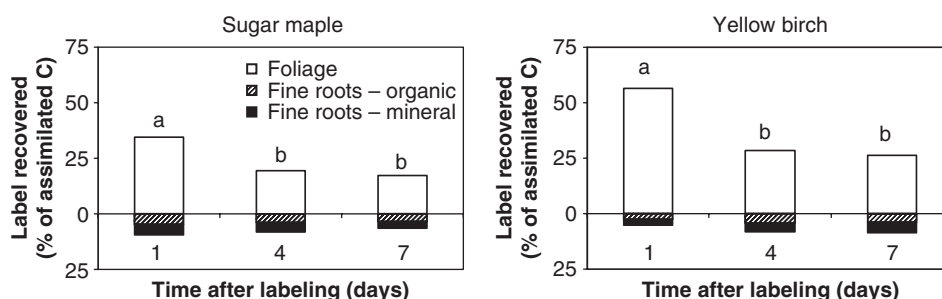


Fig. 1 Recovery of excess ¹³C in foliage and fine roots (organic and mineral horizons) for sugar maple and yellow birch saplings as a percentage of ¹³CO₂ assimilated. Different letters indicate differences between days (within a species) of the sum total of ¹³C recovered in all three fractions.

varied during labeling, none of the variables was significantly different between species, and thus species differences in RCF are unlikely to be artifacts of the labeling process. The percentage of ¹³CO₂ assimilated by plants ranged from 56% to 89% of the total amount added but did not differ significantly between the two species. As expected, average net C assimilation rates were greater in yellow birch (6.3 μmol CO₂/m² s⁻¹) than in sugar maple (3.8 μmol CO₂ m⁻² s⁻¹; Table 2). Higher net C assimilation rates in yellow birch than sugar maple are consistent with several other studies (Amthor *et al.*, 1990; Beaudet *et al.*, 2000; Delagrangue *et al.*, 2004).

Label recovery in plant and soil pools

Recovery of excess ¹³C in foliage and fine root pools differed significantly between the two tree species (Fig. 1). In both species, excess ¹³C recovered in foliar pools decreased over time (*P* < 0.0001) with 32% and 30% decreases in sugar maple and yellow birch between days 1 and 4, respectively. In addition, there was a significant interaction between species and tissue fractions (*P* < 0.0001) resulting from the temporary decreasing recovery of excess ¹³C in sugar maple roots but increasing recovery in yellow birch roots (Fig. 1).

The proportion of excess ¹³C in aboveground and belowground pools also differed between species. In sugar maple, the proportion of the label in the foliar and fine root pools did not change over time. In contrast, the proportion of label in yellow birch foliage dropped from 87% to 55% between days 1 and 7, and the proportion of label in belowground pools increased from 13% to 45% over the same interval. At harvest on day 8, the proportion of excess ¹³C recovered in branch and stem did not differ between species while the proportion of excess ¹³C recovered in coarse roots was nearly twofold greater in sugar maple than in yellow birch (*P* < 0.05; Fig. 2). In contrast, the proportion of excess ¹³C recovered in SOM was nearly twofold greater in yellow birch than in sugar maple (*P* < 0.05; Fig. 2).

Significant differences were also observed between species in the amount of excess MB-¹³C and labile ¹³C pools over the chase period (Fig. 3). For the labile ¹³C pool, a significant species by time interaction was observed (*P* < 0.001) as labile ¹³C in sugar maple soils decreased by an average of 68% from day 1 to day 7, but labile ¹³C in yellow birch soils was not significantly different between sampling dates (Figs 3a and b). Similarly, a species by time interaction was observed for MB-¹³C (*P* < 0.001) although changes in label recovery from day 1 to day 7 were much less dramatic than for

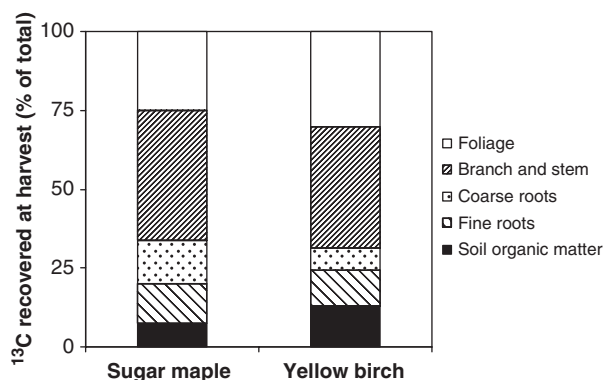


Fig. 2 Percentage of ^{13}C recovered in plant tissue and soil organic matter pools at harvest (day 8) in sugar maple and yellow birch saplings ($n = 5$).

labile ^{13}C (Figs 3c and d). For both species, recovery of labile ^{13}C and MB- ^{13}C was significantly greater in rhizosphere than bulk soil irrespective of sampling date ($P < 0.05$).

A significant species by time interaction ($P = 0.015$) was observed for the percentage of assimilated ^{13}C recovered in soil (Fig. 4). The percentage of labile ^{13}C decreased significantly in sugar maple soils from 1.4% on day 1 to 0.5% on day 7 but did not change over time in yellow birch soils. Similarly the percentage of MB- ^{13}C decreased in sugar maple soils from 2.6% on day 1 to 1.1% on day 7 but also did not change over time in yellow birch soils. The remaining excess ^{13}C recovered in soil as 'other ^{13}C ' (i.e. the difference between SOM- ^{13}C and the sum of labile ^{13}C and MB- ^{13}C at the end of the chase period) showed a different pattern. In sugar maple soils, this pool accounted for 3.4% of total assimilated C and did not differ significantly over the chase period, whereas it increased from 1.3% of total assimilated C on day 1 to 8.7% on day 7 in yellow birch soils.

Recovery of $^{13}\text{CO}_2$ from belowground respiration

We found few significant differences between species in respiratory fluxes of CO_2 and recovery of excess ^{13}C (Table 3). Estimated TSR for both species averaged $\sim 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ over the chase period which is fairly representative of mid-summer soil CO_2 fluxes from yellow birch and sugar maple stands at HBEF (Groffman *et al.*, 2001). RR, calculated as a percentage of TSR, was significantly greater in sugar maple (26%) than yellow birch (19%). These values were remarkably similar with estimates derived from an isotope mixing model (Andrews *et al.*, 1999), in which the percentage of TSR from RR was estimated as 26% in sugar maple and 21% in yellow birch. Recovery of excess ^{13}C in RMR

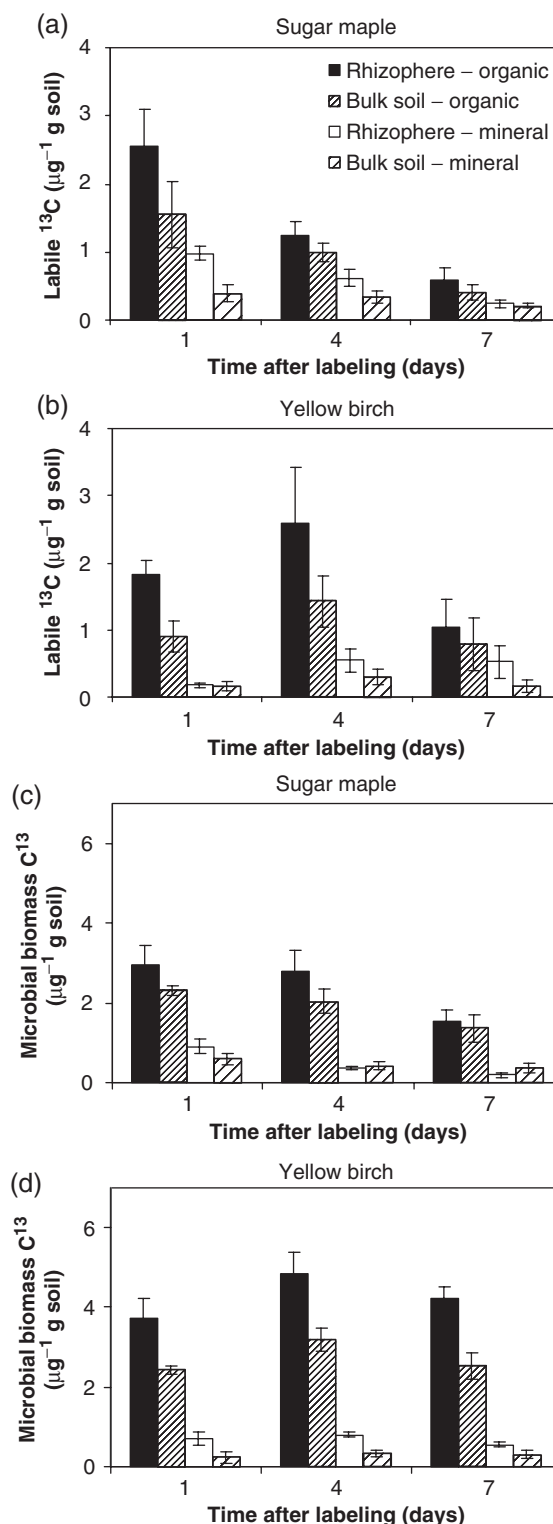


Fig. 3 Recovery of labile- ^{13}C (a and b) and microbial biomass- ^{13}C (c and d) in rhizosphere and bulk soil from organic and mineral horizons of sugar maple (a and c) and yellow birch (b and d) pots ($n = 5$). Error bars represent standard errors of the mean.

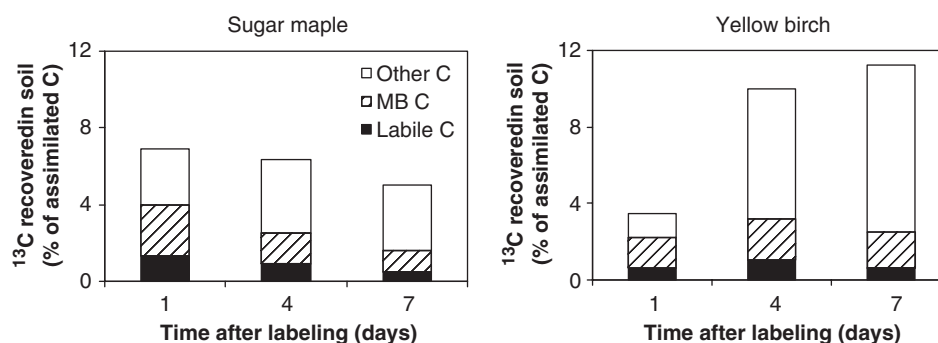


Fig. 4 Distribution of ^{13}C in soil organic matter in sugar maple and yellow birch soils as a percentage of net assimilated C ($n = 5$).

Table 3 Mean (and standard error) for total soil respiration (TSR), percentage of TSR attributed to root respiration (RR), excess ^{13}C released in RR as a percentage of ^{13}C assimilated, and excess ^{13}C released in rhizo-microbial respiration (RMR) as a percentage of ^{13}C assimilated during the 7-day chase period

Species ($n = 5$)	TSR ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	RR/TSR (%)	^{13}C in RR (% assimilated C)	^{13}C in RMR (% assimilated C)
Sugar maple	4.9 (0.2)	26.3* (2.0)	2.0 (0.1)	1.3 (0.3)
Yellow birch	5.3 (0.2)	18.6 (1.2)	1.6 (0.2)	1.7 (0.2)

*Significant differences between tree species for each variable where $P < 0.05$.

Table 4 Estimates of rhizosphere C flux (RCF) in sugar maple and yellow birch as a percentage of $^{13}\text{CO}_2$ assimilated

Species ($n = 5$)	% of assimilated $^{13}\text{CO}_2$			
	RCF _{min} (1)	RMR- $^{13}\text{C}_{\text{total}}$ (2)	RMR- $^{13}\text{C}_{\text{day}}$ (3)	RCF _{max} (1) + (3)
Sugar maple	6.9 (1.0)	1.34 (0.33)	0.19	7.08 (0.95)
Yellow birch	11.2 (2.7)	1.74 (0.19)	1.74 (0.19)	12.97 (2.66)

RCF_{min} is the maximum ^{13}C recovered in SOM during the 7 day chase period, RMR- $^{13}\text{C}_{\text{total}}$ is an estimate of the percentage of ^{13}C lost as rhizomicrobial respiration throughout the chase period, and RMR- $^{13}\text{C}_{\text{day}}$ is the percentage of ^{13}C lost as rhizomicrobial respiration prior to the day of maximum ^{13}C recovery in SOM. RCF_{max} is the sum of RCF_{min} and RMR- $^{13}\text{C}_{\text{day}}$. Values are means (and 1 SE).

(calculated as the difference between excess ^{13}C recovered in TSR and RR) did not differ significantly between species. Average recovery of excess ^{13}C in RMR for both species represented 1.5% of total assimilated C. However, RMR- ^{13}C may represent as much as 2.5% of total assimilated C if an upper estimate of TSR (based on the 95% prediction interval around the regression of TSR and soil temperature) is used in the calculation.

RCFs at HBEF was calculated based on the percentage of assimilated label recovered in SOM in the pots. RCF_{min}, which represents the maximum ^{13}C recovered in SOM- ^{13}C during the chase period, accounted for 6.9% of assimilated C in sugar maple and 11.2% of assimilated C in yellow birch (Table 4;

Fig. 4). RCF_{max}, defined as sum of RCF_{min} and the maximum respiratory loss of rhizodeposits accounted for 7.1% of assimilated C in sugar maple soils and 13.0% of assimilated C in yellow birch soils (Table 4). Using an average net C assimilation value from the HBEF of $800 \text{ gC m}^{-2} \text{ yr}^{-1}$ (Fahey *et al.*, 2005), these fluxes represent about 55 to $104 \text{ gC m}^{-2} \text{ yr}^{-1}$ from sugar maple and yellow birch roots, respectively.

Discussion

Belowground C allocation and RCF

The allocation of recently fixed C to belowground pools differed significantly between sugar maple and yellow

birch. In sugar maple, recovery of excess ^{13}C decreased continuously after the first sampling (i.e. 24 h after labeling) in both foliar and fine root pools, whereas in yellow birch excess ^{13}C decreased in foliage but increased in fine roots over the chase period (Fig. 1). These contrasting patterns indicate that the process of C allocation from new photosynthate to fine roots differs between these two species. In sugar maple the initially proportional allocation of excess ^{13}C to foliage and roots suggests that the label was distributed rapidly throughout the plant system and respiratory losses were mostly responsible for decreases in excess ^{13}C over time. In contrast, allocation to belowground tissues in yellow birch was initially low and increased over the following days. The continuing belowground C allocation in yellow birch may have been driven by the strong sink for recently assimilated C provided by its EM (Norton *et al.*, 1990; Rygiewicz & Andersen, 1994; Rouhier & Read, 1998).

Patterns of C release from fine roots to soil appear to be related to species differences in C allocation to fine roots and mycorrhizae. In both sugar maple and yellow birch, recovery of excess ^{13}C in soil followed the same pattern as recovery in fine roots; hence, although belowground C allocation patterns differed between species, recovery of excess ^{13}C in roots and in rhizosphere SOM was strongly correlated in both species ($r = 0.75$) suggesting that the sink strength of fine roots is an important determinant of RCF (Whipps, 1990). Root sink strength is strongly influenced by the sink strength of the mycorrhizal fungi, and C flux through hyphae influences C flux to soil for both EM (Norton *et al.*, 1990; Rouhier & Read, 1998) and AM (Wright *et al.*, 1998; Johnson *et al.*, 2002). However, it is impossible in practice to exclude severed mycorrhizal hyphae from the rhizosphere pool, and hyphal contamination probably contributed to the high correlation between fine root and rhizosphere soil ^{13}C . In sugar maple soils recovery of ^{13}C was greatest in the 16:1 ω 5 lipid (R. Phillips, T. Balser, unpublished data), a lipid unique to AM (Olsson *et al.*, 1995; Gavito & Olsson, 2003), and in yellow birch soils recovery of ^{13}C was greatest in the fungal lipid 18:2 ω 6,9, a lipid common in ectomycorrhizal fungi (Olsson, 1999). Of course, the issue of hyphal contamination is largely a matter of definition as C flux to extramatrical hyphae could legitimately be considered part of RCF. In this experiment, many severed hyphae were likely respired by soil microbes (during the 10-day incubation) and recovered as labile- ^{13}C , whereas hyphae not respired were likely recovered as MB- ^{13}C (Norton *et al.*, 1990).

Rapid mobilization of C into belowground soil pools was observed in sugar maple but less so in yellow birch. Rapid C flux from roots to soil in the first four

hours after labeling has been observed in many species including sugar maple (Topa *et al.*, 2004), and this C is believed to be mostly water soluble exudates (Kuz'yakov, 2002). Sugar maple and yellow birch roots release a wide range of simple sugars and organic acids (Smith, 1976) which eventually become C substrates for rhizosphere microbes. One day after labeling, 60% and 80% of the SOM- ^{13}C was either respired by microbes (labile ^{13}C) or incorporated into microbial cells (MB- ^{13}C) in sugar maple and yellow birch, respectively. A possible explanation for the lower percentage in sugar maple is that the AM released to soil more recalcitrant forms of C such as glomalin (Wright & Upadhyaya, 1998), although the temporal dynamics of glomalin production and release are poorly understood.

Differences in the recovery of labile ^{13}C in sugar maple and yellow birch suggest species differences in the timing of RCF. In sugar maple, recovery of labile ^{13}C in soil peaked 1 day after labeling and decreased over the chase period. This pattern may have resulted from a single pulse of exudates in the first 24 h after labeling, and the subsequent respiration of these substrates by rhizosphere microbes (Kuz'yakov, 2002). In contrast recently fixed C in yellow birch appears to have been released continuously to the rhizosphere throughout the chase period. However, some of the label recovered as labile ^{13}C may have resulted from severed mycorrhizal hyphal tissues in rhizosphere soil, so that differences in the timing of RCF between species might be attributed, in part, to differences in the timing of C allocation to extramatrical hyphae. The substantial recovery of labile ^{13}C in the bulk soil pools (data not shown) suggests that hyphae were probably important conduits for C flux to areas beyond the rhizosphere.

We fit the MB- ^{13}C data to a negative exponential model to estimate turnover time of recently assimilated C in the MB pools (Butler *et al.*, 2004). The average turnover time of ^{13}C in MB in sugar maple soils were 2.4 and 4.4 days in rhizosphere and bulk soil pools, respectively. Faster turnover in the rhizosphere is consistent with fast growing microbial populations in the rhizosphere (Grayston *et al.*, 1996). The rapid turnover is also consistent with the high turnover rate of arbuscular mycorrhizal hyphae which live on average for 5–6 days (Staddon *et al.*, 2003). Estimating turnover times for yellow birch was more problematic because incorporation of the label into MB-C peaked 4 days after labeling; however, a negative exponential function for days 4–7 resulted in estimated turnover time of ^{13}C in MB of 6.4 and 4.8 days in rhizosphere and bulk soil, respectively. A longer turnover time in yellow birch soils would be consistent with the slower turnover of ectomycorrhizal hyphae (Soderstrom & Read, 1987; Norton *et al.*, 1990; Rygiewicz & Andersen, 1994).

Respiratory losses

The recovery of excess ^{13}C from root (RR) and RMR did not differ significantly between species despite the differences in RCF and temporal patterns of root C allocation between species. However, our sampling design did not allow continuous calculation of respiratory losses throughout the chase period. In a similar pulse chase study, Kuzyakov (2002) found that the peak respiratory losses of labeled C from roots and rhizosphere microbes were offset in time even though the total amounts recovered from RR and RMR were similar at the end of the chase period. In that experiment, nearly all labeled CO_2 recovered in the first 4 days after labeling came from RR which peaked 2–3 days after labeling. In contrast, recovery of labeled CO_2 from RMR did not represent a significant contribution to respiratory losses until 5–7 days after labeling. Thus, the lack of differences between species in recovery of RR- ^{13}C and RMR- ^{13}C in our experiment may have resulted from the short length of the chase period. Recovery of RMR- ^{13}C would likely have been greater in yellow birch than sugar maple soils if the chase period was extended for several more days.

The contribution of RR to TSR was estimated for each pot. Respiration from sugar maple roots accounted for a significantly higher proportion of TSR (26%) than for yellow birch (19%; Table 2), and the values are consistent with other values for sugar maple (35%; Edwards & Norby, 1999). The values obtained in the pulse labeling experiment are also similar to estimates of RR/TSR for mature trees at the HBEF after correcting for fine root density; that is the average contribution of RR to TSR in the pots (25%) was lower than in mature forests at HBEF (37%; Fahey *et al.*, 2005) but average fine root density in the pots (348 g m^{-2}) also was lower than the average fine root density in the mature forests at HBEF (522 g m^{-2}) by a similar proportion (Fahey *et al.*, 2005). These comparisons support the contention that root and soil dynamics in the pots were qualitatively similar to those in the mature forest at the HBEF.

RCF in forests

Estimates of RCF in mature forests are rare despite the importance of RCF in ecosystem C budgets (Grayston *et al.*, 1996; Hanson *et al.*, 2000; Kuzyakov & Domanski, 2000). Nearly all studies of RCF in trees have been conducted with seedlings growing in nonnative soil (Norton *et al.*, 1990; Rygielwicz & Andersen, 1994; Rouhier *et al.*, 1996; Kelting *et al.*, 1998; Dyckmans *et al.*, 2000), and their reliability for scaling up to the forest level is questionable because of physiological differences between trees and seedlings, and differences in soils and

their microbial communities. Estimates of RCF in mature forests can be derived by mass balance, but this approach is limited to ecosystems with detailed, long-term measurements of both aboveground and belowground C dynamics. Moreover, the accuracy of mass balance estimates is limited by the low precision of certain flux measurements (e.g. RR; Fahey *et al.*, in press).

In one of the few studies of its kind, Smith (1976) trained roots from mature sugar maple and yellow birch trees at HBEF to grow into a sterilized medium in order to characterize the quantity and chemical quality of root exudates. He reported that yellow birch roots release nearly three times more organic substrates (carbohydrates, amino acids and low molecular weight organic acids) to soil than sugar maple roots, and calculated annual flux of C from root exudation at HBEF as $0.4\text{ g C m}^{-2}\text{ yr}^{-1}$. However, Smith's approach would underestimate RCF because (1) only root exudates and not other components of rhizodeposition were measured, (2) the medium was probably not entirely sterile (sterility was assessed by microbial growth on agar), and thus some exudates may have been respired and (3) C flux from mycorrhizal roots and hyphae, which is greater in magnitude than C flux from root exudates (Norton *et al.*, 1990; Rygielwicz & Andersen, 1994; Johnson *et al.*, 2002), was absent. Despite the artificiality of the medium and absence of soil microflora, Smith's results are consistent with our findings of greater release of labile C from yellow birch than sugar maple roots.

For the present study, RCF ranged from 7% to 13% of net assimilated C. Several methodological factors could have biased these estimates. Overestimation could have resulted from inclusion of severed root fragments in the SOM- ^{13}C pools. Despite our efforts to pick out root fragments from soil samples, a small portion of the fragments likely remained. We evaluated the potential contribution of root fragments to soil pools using an isotope mixing model (Staddon, 2004) which suggests that a maximum of 2.1% and 2.8% of assimilated C could have been derived from fine root fragments in sugar maple and yellow birch soils, respectively. The primary source of bias in underestimating RCF is the low recovery of $^{13}\text{CO}_2$ from TSR which would subsequently result in an underestimation of RMR- ^{13}C . We assessed the relative magnitude of this bias by calculating 95% prediction intervals around estimates of TSR (based on the regression of TSR and soil temperature). Using maximum estimates for TSR, RMR- ^{13}C could have been as much as 2.5% of assimilated C, or nearly double our estimate used to calculate RCF_{max}. Together these results suggest that our estimates of RCF are unlikely to be strongly biased in one direction or another.

In order to compare RCF between the two species and estimate RCF at the ecosystem level several assumptions were necessary. First, the phenological stages of these two species were assumed to be comparable at the time of labeling, so that species differences were not the result of asynchronous phenology. This assumption was based on the lack of differences between sugar maple and yellow birch in fine root production, mortality or root length in a previous investigation of the same pots used in this study (G. Tierney, T. Fahey, unpublished). Second, we assumed that the growth stage of the plants at the time of labeling was representative of the entire growth period at the HBEF. At the HBEF, fine root production and mortality of sugar maple and yellow birch have been shown to be relatively constant throughout the growing season (Tierney *et al.*, 2001). Although we have no data for how RCF may vary throughout the growing season, RCF is intimately linked to photosynthesis (Kuziyakov & Cheng, 2004), and thus any variability in RCF should not be affected by our scaling as long as the variability in photosynthesis is proportional. Finally, we assumed that the rooting environment in the pots was relatively similar to that in the mature forest at HBEF. Although root densities were lower in the pots than those at the HBEF, mycorrhizal colonization of sapling roots in this experiment (32% of root length in sugar maple; 49% in yellow birch) was similar to literature values reported for mature trees of these two species (~30% in sugar maple; ~65% in yellow birch; DeBellis *et al.*, 2002).

Implications

Our estimate of RCF at the HBEF ($55\text{--}104\text{ gC m}^{-2}\text{ yr}^{-1}$) is comparable to estimates derived by difference in a mass balance of the terrestrial C budget at HBEF ($60\text{--}80\text{ gC m}^{-2}\text{ yr}^{-1}$; Fahey *et al.*, 2005). These values represent nearly one third of the C that enters the forest floor at HBEF in aboveground litterfall (Fahey *et al.*, 2005), suggesting that RCF is an important driver of soil microbial activity. Differences in patterns of RCF between yellow birch and sugar maple may contribute to spatial variability in C and N cycling in northern hardwood forests. For example, at HBEF C and N cycling differ dramatically between stands dominated by yellow birch vs. sugar maple. Yellow birch stands have more labile soil C and more available N than sugar maple stands (Nielsen *et al.*, 2001), yet yellow birch leaf litter is considered to be of lower chemical quality (i.e. higher lignin:N ratios) than sugar maple litter (Melillo *et al.*, 1982). Thus, C inputs from roots and mycorrhizal fungi are likely important sources of labile C in these soils, and may contribute to the observed

elevated net N mineralization rates in yellow birch stands and elevated nitrate loss in sugar maple stands (Fitzhugh *et al.*, 2001). The differential effects of yellow birch and sugar maple on C and N cycling may have important implications for ecosystem dynamics if declining sugar maples are replaced by yellow birch (or other ectomycorrhizal species) as dominant tree species in northern hardwood forests.

Conclusion

Despite its importance in the terrestrial C cycle, RCF has rarely been quantified in mature forests, and even less is known about the role of tree species and mycorrhizal associations in mediating this flux (Jones *et al.*, 2004). The temporal pattern of C allocation to roots and the magnitude of RCF ($55\text{--}104\text{ gC m}^{-2}\text{ yr}^{-1}$) differed markedly between AM sugar maple and EM yellow birch. Whether these differences reflect species-specific differences in patterns of RCF or general differences between AM and EM tree species warrants further investigation. Overall, the results support the notion that RCF in forests exerts a substantial influence on soil microbial activity, nutrient dynamics and C storage (Grayston *et al.*, 1996; Cheng, 1999; Kuziyakov, 2002; Paterson, 2003).

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References

- Amthor JS, Gill DS, Bormann FH (1990) Autumnal leaf conductance and apparent photosynthesis by saplings and sprouts in a recently disturbed northern hardwood forest. *Oecologia*, **84**, 93–98.
- Andrews JA, Harrison KG, Matamala R *et al.* (1999) Separation of root respiration from total soil respiration using carbon-13 labeling during free-air carbon dioxide enrichment (FACE). *Soil Science Society of America Journal*, **63**, 1429–1435.
- Bailey SW, Hornbeck JW (1992) *Lithologic Composition and Rock Weathering Potential of Forested, Glacial-Till Soils. Research Paper NE-662*. U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station, Radnor, PA.

- Bailey SW, Horsley SB, Long RP *et al.* (2004) Influence of edaphic factors on sugar maple nutrition and health on the Allegheny Plateau. *Soil Science Society of America Journal*, **68**, 243–252.
- Beaudet M, Messier C, Hilbert DW *et al.* (2000) Leaf- and plant-level carbon gain in yellow birch, sugar maple, and beech seedlings from contrasting forest environments. *Canadian Journal of Forest Research*, **30**, 390–404.
- Brundrett M, Melville L, Peterson L (1994) *Practical Methods in Mycorrhiza Research* pp. 161, Mycologue Publications, Guelph, Ontario.
- Burton AJ, Pregitzer KS, Ruess RW *et al.* (2002) Root respiration in North American forests: effects of nitrogen concentration and temperature across biomes. *Oecologia*, **131**, 559–568.
- Butler JL, Bottomley PJ, Griffith SM *et al.* (2004) Distribution and turnover of recently fixed photosynthate in ryegrass rhizospheres. *Soil Biology and Biochemistry*, **36**, 371–382.
- Cheng W (1999) Rhizosphere feedbacks in elevated CO₂. *Tree Physiology*, **19**, 313–320.
- DeBellis T, Widden P, Messier C (2002) Effects of selective cuts on the mycorrhizae of regenerating *Betula alleghaniensis* and *Acer saccharum* seedlings in two Quebec mixed deciduous forests. *Canadian Journal of Forest Research*, **32**, 1094–1102.
- Delagrange S, Messier C, Lechowicz MJ *et al.* (2004) Physiological, morphological and allocational plasticity in understory deciduous trees: importance of plant size and light availability. *Tree Physiology*, **24**, 775–785.
- Dyckmans J, Flessa H, Polle A *et al.* (2000) The effect of elevated CO₂ on uptake and allocation of ¹³C and ¹⁵N in beech (*Fagus sylvatica* L.) during leafing. *Plant Biology*, **2**, 113–120.
- Edwards NT, Norby RJ (1999) Belowground respiratory responses of sugar maple and red maple saplings to atmospheric CO₂ enrichment and elevated air temperature. *Plant and Soil*, **206**, 85–97.
- Fahey TJ, Tierney GL, Fitzhugh RD *et al.* (2005) Soil respiration and soil carbon balance in a northern hardwood forest ecosystem. *Canadian Journal of Forest Research*, **35**, 244–253.
- Fitzhugh RD, Driscoll CT, Groffman PM *et al.* (2001) Effects of soil freezing disturbance on soil solution nitrogen, phosphorus, and carbon chemistry in a northern hardwood ecosystem. *Biogeochemistry*, **56**, 215–238.
- Gavito ME, Olsson EA (2003) Allocation of plant carbon to foraging and storage in arbuscular mycorrhizal fungi. *FEMS Microbiology Ecology*, **45**, 181–187.
- Grayston SJ, Vaughn D, Jones D (1996) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. *Applied Soil Ecology*, **5**, 29–56.
- Groffman PM, Driscoll CT, Fahey TJ *et al.* (2001) Effects of mild winter freezing on soil nitrogen and carbon dynamics in a northern hardwood forest. *Biogeochemistry*, **56**, 191–213.
- Hanson PJ, Edwards NT, Garten CT *et al.* (2000) Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry*, **48**, 115–146.
- Harris D, Horwath WR, van Kessel C (2001) Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. *Soil Science Society of America Journal*, **65**, 1853–1856.
- Harris D, Porter LK, Paul EA (1997) Continuous flow isotope ratio mass spectrometry of carbon dioxide trapped as strontium carbonate. *Communications in Soil Science and Plant Analysis*, **28**, 747–757.
- Horwath WR, Paul EA, Harris D *et al.* (1996) Defining a realistic control for the chloroform fumigation-incubation method using microscopic counting and ¹⁴C-substrates. *Canadian Journal of Soil Science*, **76**, 459–467.
- Hu S, Firestone MK, Chapin FS III (1999) Soil microbial feedbacks to atmospheric CO₂ enrichment. *Trends in Ecology and Evolution*, **14**, 433–437.
- Hungate BA, Holland EA, Jackson RB *et al.* (1999) The fate of carbon in grasslands under carbon dioxide enrichment. *Nature*, **388**, 576–579.
- Hütsch BW, Augustin J, Merbach W (2002) Plant rhizodeposition – an important source for carbon turnover in soils. *Journal of Plant Nutrition and Soil Science*, **165**, 397–407.
- Iverson LR, Prasad AM (2002) Potential redistribution of tree species habitat under five climate change scenarios in the eastern US. *Forest Ecology and Management*, **155**, 205–222.
- Johnson D, Leake JR, Ostle N *et al.* (2002) In situ ¹³CO₂ pulse-labeling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelium to the soil. *New Phytologist*, **153**, 327–334.
- Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. *Tansley Reviews, New Phytologist*, **163**, 459–480.
- Kelting DL, Burger JA, Edwards GS (1998) Estimating root respiration and microbial respiration in the rhizosphere, and root-free soil respiration in forest soils. *Soil Biology and Biochemistry*, **30**, 961–968.
- Kuzyakov Y (2002) Separating microbial respiration of exudates from root respiration in non-sterile soils: a comparison of four methods. *Soil Biology and Biochemistry*, **34**, 1621–1631.
- Kuzyakov Y, Cheng W (2004) Photosynthesis controls of CO₂ efflux from maize rhizosphere. *Plant and Soil*, **263**, 85–99.
- Kuzyakov Y, Domanski G (2000) Carbon input by plants into soil. Review. *Journal of Plant Nutrition and Soil Science*, **163**, 421–431.
- Langley JA, Hungate BA (2003) Mycorrhizal controls on below-ground litter quality. *Ecology*, **84**, 2302–2312.
- Leyval C, Berthelin J (1993) Rhizodeposition and net release of soluble organic-compounds by pine and beech seedlings inoculated with rhizobacteria and ectomycorrhizal fungi. *Biology and Fertility of Soils*, **15**, 259–267.
- Luo Y, Wu L, Andrews JA *et al.* (2001) Elevated CO₂ differentiates ecosystem carbon processes: deconvolution analysis of Duke Forest FACE data. *Ecological Monographs*, **71**, 357–376.
- Meharg AA (1994) A critical review of labeling techniques used to quantify rhizosphere carbon-flow. *Plant and Soil*, **166**, 55–62.
- Melillo JM, Aber J, Muratore JF (1982) Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology*, **63**, 621–626.
- Neilsen CB, Groffman PM, Hamburg SP *et al.* (2001) Freezing effects on carbon and nitrogen cycling in Northern Hardwood forest. *Soil Science Society of America Journal*, **65**, 1723–1730.
- Norby RJ, O'Neil EG, Hood WG *et al.* (1987) Carbon allocation, root exudation, and mycorrhizal colonization of *Pinus echinata*

- seedlings grown under CO₂ enrichment. *Tree Physiology*, **3**, 203–210.
- Norton JM, Smith JL, Firestone MK (1990) Carbon flow in the rhizosphere of ponderosa pine seedlings. *Soil Biology and Biochemistry*, **22**, 449–455.
- Olsson PA (1999) Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiology Ecology*, **29**, 303–310.
- Olsson PA, Baath E, Jakobsen I *et al.* (1995) The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. *Mycological Research*, **99**, 623–629.
- Paterson E (2003) Importance of rhizodeposition in the coupling of plant and microbial productivity. *European Journal of Soil Science*, **54**, 741–750.
- Paterson E, Hall JM, Rattray EAS *et al.* (1997) Effect of elevated CO₂ on rhizosphere carbon flow and soil microbial processes. *Global Change Biology*, **3**, 363–377.
- Pendall E, Mosier AR, Morgan JA (2004) Rhizodeposition stimulated by elevated CO₂ in a semiarid grassland. *New Phytologist*, **162**, 447–458.
- Rouhier H, Billès G, Billès L *et al.* (1996) Carbon fluxes in the rhizosphere of sweet chestnut seedlings (*Castanea sativa*) grown under two atmospheric CO₂ concentrations: ¹⁴C partitioning after pulse labeling. *Plant and Soil*, **180**, 101–111.
- Rouhier H, Read DJ (1998) Plant and fungal responses to elevated atmospheric carbon dioxide in mycorrhizal seedlings of *Pinus sylvestris*. *Environmental and Experimental Botany*, **40**, 237–246.
- Rygielwicz PT, Andersen CP (1994) Mycorrhizae alter quality and quantity of carbon allocated belowground. *Nature*, **369**, 58–60.
- SAS Institute Inc. (2003) *SAS for Windows, Version 9.1*. SAS Institute Inc., Cary, NC.
- Smith WH (1976) Character and significance of forest tree root exudates. *Ecology*, **57**, 324–331.
- Soderstrom B, Read DJ (1987) Respiratory activity of intact and excised ectomycorrhizal mycelial systems growing in unsterilized soil. *Soil Biology Biochemistry*, **19**, 231–236.
- Staddon PL (2004) Carbon isotopes in functional soil ecology. *Trends in Ecology and Evolution*, **19**, 148–154.
- Staddon PL, Ramsey CB, Ostle N *et al.* (2003) Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of ¹⁴C. *Science*, **300**, 1138–1140.
- Svejar TJ, Boutton TW, Trent JD (1990) Assessment of carbon allocation with stable carbon isotope labeling. *Agronomy Journal*, **82**, 18–21.
- Tierney GL, Fahey TJ, Groffman PM *et al.* (2001) Soil freezing alters fine root dynamics in a northern hardwood forest. *Biogeochemistry*, **56**, 175–190.
- Topa MT, McDermitt DJ, Yun SC *et al.* (2004) Do elevated ozone and variable light alter carbon transport to roots in sugar maple? *New Phytologist*, **162**, 173–186.
- Whipps JM (1990) Carbon economy. In: *The Rhizosphere* (ed Lynch JM), pp. 59–98. John Wiley and Sons Inc., NY, USA.
- Wollum AG (1994) Soil sampling for microbiological analysis. In: *Methods of Soil Analysis: Part 2. Microbiological and Biochemical Properties* (eds Weaver RW, Angle S, Bottomley P, Bezdicek D, Smith S, Tabatabai A, Wollum A), pp. 59–79. Soil Science Society of America, Madison, WI.
- Wright DP, Read DJ, Scholes JD (1998) Mycorrhizal sink strength influences the whole plant carbon balance of *Trifolium repens* L. *Plant Cell and the Environment*, **21**, 881–891.
- Wright SE, Upadhyaya A (1998) A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil*, **198**, 97–107.
- Zak DR, Pregitzer KS, Curtis PS *et al.* (1993) Elevated atmospheric CO₂ and feedback between carbon and nitrogen cycles. *Plant and Soil*, **151**, 105–117.