

Allocation and residence time of photosynthetic products in a boreal forest using a low-level ^{14}C pulse-chase labeling technique

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Abstract

Much of our understanding about how carbon (C) is allocated in plants comes from radiocarbon (^{14}C) pulse-chase labeling experiments. However, the large amounts of ^{14}C required for decay-counting mean that these studies have been restricted for the most part to mesocosm or controlled laboratory experiments. Using the enhanced sensitivity for ^{14}C detection available with accelerator mass spectrometry (AMS), we tested the utility of a low-level ^{14}C pulse-chase labeling technique for quantifying C allocation patterns and the contributions of different plant components to total ecosystem respiration in a black spruce forest stand in central Manitoba, Canada. All aspects of the field experiment used ^{14}C at levels well below regulated health standards, without significantly altering atmospheric CO_2 concentrations. Over 30 days following the label application in late summer (August and September), we monitored the temporal and spatial allocation patterns of labeled photosynthetic products by measuring the amount and ^{14}C content of CO_2 respired from different ecosystem components. **The mean residence times (MRT) for labeled photosynthetic products to be respired in the understory (feather mosses), canopy (black spruce), and rhizosphere (black spruce roots and associated microbes) were <1, 6, and 15 days, respectively.** Respiration from the canopy and understory showed significantly greater influence of labeled photosynthates than excised root and intact rhizosphere respiration. After 30 days, ~65% of the label assimilated had been respired by the canopy, ~20% by the rhizosphere, and ~9% by the understory, with ~6% unaccounted for and perhaps remaining in tissues. Maximum ^{14}C values in root and rhizosphere respiration were reached 4 days after label application. The label was still detectable in root, rhizosphere and canopy respiration after 30 days; these levels of remaining label would not have been detectable had a ^{13}C label been applied. Our results support previous studies indicating that a substantial portion of the C fueling rhizosphere respiration in the growing season may be derived from stored C pools rather than recent photosynthetic products.

Keywords: accelerator mass spectrometry, allocation, black spruce, ^{14}C , pulse-chase labeling, radiocarbon, root respiration

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Introduction

Uncertainties in how plant carbon (C) is allocated to and utilized by different ecosystem components currently limit progress in predicting ecosystem respiration fluxes. Of particular significance is the transport of C belowground by plant roots, and the subsequent trans-

fer of this C to microbial and soil pools (Nadelhoffer & Raich, 1992). Most methodologies used to distinguish the autotrophic (root metabolism) and heterotrophic (microbial decomposition) contributions to belowground C dynamics are limited by our ability to observe plant–soil processes *in situ* (Norby & Jackson, 2000). Methods applied, including trenching, girdling, coring techniques, minirhizotrons, and respiration partitioning with C isotopes have provided insights about belowground C processes and fluxes (Hanson *et al.*, 2000;

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Ryan & Law, 2005; Subke *et al.*, 2006). Yet, these techniques fail to address whole-plant C allocation patterns, which ultimately constrain the amount of C available for above- and belowground respiration.

Pulse-chase labeling (tracer) studies with C isotopes are one way to follow the allocation of recent assimilates into respiration, growth, and storage pools (Isebrands & Dickson, 1991; Hanson *et al.*, 2000; Kuzyakov & Domanski, 2000). In particular, radiocarbon (^{14}C) studies in the 1960s and 1970s provided much of the current understanding of how C is allocated by plants (summarized in Isebrands & Dickson, 1991). Perennial plants were shown to have distinct seasonal allocation patterns, including the ability to store excess assimilates as carbohydrates that fuel maintenance respiration in the dormant season, new growth in the spring, and growth flushes throughout the growing season (Dickson, 1991). However, in order to obtain sufficient ^{14}C for measurement by available decay counting methods, these early labeling studies used large amounts (MBq) of ^{14}C . This meant that the majority of studies were limited to juvenile, short-stature vegetation, in greenhouses or growth chambers, with very few studies conducted on mature woody plants and under field conditions (Isebrands & Dickson, 1991). In recent years, the use of ^{14}C in field studies has become even more limited due to stricter health and safety regulations for radioactive tracer applications (for example King *et al.*, 2002; W. S. Reeburgh, personal communication).

In the United States, the use of ^{14}C in the environment is regulated by individual states. In the state of California, ^{14}C levels below 0.037 Bq mL^{-1} (in air) and 296 Bq mL^{-1} (in liquid) are considered exempt quantities (California Code of Regulations, 2005). Because accelerator mass spectrometry (AMS) technology detects individual ^{14}C atoms instead of decay-counting, it allows for 10^3 – 10^9 times more sensitive measurements of ^{14}C ; and the ability to use small (milligrams of material) sample sizes to measure ^{14}C at natural levels (Turteltaub & Vogel, 2000). The natural abundance of ^{14}C in the atmosphere is very low ($^{14}\text{C}/^{12}\text{C} \sim 10^{-12}$) or $\sim 4.7 \times 10^{-8}\text{ Bq mL}^{-1}$, at standard temperature and pressure and with a CO_2 concentration of 380 ppm. A ^{14}C label with an activity of $\sim 3.7 \times 10^{-5}\text{ Bq mL}^{-1}$ air will increase the ^{14}C content to $\sim 10^3$ times. This level is still easily detectable by AMS, and the atmospheric radioactivity produced will be three orders of magnitude below-regulated levels. Depending on how fast it is diluted, a low-level ^{14}C pulse-label signal could potentially be followed for hours to years revealing allocation to longer-lived plant C pools such as growth and storage. Such low-level ^{14}C methods have been developed and used in biomedical AMS applications over the past decade (e.g. Turteltaub & Vogel, 2000) but

have yet to be applied in the environmental sciences. One notable exception to this is an opportunistic study reported by Trumbore *et al.* (2002) in Oak Ridge, Tennessee, where ^{14}C enriched CO_2 released by hazardous waste incinerators was taken up by the vegetation. Researchers are tracing this low-level pulse through the ecosystem with AMS technology (Hanson *et al.*, 2005; Swanston *et al.*, 2005; Cisneros-Dozal *et al.*, 2006).

In recent years, the stable isotope (^{13}C) has been more commonly used for pulse-chase labeling experiments (Thompson, 1996; Stewart & Metherell, 1999; Bromand *et al.*, 2001; Niklaus *et al.*, 2001; Staddon *et al.*, 2003; Phillips & Fahey, 2005; Wiegner *et al.*, 2005). The ^{13}C label is useful to follow the allocation of C into fast-cycling pathways (hours to days) such as plant respiration. It is advantageous because it is unregulated and safe. Additionally, analyses are inexpensive and require less sample preparation in comparison with ^{14}C measurement by AMS. However, due to the high natural abundance of ^{13}C ($^{13}\text{C}/^{12}\text{C} \sim 0.01$) it is a less-sensitive tracer (Vogel, 2000). Thus, to enhance the signal strength of a ^{13}C label, applications require increasing CO_2 concentrations significantly above ambient concentrations, or alternatively, labeling for an extended (days to years) period of time (continuous labeling). Such continuous isotope labeling applications have been used to follow the allocation and cycling of ecosystem C pools (Pataki *et al.*, 2003; Pendall *et al.*, 2004). Experiments like the Free-Air CO_2 Enrichment (FACE) studies elevate atmospheric CO_2 concentrations using fossil fuel-derived CO_2 , which has ^{13}C and ^{14}C signatures that are distinct (depleted) in comparison with the background atmosphere. This dual isotope label can be followed over long timescales because it is continuously administered (e.g. into roots, Matamala *et al.*, 2003; soil CO_2 , Sørensen *et al.*, 2004; allocation by trees, Körner *et al.*, 2005). However, obtaining a sufficiently distinct ^{13}C signature in the field requires substantially increased CO_2 concentrations and results from such studies cannot be used to assess how C cycles through plants and ecosystems under ambient CO_2 concentrations.

We initiated this study in a black spruce forest in Manitoba, Canada to develop and test a low-level ^{14}C pulse-chase field labeling technique. We had two main goals: (1) to test the efficacy of this method for *in situ* field determination of allocation patterns and the fate of photosynthetic products at ambient CO_2 concentrations; (2) to determine how C gets allocated and subsequently respired in late summer. We had particular interest in the allocation to roots, because recent studies (Czimczik *et al.*, 2006; Schuur & Trumbore, 2006) have shown that excised black spruce fine roots respire CO_2 with ^{14}C signatures that are significantly higher than those expected for current photosynthetic products.

Radiocarbon levels in atmospheric CO₂ have declined since the cessation of atomic bomb testing in the atmosphere in 1963. Elevated ¹⁴C signatures therefore indicated that the C being respired by the black spruce roots was fixed from the atmosphere on average several years before, (i.e. when ¹⁴C signatures in the atmosphere were higher). These data imply longer-lived (3–8-year old) storage pools as a major source of root respired C. This finding is inconsistent with the majority of studies in woody plants that indicate that a large portion of C allocated to fine roots is returned to the atmosphere within 1 year (Gill & Jackson, 2000). The girdling studies in boreal forests in Sweden (Högberg *et al.*, 2001) and trenching studies in Canada (Bond-Lamberty *et al.*, 2004) indicate that cutting the supply of fresh photosynthetic products to boreal conifer tree roots results in a rapid (days to weeks) decline in soil-respired CO₂, and clearly demonstrate a link between the canopy and soil respiration. However, continued declines beyond the first year indicate that pools with longer residence times may also fuel respiration (Bhupinderpal-Singh *et al.*, 2003).

In this study, we attempted to resolve the relative roles of new photosynthetic products vs. stored C as sources of root, soil, and aboveground plant respiration, while demonstrating the effectiveness of this novel ¹⁴C labeling technique. Based on previous tree C allocation studies summarized by Dickson (1991) and more recent experiments by Horwath *et al.* (1994), Hansen & Beck (1994), the timing of the label application (late summer) was designed to maximize current photosynthate and minimize reserves contributing to root respiration. We hypothesized that based on the work of Czimczik *et al.* (2006) and Schuur & Trumbore (2006) that root-respired CO₂ would be derived from both current photosynthate and reserves, even in late summer.

Materials and methods

Study site

The study took place in August and September of 2004 near the BOREAS Northern Study Area outside of Thompson, Manitoba, Canada (55°53'N, 98°20'W). The research site was a poorly drained black spruce forest, and the time elapsed since the last stand killing fire was 40 years. The vegetation was dominated by 31–36-year-old black spruce (*Picea mariana* B. S. P.) up to 4 m tall. The understory included feather mosses (*Pleurozium* MITT and *Hylocomium* B. S. G.), grasses, and sparse, small shrubs including Labrador tea (*Ledum groenlandicum* OEDER). The soils were poorly drained clay with underlying discontinuous permafrost (Rapalee *et al.*, 1998).

Experimental setup

Three separate plots were established within a 30 m² area. The control plot and ecosystem label plot (EL) were 11.3 m² in area and included black spruce trees (seedlings to 4 m tall), shrubs, feather mosses, and grasses. A separate label plot (moss only, ML) was established in the interspace of the spruce trees, with only moss and grass understory vegetation (lacking trees). This plot was smaller, 1 m² in area. The control and EL plots were each instrumented with two soil surface collars to provide a base for chamber measurements of soil respiration and four soil gas probes to sample soil CO₂. The ML plot contained one soil surface collar and two soil gas probes. The collars were made from PVC (25 cm diameter) and inserted ~10 cm in the moss layer. Because the collars were not placed into the mineral soil, a secondary flexible aluminum collar was placed around the PVC collar, and the ring (3–4 cm) between the aluminum and PVC was filled with fine sand to create a diffusion barrier. Care was taken to avoid cutting roots during the placement of the collars. Each soil gas probe was made from a 15 cm piece of 6.3 mm OD porous Teflon tubing (International Polymer Engineering, Tempe, AZ, USA) attached to 1.6 mm stainless-steel tubing with a stainless-steel fitting. Gas probes were inserted horizontally in the moss layer (5 cm) and in the organic layer (10 cm) in each plot. Belowground temperature at 5 and 10 cm, leaf and air temperature, relative humidity, and photosynthetically active radiation (PAR) were continually monitored and logged in the EL and control plots with a CR10-X data logger (Campbell Scientific, Logan, UT, USA).

Labeling chambers

The EL chamber was adapted from a dome-shaped portable polyethylene yurt made by Shelter Systems Inc. (Menlo Park, CA, USA; described in Arnone & Obrist, 2003) so that the final dimensions were 4.2 m tall, by 3.8 m diameter, with a volume of ~37 m³. Poly Tarp Tape (BAC Industries Inc., Minneapolis, MN, USA) was used to seal the panels of the dome. To accommodate trees, we increased the height of the dome by installing six (2.5 m long, 5.5 cm diameter) steel posts into the ground and attached the dome base to the posts with structural pipe-fittings. A 1.5 m tall cylindrical skirt (made from the same material as the dome) was inserted into the ground to approximately 30 cm depth (into the local water table) to prevent leakage from the bottom. The top of the skirt was sealed to the base of the dome with 2.5 cm wide Velcro to complete the enclosure. Six battery-operated fans (15 cm diameter) were installed at different heights within the

dome chamber for mixing. The translucent polyethylene fabric admits greater than 70% of PAR (Arnone & Obirst, 2003). For determination of dark respiration, we covered the dome with an opaque plastic material.

The ML chamber was smaller, ~500 L (0.5 m tall, 1 m × 1 m) and made from the same material as the EL chamber and a 2.5 cm diameter PVC tubing frame. A similar plastic skirt was inserted into the ground, and attached to the main chamber with Velcro. One fan was used for mixing within the chamber.

Each labeling chamber had five ports for gas sampling. Plastic tubing (Bevaline IV 6.3 mm OD) connected each port to exterior instruments. Inside the chambers, the ports were connected to tubing distributed evenly throughout the upper portion of the chamber to allow for well-mixed gas sampling. Individual ports were used for ^{14}C label addition, CO_2 concentration and ^{14}C sampling, and secondary tracer (propane) addition and subsequent sampling.

^{14}C labeling procedure

EL and ML plots were labeled with $^{14}\text{CO}_2$ on August 17 and 18, 2004, respectively. To make the label, we diluted commercially purchased sodium bicarbonate enriched in ^{14}C ($9.25 \times 10^7 \text{ Bq g}^{-1} \text{ NaHCO}_3$, MP Biomedicals LLC, Irvine, CA, USA) with 0.1 M bicarbonate solution until the final solution had a concentration of $\sim 750 \text{ Bq g}^{-1} \text{ NaHCO}_3$. The dilution was performed in a laboratory licensed for radiochemical use. Carbon dioxide was released into the labeling chambers by acidifying this label solution with acetic acid. The label solution was put in a 1 L mason jar and sealed with a lid containing two valves and a septum port. Plastic tubing connected the mason jar lid with valves to the chamber inlet and outlet ports inline with a pump. The acetic acid was added to the jar through the septum port with a syringe, the valves were opened, and chamber air was circulated ($\sim 3 \text{ L min}^{-1}$) through the mason jar and back to the chamber. The EL chamber received 138 Bq of ^{14}C (which yielded 0.01 moles of CO_2), and the ML chamber received 9 Bq (7×10^{-4} moles of CO_2). Label ^{14}C amounts were well below exempt quantity levels for ^{14}C use in Canada. The release of the label produced a ^{14}C signature for CO_2 in the chambers that was ~ 80 – 120 times background abundance for CO_2 (or a $\Delta^{14}\text{C}$ of 80 000–120 000‰, compared with ~ 60 ‰ for background air CO_2). The label increased CO_2 concentrations by < 6 ppm within the EL chamber.

We left the EL chamber on for 1 h and the ML for 1.5 h to ensure that sufficient quantities of the label were taken up by the plants (based on photosynthetic measurements and allocation estimates). The CO_2 concentration within both chambers was continually

monitored and recorded with a LI-800 infrared gas analyzer and LI-1400 data logger (LiCor, Lincoln, NE, USA). Owing to significant photosynthetic drawdown of CO_2 by trees in the EL chamber, additional unlabeled pure CO_2 was released (by acidifying sodium bicarbonate) into the chamber three times during labeling (total of 0.08 moles CO_2) to keep levels of CO_2 in the chamber close to ambient concentrations. Samples of the ^{14}C content of CO_2 in the dome air space were taken at the beginning, middle, and end of the labeling period and combined with the CO_2 concentration data to quantify the amount of label taken up by the vegetation.

A secondary tracer, propane, was used to (1) determine the chamber volume and (2) observe potential leakage from the chambers. We assumed propane was an inert gas over the 1 h time period when the chamber was in place. Three liters of propane were measured into a Tedlar bag, which was then emptied over the period of ~ 1 min by pumping into the EL chamber. The propane was injected simultaneously with the ^{14}C label into the large chamber. The bag was subsequently disconnected from the chamber, and the tubing was opened to the atmosphere and flushed for ~ 1 min. Air samples (30 mL) were taken by syringe from the same tubing immediately, and approximately every 10 min during labeling to quantify chamber volume and leak rate. Propane samples were introduced into septum-sealed vials (pre-evacuated) which were returned to the University of California, Irvine (UCI), and analyzed by gas chromatography. We found that the amount of time used to flush the tubing was insufficient to clean sorbed propane from the tubing, which led to unrealistically high values in the first few samples of each time series. Subsequent results gave an average EL chamber volume and indicated leakage was only an issue one very windy day (day 2).

Flux measurements and ^{14}C sampling

The CO_2 fluxes and ^{14}C content of respired CO_2 were measured in all plots to quantify the allocation of the label. All dark respiration measurements were made during the daytime between 10:30 and 18:00 hours. Sampling occurred before labeling, 4 h after labeling, intensively for 1–8 days, and again 30 days after labeling (Table 1). Soil respiration was sampled with soil surface chambers, which included (a) respiration from mosses, grasses and small shrubs – henceforth referred to as understory, (b) rhizosphere respiration, and (c) decomposition of soil organic matter. Soil respiration fluxes were measured from the ground surface by placing a 10 L PVC chamber lid with a silicon tubing gasket on the soil surface collar, and sealing together using four large binder clips. Aboveground respiration

Table 1 Measurements and sampling periods for control, ecosystem label (EL), moss only label (ML) plots, and air

Plot	Measurement	CO ₂ Flux	¹⁴ C Content	Sampling Period (Days/hours since label)
Control	Soil surface	X	X	-1, 3, 7
Control	Branch bag	X	X	-1, 3, 7
Control	Soil gas		X	-1, 3, 7
Control	Moss incubation		X	-1, 30
Control	Root incubation		X	-1, 30
EL	Soil surface	X	X	-1, 4 h, 1, 2, 4, 6, 30
EL	Branch bag	X	X	-1, 4 h, 1, 2, 4, 6, 30
EL	Ecosystem	X	X	-1, 4 h, 1, 2, 4, 6
EL	Soil gas		X	-1, 4 h, 1, 2, 4, 6, 30
EL	Moss incubation		X	-1, 1, 4, 6
EL	Root incubation		X	-1, 1, 4, 6, 30
ML	Soil surface	X	X	-4 h, 4 h, 1, 2, 4, 6, 8
ML	Soil gas		X	-4 h, 4 h, 1, 2, 4, 6, 8
ML	Moss incubation		X	-4 h, 4 h, 4, 8
ML	Root incubation		X	-4 h, 4 h, 4, 8
All	Air		X	-1, 0, 3, 5, 8, 30

Sampling occurred between 10:30 and 18:00 hours.

Measurement taken represented with "X"

was sampled with branch bags, which included black spruce branches (needles and stems) – henceforth referred to as canopy. Aboveground fluxes from trees in the EL and control plots were measured with ~10 L branch bags constructed of white (outside) and black (inside) 6 mil polyethylene plastic (Discount Hydroponics, Riverside, CA, USA). Each bag had inlet and outlet plastic tubing and was sealed to the black spruce branches with putty and a bungee cord. Ecosystem respiration measurements were made in the EL plot only, by covering the EL chamber with the same opaque white and black plastic. No EL measurement was made for the 30-day sampling period.

Gas exchange rates were determined by measuring the change in CO₂ concentration over ~5 min by circulating air (0.5 L min⁻¹) from the chamber/bag to the LI-800. All respiration measurements were taken ~3–5 min after darkening the source, to minimize the effects of postillumination burst (PIB) and light-enhanced dark respiration (LED_R; Atkin *et al.*, 1998). The chamber/bag volume for each flux was determined by injecting a known volume of pure tracer (propane) with a syringe through a septum port into the chamber/bag, allowing the tracer to mix well, and extracting a 30 mL sample. Black spruce branches sampled repeatedly for fluxes were harvested at the end of the sampling and brought back to UCI where the needles were removed from the branch, optically scanned with a flatbed scanner, dried at 60 °C for 3 days, and weighed. Specific leaf area (m² leaf g⁻¹ leaf) was calculated with Image J 1.32j

(National Institutes of Health, USA). Branch bag fluxes were calculated per m² ground area by calculating the leaf area index using site-specific allometric equations reported by Bond-Lamberty *et al.* (2002a,b). A secondary measure of leaf area was taken in the EL plot with a LAI-2000 meter (LiCor).

Field incubations were used to determine ¹⁴C signatures from root respiration and understory respiration using methods described in Czimeczik *et al.* (2006). Briefly, fine roots (<2 mm; but excluding extremely fine <0.2 mm roots) from black spruce were hand-picked from soil blocks 20 cm × 20 cm square, and 10–20 cm deep, excavated from three to four locations within each plot. Roots were extracted from the ground, rinsed with water and placed in an air tight, dark plastic incubation container of 2020 cm³ volume with gas in- and outlets on the lid. Understory samples of living moss and grasses were also cut in 20 cm × 20 cm squares and placed in the incubation container. The containers were returned to the original sample location (below-ground for roots, surface for understory) to maintain temperatures close to *in situ* conditions. The container was flushed (0.5 L min⁻¹) with CO₂-free air for 10 min, allowed to accumulate CO₂ for 2–4 h, then sampled for ¹⁴C content as discussed below.

The ¹⁴C content of respired CO₂ was sampled after measuring the CO₂ flux from the chambers and bags. We let CO₂ accumulate within the chamber/bag (~1 h or ~1000 ppm) or incubation container (~2–4 h). We measured and recorded the final CO₂ concentration in each before sampling. The CO₂ in the chamber was sampled by circulating the air from the chamber through Drierite to an activated molecular sieve 13 × trap that quantitatively removed CO₂, then back to the chamber for 15 min at 0.5 L min⁻¹ (Cisneros-Dozal *et al.*, 2006; Czimeczik *et al.*, 2006). Ambient air and soil gas probes were sampled by pumping air through Drierite to an activated molecular sieve trap for 15 min at 0.5 and 0.1 L min⁻¹, respectively.

¹⁴C preparation and analytical analysis

All samples collected from the ¹⁴C labeled plots were stored and analyzed separately from control plot samples to avoid potential contamination problems in the AMS facility. The CO₂ collected in each molecular sieve trap was desorbed and purified cryogenically on a vacuum line; labeled samples were processed in a laboratory in a building separate from the one housing the AMS.

The molecular sieve traps were heated to 650 °C for 45 min to desorb the CO₂, which was dried using an ethanol/dry ice bath to remove water vapor and frozen into a liquid nitrogen trap, which allowed O₂ and N₂ to

be evacuated. The amount of CO₂ was measured barometrically. An amount equivalent to ~1 mg C was frozen and sealed into an evacuated 9 mm Pyrex tube. This tube contained 25 mg zinc powder and 17 mg titanium hydride powder at the bottom, with 5 mg cobalt catalyst in a 6 mm Pyrex tube suspended above (Vogel, 1992). The tube was heated for 5 h to a maximum temperature of 550 °C, causing a catalyzed reduction of CO₂ to form graphite coating the cobalt powder. The ¹⁴C content of the graphite was measured using AMS (NEC 0.5MV 1.5SDH-2 AMS system) at the W.M. Keck-CCAMS facility of UCI (Southon *et al.*, 2004).

In order to decrease the count rates of highly labeled samples, the ¹⁴C content of CO₂ collected during the 1 h labeling application, was diluted (~50 ×) using 10–50 mg C ¹⁴C-free' (–1000‰) CO₂ derived by acidifying CaCO₃. Aliquots (1 mg C) of the dilution were prepared for AMS analysis as described above. The overall instrument error for ¹⁴C analyses was 3‰. All samples measured on the AMS for this study had ¹⁴C signatures less than three times background ($\Delta^{14}\text{C} < 3000\text{‰}$), well within the range of what could be measured by the AMS. Frequent measurement of ¹⁴C background materials tested the levels of contamination in our vacuum lines and potential sample cross-contamination in the AMS source. All of these showed no contamination from the levels of ¹⁴C for which we designed this study.

The radiocarbon data ($\Delta^{14}\text{C}$) are reported in per mil (‰), the deviation (in parts per thousand) of the ratio of ¹⁴C/¹²C in a sample divided by that of a standard of fixed isotopic composition (0.95 times the ¹⁴C/¹²C of oxalic acid I standard, decay corrected to 1950). All data are corrected for the effects of mass-dependent isotope fractionation by correcting to a common $\delta^{13}\text{C}$ value (–25‰) and assuming ¹⁴C is fractionated twice as much as ¹³C (Stuiver & Polach, 1977). Respiration measurement $\Delta^{14}\text{C}$ data were also corrected for air contribution to samples with a mass balance approach

$$\Delta^{14}\text{C}_S \times [\text{CO}_2]_S = \Delta^{14}\text{C}_A \times [\text{CO}_2]_A + \Delta^{14}\text{C}_R \times [\text{CO}_2]_R, \quad (1)$$

$$\Delta^{14}\text{C}_R = ((\Delta^{14}\text{C}_S \times [\text{CO}_2]_S) - (\Delta^{14}\text{C}_A \times [\text{CO}_2]_A)) / [\text{CO}_2]_R, \quad (2)$$

where $\Delta^{14}\text{C}_S$, $\Delta^{14}\text{C}_A$, $\Delta^{14}\text{C}_R$ are the signatures of the sample, air, and respiration respectively, and $[\text{CO}_2]_S$, $[\text{CO}_2]_A$, $[\text{CO}_2]_R$ are the CO₂ concentrations of the sample, air, and respiration, respectively, measured in the field at the time of sampling. A similar approach was used to calculate the contribution of label to total respiration in each measurement

$$\Delta^{14}\text{C}_S = (1 - f) \times (\Delta^{14}\text{C}_B) + (f) \times (\Delta^{14}\text{C}_L), \quad (3)$$

where

$$f = \frac{(\Delta^{14}\text{C}_B - \Delta^{14}\text{C}_S)}{(\Delta^{14}\text{C}_B - \Delta^{14}\text{C}_L)}, \quad (4)$$

and $\Delta^{14}\text{C}_S$ is the sample signature, $\Delta^{14}\text{C}_B$ is the background signature (prelabel), $\Delta^{14}\text{C}_L$ is the label signature, and f is the fraction of respiration from the label.

Analysis of ¹⁴C in respiration

For the period of 30 days following the label application, CO₂ fluxes for each ecosystem respiration component were estimated (on days when they were not measured) using empirically derived exponential temperature relationships and half-hourly air temperatures. The ¹⁴CO₂ emissions per milligram respired CO₂ for each component were combined with CO₂ flux estimates to model (SPSS Inc., Chicago, IL, USA) the total amount of ¹⁴C respired by each different ecosystem component. We assumed ¹⁴C concentrations varied linearly between days when observations were available. Ecosystem respiration was divided into canopy (black spruce), understory (moss and grass), and rhizosphere (root and associated microbial) respiration. The rhizosphere contribution to ¹⁴CO₂ flux from the soil surface was calculated as the difference between the label appearance in the EL soil surface chamber and the ML soil surface chamber (where black spruce roots were present, but not labeled). We estimated errors in the total allocation of C over the 1-month period following the labeling event by propagating errors (± 1 SD) from CO₂ flux measurements (if replicated), $\Delta^{14}\text{C}$ values (if replicated), and/or AMS instrument error. When replicates were not available for flux measurements, $\pm 10\%$ was used as the error.

Calculation of mean residence time (MRT)

The MRT of the label in each ecosystem component was calculated by fitting exponential decay functions to the observed fraction of respiration from the label. The MRT represents the time required for the fraction of the label to be reduced to 1/e times its value in the initial sampling point (4 h). For the root and rhizosphere components, the maximum contribution sampling point (4 days) was used as the 'time zero' for the exponential curve fit.

Results

Chamber labeling conditions

Air temperature within the EL chamber increased to a maximum of 29.9 °C during the labeling period compared with 18.3 °C at ambient. Increases in leaf, 5 cm

moss, and 10 cm moss⁻¹ soil temperatures were 8.1, 4.8 and 3.1 °C, respectively above levels outside the chamber. Relative humidity within the chamber ranged from 68% to 82% compared with 45 to 68% ambient levels outside the chamber. PAR within the chamber averaged 439 $\mu\text{mol m}^{-2} \text{s}^{-1}$ while ambient PAR ranged between 420 and 604 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Environmental conditions were not monitored in the ML chamber during labeling, but ambient temperature was 11.9 °C, relative humidity 59%, and PAR was 422 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

CO₂ fluxes

All dark respiration flux measurements exhibited relationships with temperature (Fig. 1). Fitted exponential functions resulted in Q_{10} values of 2.6 ($R^2 = 0.72$) and 3.5 ($R^2 = 0.56$) for canopy and soil respiration, respectively (Fig. 1a). Aboveground fluxes (ecosystem and canopy respiration) were positively correlated with average daily temperature ($R^2 = 0.36$ and 0.52, respectively). Soil respiration fluxes were more strongly correlated with 5 cm moss⁻¹ soil temperature ($R^2 = 0.62$) than air temperature ($R^2 = 0.49$). Total ecosystem respiration fluxes ranged between 143 and 196 $\text{mg C m}^{-2} \text{h}^{-1}$. Canopy respiration measured between 67 and 124 $\text{mg C m}^{-2} \text{h}^{-1}$. Soil respiration measurements were the lowest ranging from 44 to 92 $\text{mg C m}^{-2} \text{h}^{-1}$. Over the measurement period, canopy respiration averaged $57 \pm 13\%$ and soil respiration averaged $42 \pm 10\%$ of total ecosystem respiration (Fig. 1b).

Background and label ¹⁴C measurements

Radiocarbon contents of respiration collected in the EL and ML plots before labeling and in the control plot over the course of the sampling period were consistent and well below labeled values (Fig. 2a–f). Background soil respiration (Fig. 2a and b) measurements averaged

$71 \pm 7\%$. Background moss and root incubation (Fig. 2c and d) $\Delta^{14}\text{C}$ values over the sampling period were similar, $76 \pm 4\%$ and $76 \pm 23\%$, respectively, with roots displaying much greater variation. Background canopy respiration $\Delta^{14}\text{C}$ signatures ($50 \pm 5\%$, Fig. 2e) were similar to those of the background atmospheric CO₂, which averaged $57 \pm 9\%$. Labeled plot respiration measurements produced $\Delta^{14}\text{C}$ signatures that were clearly greater than background levels 30 days after labeling.

Label contribution to respiration

The fraction of respiration derived from the ¹⁴C pulse-label varied with ecosystem respiration sources and time (Fig. 3). The ML soil surface chamber measurements (Fig. 3a) displayed the greatest initial fraction of C derived from the ¹⁴C label but decreased the most rapidly with time. Linear extrapolation predicted no label contribution to soil respiration by day 20. The ML moss incubations showed slightly higher values, but the same pattern as observed with the ML soil surface chambers (which include living mosses). ML root incubations show no incorporation of the label; black spruce roots in this chamber originated outside the area of label application.

EL soil surface chamber measurements (Fig. 3b) declined in a manner similar to the ML soil surface chamber for the first 48 h after labeling, but then increased again by day 4. There was a decline from days 4 to 6, but the label content on days 6 and 30 were similar. The CO₂ respired by branches and leaves in the EL plot declined more gradually than mosses and soil respiration. However, by day 30, the fraction of respired CO₂ in branch bags that was derived from the label was less than that of the EL soil surface chamber. EL root incubations reached a maximum in label content on day 4, coincident with the secondary peak observed in

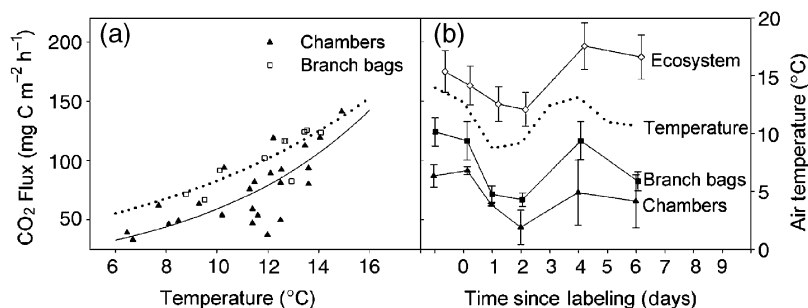


Fig. 1 (a) Soil surface chamber and branch bag CO₂ flux measurements plotted against air temperature at time of measurement for all plots. Soil surface chambers from all plots are \blacktriangle , with the Q_{10} (3.5) function in solid black line. Branch bags from all plots are \square , with the Q_{10} (2.6) function in dotted black line. (b) Dark respiration CO₂ fluxes measured daily over the intensive sampling period (8 days). Soil surface chamber measurements (\blacktriangle), branch bag measurements (\blacksquare), and ecosystem label (EL) chamber (whole ecosystem) measurements (\diamond). Mean daily (12 h period) air temperature is the dotted line. Error bars represent ± 1 SD for chamber measurements. There was no replication for EL chamber and branch bag measurements, hence, error estimates represent $\pm 10\%$.

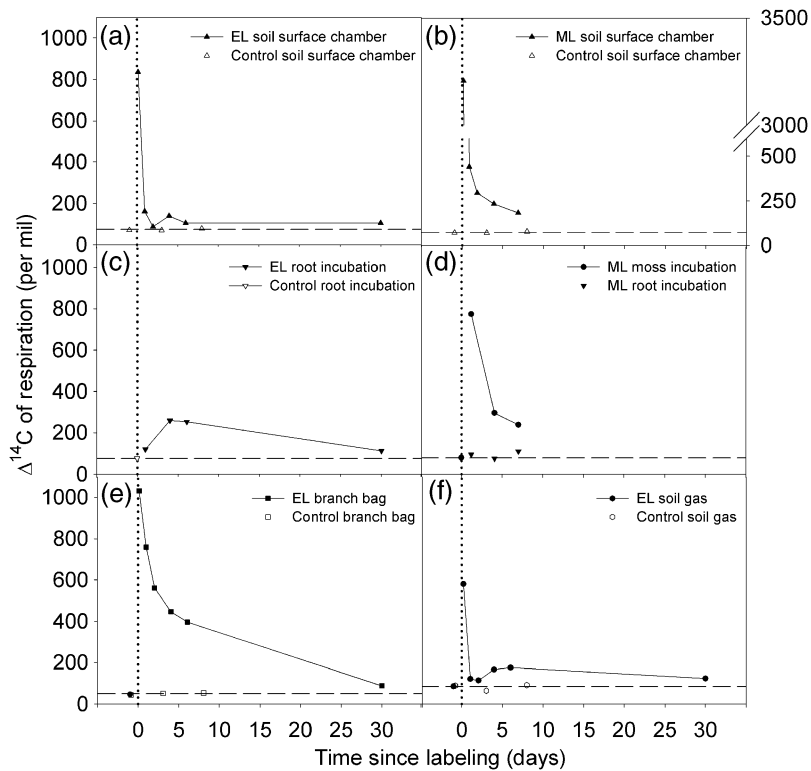


Fig. 2 $\Delta^{14}\text{C}$ values of respiration for ecosystem components vs. time since labeling. Closed symbols represent measurements taken in labeled plots (before and after labeling), open symbols represent control plot measurements. (a) Ecosystem label (EL) and control soil surface chambers; (b) moss only label (ML) and control soil surface chambers; (c) EL and control root incubations; (d) ML and control moss incubations in circles, ML root incubation in inverted triangles; (e) EL and control branch bags; (f) EL and control soil gas.

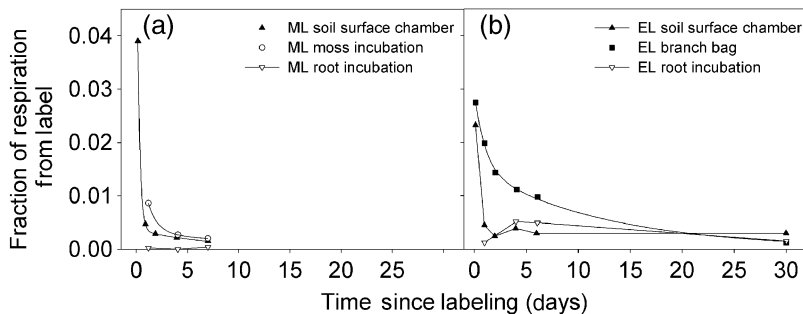


Fig. 3 Fraction of respiration from label ($\text{mg C label mg}^{-1}\text{ C respiration}$) plotted with time since label application for the moss only label (ML) (a) and ecosystem label (EL) (b) plots. Soil surface chamber measurements are closed triangles, branch bag (EL only) measurements are closed squares, moss incubations (ML plot only) are open circles, root incubations are open inverted triangles.

EL soil surface chamber and soil gas measurements (Figs 3b and 2a, c and f). The label was still present in the respiration of roots at day 30 (Figs 3b and 2c).

Allocation of label

The flux weighted label content in respiration also varied with time and ecosystem component. After 1 day, $\sim 15\%$ of the total label assimilated had been respired by the ecosystem. By day 10, $\sim 50\%$ had been

respired. Within the 30-day sampling period, the canopy respired $\sim 65 \pm 4\%$ of the applied label, the rhizosphere respired $\sim 20 \pm 3\%$, the understory respired $\sim 9 \pm 3\%$ and the remaining $\sim 6 \pm 6\%$ of the label was unrecovered (Fig. 4).

The MRT for dilution or loss by respiration of the initial label was the longest for the rhizosphere at >15 days. Label respired by the canopy had a MRT of 6 days. The shortest MRT (<1 day) was observed in the understory/moss respiration. By day 20, there was no

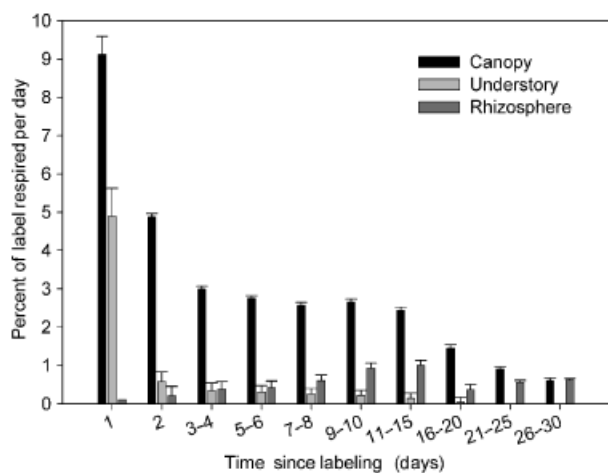


Fig. 4 Percent of label respired by individual ecosystem components per day (24 h) over the sampling period. Canopy label respiration ($65 \pm 4\%$) is shown in black, the understory ($9 \pm 3\%$) in light gray, and the rhizosphere ($20 \pm 3\%$) in dark gray. There was $6 \pm 6\%$ of label unaccounted for by respiration.

label present in the respiration of the understory/moss or discernable in the soil respiration. The label was present in rhizosphere respiration by day 2, but peaked later (days 11–15) in the sampling period concurrent with average daily temperatures.

Discussion

CO₂ fluxes and their sources

Respiration fluxes from both above- and belowground sources covaried with air temperature, which we attribute primarily to aboveground and understory autotrophic sources, and their responses to air temperature. The soil respiration fluxes were lower on a per-unit-area basis than the canopy respiration measurements, but displayed a greater temperature response. As our measurements of soil respiration include moss respiration, we suggest that heterotrophic sources were a minor contributor to the observed temperature response (Czimczik *et al.*, 2006)

Canopy respiration isotopic signatures in the control (unlabeled) plot had $\Delta^{14}\text{C}$ values close to those of atmospheric CO_2 , indicating recent photosynthetic products were the dominant source of leaf and stem respiration. Moss, root and soil respiration signatures were significantly ($P < 0.01$) higher than atmospheric values indicating potential contribution of older (bomb) C.

Allocation of the label

The CO_2 respired by aboveground sources (canopy and understory) had a greater influence of recent photosynthetic products and declined faster than C respired by

roots. The understory, in particular, demonstrated rapid cycling of labeled assimilates, most likely attributable to plant physiological differences, such as lack of complex storage and root structures in mosses. The majority of the label was allocated to and respired by the canopy, where the MRT was longer than for the understory vegetation. Less than one-third as much of the label ^{14}C was allocated to, and respired by, the rhizosphere, which also had the longest MRT. The results presented here are for a time relatively late in the growing season; a pulse-labeling experiment early in the season would most likely show different allocation patterns.

We attribute the time lag of 4 days in the appearance of the maximum label content in rhizosphere respiration to translocation from the needles to the roots. This result is similar to the 2-day time lag observed by Horwath *et al.* (1994) in 2-year-old hybrid poplar trees of comparable size. It is also in agreement with studies that correlate changes in $\delta^{13}\text{C}$ of assimilation (associated with stomatal response to relative air humidity) to that of soil respiration (Ekblad & Högberg, 2001; Bowling *et al.*, 2002). This result also agrees with a girdling experiment that showed 37% decrease in soil respiration 5 days after girdling (Högberg *et al.*, 2001).

The maximum relative contribution of label to respiration in the roots and rhizosphere was approximately five times less than that observed in the canopy and understory. Because current photosynthetic products are the overwhelming source of C for aboveground respiration, we can infer that at the time of our measurements, they are a less important source for root respiration, which demonstrated a much lower contribution of the ^{14}C label overall. Therefore, a significant amount of root respiration could be derived from an additional older C source. We cannot say how old this C source is, only that it predates the labeling event. Our measurements of ^{14}C in root respiration in the control plots, although not as enriched in ^{14}C compared with current atmospheric $^{14}\text{CO}_2$ as those reported by Czimczik *et al.* (2006) and Schuur & Trumbore (2006), are nonetheless consistent with a source of root respiration that is several years (3–5 years) old. This result supports the hypothesis that the C respired by black spruce roots is derived from multiple sources: storage and recent photosynthetic products. This also suggests that initial declines in soil respiration in girdling experiments (Högberg *et al.*, 2001) may underestimate root respiration rates, and nonstructural carbohydrate pools may be sufficient to fuel root respiration longer than previously observed (Bhupinderpal-Singh *et al.*, 2003).

These results are interesting in that they are for a time relatively late in the growing season when previous studies have shown that woody plants allocate C to starch reserves (Horwath *et al.*, 1994 and references

there in). Additionally, previous pulse-chase labeling studies have shown that conifers in some environments rely less on reserves in comparison with deciduous trees due to their extended growing season for photosynthesis (Kozlowski, 1992). Other studies have also shown that recent assimilates in conifers can be allocated to storage pools and used belowground when large C demand by shoots prevents allocation to roots (Hansen & Beck, 1994; Hansen *et al.*, 1996). The apparent contradictions among these observations suggest that whole plant source–sink linkages between photosynthetic assimilation, C allocation, and root respiration in black spruce trees are complex and act on timescales longer than previously thought.

Advantages of a ^{14}C label

Using the ^{14}C label vs. a ^{13}C label means that very little $^{14}\text{CO}_2$ is required to increase the longevity/sensitivity of the label signal. Our ^{14}C label was applied without significantly altering ambient CO_2 concentrations within the enclosure. In this study, the root respiration signal was particularly low in label content, and therefore, may not have been detectable at all if ^{13}C was used. Two ways to increase the sensitivity of a ^{13}C signal would be: (1) to increase the period of time over which the vegetation is exposed to the label, which will inevitably alter environmental conditions within the chamber; or (2) to increase the amount of label within the enclosure. If we increase the amount of $^{13}\text{CO}_2$ label to a level that would be observable for 30 days (100%), the CO_2 concentrations within the chamber would be increased significantly above ($\sim 10^2$ times) ambient concentrations. Furthermore, such large amounts of ^{13}C can be costly. All of these disadvantages of a ^{13}C label can be avoided by using ^{14}C .

The major advantage of this technique is that the radioactivity in a low-level ^{14}C label is well below levels considered 'exempt' by state and federal governments (USA and Canada), allowing *in situ* field labeling without using hazardous amounts of radiation (Vogel, 2000). We emphasize that because the laws governing the use of radioisotopes in the United States vary from state-to-state, researchers who plan to use low-level labeling techniques should discuss their specific plans with regulators in their area.

The disadvantages of ^{14}C are higher associated measurement costs (currently \$100–200 per sample) and increased time for sample processing (^{13}C in CO_2 can be measured directly without the need for conversion to graphite). However, as additional small AMS facilities become available (a number are now being manufactured and sold for biomedical labeling applications of AMS) the cost of ^{14}C measurements will decrease. We

predict that this method will become more cost-effective in the future. In the meantime, future experiments might combine ^{13}C and ^{14}C tracers to take maximum advantage of the benefits of each. Together these tracers permit tracing the fate of C in the short term (with ^{13}C) and longer term (with ^{14}C) and minimize the cost and time associated with ^{14}C measurements.

Methodological issues

As with most new techniques, there are some methodological issues with our field-labeling technique that should be discussed. Obvious (and predicted) difficulties stem from the environmental conditions within the large chamber while labeling. We tried to minimize these effects by labeling for a short period of time at mid-morning on a cool, sunny day (Kajji *et al.*, 1993). Plant physiological mechanisms were potentially affected by increased temperature and humidity, decreased PAR, and more diffuse radiation in the chamber. Lack of replication in our measurements is an apparent weakness in this experiment; our data represent the response of a single stand of trees at a given point in time. We did not attempt to capture diurnal and spatial variation in respiration fluxes and isotopic signatures. This could be a significant source of error in our estimates that is unquantifiable. We stress that the goal of this experiment was to test the feasibility of a method, and to assess the relative importance of recent photosynthetic products in fueling root respiration. We note that most methodological problems discussed above could be fixed or improved in future experiments.

Conclusions

This study demonstrates that a low-level ^{14}C label is a potentially useful technique for studying plant allocation patterns and partitioning ecosystem respiration sources in the field. Our data support previous findings that the respiration of recently assimilated C belowground lagged by several days that of aboveground sources. Most of the C assimilated in late summer was respired relatively quickly by canopy and understory vegetation and only $\sim 20\%$ by the rhizosphere in the first month following assimilation. Our data support the idea, based on the ^{14}C signatures of CO_2 respired from the control (unlabeled roots), that a major source of the C respired by the roots is from stored (as opposed to recent) photosynthetic products.

The data we collected in this study were limited to vegetation that we could enclose in a large portable dome. Small-scale plot applications with the portable chamber can reveal differences in C allocation and cycling between short stature plant functional types in

response to environmental stresses or experimental manipulations. How the timing and C sources of root respiration differ across the boreal landscape (with stand age and environmental stresses) can only be addressed with future large-scale free-air (without enclosures) pulse-chase labeling applications. Such techniques would eliminate many of the methodological difficulties encountered in this experiment, and could provide valuable information in even larger stature ecosystems (like tropical forests) with sizeable contributions to global C fluxes.

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