Global Change Biology (2006) **12,** 165–176, doi: 10.1111/j.1365-2486.2005.01066.x

Partitioning sources of soil respiration in boreal black spruce forest using radiocarbon

EDWARD A.G. SCHUUR* and SUSAN E. TRUMBORE†

*Department of Botany, 220 Bartram Hall, University of Florida, Gainesville, FL 32611, USA,

†Department of Earth System Science, 3218 Croul Hall, University of California, Irvine, CA 92697-3100, USA

Abstract

Separating ecosystem and soil respiration into autotrophic and heterotrophic component sources is necessary for understanding how the net ecosystem exchange of carbon (C) will respond to current and future changes in climate and vegetation. Here, we use an isotope mass balance method based on radiocarbon to partition respiration sources in three mature black spruce forest stands in Alaska. Radiocarbon ($\Delta^{14}C$) signatures of respired C reflect the age of substrate C and can be used to differentiate source pools within ecosystems. Recently-fixed C that fuels plant or microbial metabolism has $\Delta^{14}C$ values close to that of current atmospheric CO_2, while C respired from litter and soil organic matter decomposition will reflect the longer residence time of C in plant and soil C pools. Contrary to our expectations, the $\Delta^{14}C$ of C respired by recently excised black spruce roots averaged 14‰ greater than expected for recently fixed photosynthetic products, indicating that some portion of the C fueling root metabolism was derived from C storage pools with turnover times of at least several years. The $\Delta^{14}C$ values of C respired by heterotrophs in laboratory incubations of soil organic matter averaged 60‰ higher than the contemporary atmosphere $\Delta^{14}CO_2$, indicating that the major contributors to decomposition are derived from a combination of sources consistent with a mean residence time of up to a decade. Comparing autotrophic and heterotrophic $\Delta^{14}C$ end members with measurements of the $\Delta^{14}C$ of total soil respiration, we calculated that 47–63% of soil CO_2 emissions were derived from heterotrophic respiration across all three sites. Our limited temporal sampling also observed no significant differences in the partitioning of soil respiration in the early season compared with the late season. Future work is needed to address the reasons for high $\Delta^{14}C$ values in root respiration and issues of whether this method fully captures the contribution of rhizosphere respiration.

Keywords: Alaska, autotrophic, black spruce, carbon, heterotrophic, isotopes, radiocarbon, respiration, soil, soil organic matter

Received 18 October 2004; Revised version received 31 August 2005 and accepted 1 September 2005

Introduction

The net ecosystem exchange (NEE) of carbon (C) is the balance between C uptake by plants (photosynthesis), and C release by plants and microbes (respiration). On an annual basis, these two component C fluxes largely define whether an ecosystem is a source or sink of C to the atmosphere (Randerson *et al*., 2002). Variations in ecosystem respiration have been identified as the process most responsible for regional patterns in NEE (Valentini *et al*., 2000). However, understanding and modeling the controls over soil and ecosystem respiration have proven difficult because of the many pathways that release C from the ecosystem back to the atmosphere. In fact, the application of empirically derived temperature relationships is the approach still most commonly used for extrapolating respiration data across temporal gaps in sampling (Raich & Schlesinger, 1992; Davidson *et al*., 2005). However, it is well known that other factors such as moisture (Burton *et al*., 1998; Davidson *et al*., 2000; Luo *et al*., 2001), and substrate supply (Gardenas, 2000; Janssens *et al*., 2001; Luo *et al*., 2001) can regulate respiration as well. While experiments and observations have demonstrated the effect of variables other than temperature, this information has

Correspondence: Edward A. G. Schuur, tel. +1 352 392 7913, fax +1 352 392 3993, e-mail: tschuur@ufl.edu

© 2006 Blackwell Publishing Ltd

yet to be fully integrated into our conceptual and numerical models of ecosystem dynamics. In part, while many of these variables have been incorporated into models, empirical limitations have made it difficult to quantitatively test model predictions. Developing a mechanistic understanding of the sources of respiration will be necessary for predicting the response of C fluxes to current and future changes in climate and vegetation.

One barrier to the incorporation of multiple driving variables is the differential response of plant and soil respiration to controlling factors. While each may be subjected to a similar set of driving variables, the response could be different for autotrophic (plant) respiration and heterotrophic (microbial) respiration. In conjunction with the multiple pathways that comprise ecosystem and soil respiration, the ability to empirically distinguish respiration arising from different component sources presents a methodological challenge. While aboveground and belowground respiration can be effectively partitioned using eddy covariance measurements combined with soil respiration measurements (e.g. Davidson *et al*., 2005), widely applicable and reliable methods for separating the contributions of plants and microbes to total soil respiration, while minimizing disturbance, are still lacking.

Partitioning soil respiration is difficult and various methods have been applied to this problem over many decades as reviewed by Hanson *et al*. (2000). One widely used approach has been root-trenching experiments to isolate blocks of soil without living roots within forest stands (Ewel *et al*., 1987; Bowden *et al*., 1993). One critique of this method is the disturbance of the treatment and how it might impact soil respiration. For example, enhanced decomposition from freshly killed roots may increase heterotrophic respiration compared with control plots in such experiments. Disturbance effects are also a concern with methods that directly measure respiration from excavated roots (Palta & Nobel, 1989; Qi *et al*., 1994; Burton *et al*., 1997) and other components of soil (Kelting *et al*., 1997). Tree girdling is a relatively new approach to this problem, and solves some of the disturbance problems by cutting the phloem of trees, effectively stopping the flow of photosynthate to the root plus mycorrhizal network while maintaining water flow through the xylem (Högberg *et al*., 2001; Scott-Denton *et al*., 2005). Of course, this is a one-time experiment for a girdled forest stand, which then dies, so repeated observations through time and in ecosystems without woody dominants, are not possible.

The past several years have seen an increase in the application of isotopic techniques to partitioning soil respiration into component sources in natural ecosystems. Isotope methods rely on the existence of a difference in isotopic signature of C respired by different sources, for example, root and microbial respiration. When this condition exists, an isotope mixing model can be applied by first determining the isotopic values of these component fluxes alone, then using these so-called 'end-member' isotopic values to determine their relative contribution to soil respiration. In the case where isotopic signatures of autotrophic and heterotrophic sources of soil respiration differ significantly, the isotopic value of the soil respiration is calculated as a proportional mixture of these two end-member isotopic values (e.g. Gaudinski *et al*., 2000; Cisneros Dozal *et al*., 2005). The key assumption with this method, which has begun to be tested (Dioumaeva *et al*., 2003), is that the isotopic value of component respiration sources is less sensitive than the actual rate of CO_2 flux to disturbances related to separating and isolating roots and soil for end-member determination.

The isotopic approach to respiration partitioning has been attempted successfully using measurements of stable isotopes of carbon ($\delta^{13}C$). In many of these studies, isotopic differences between components are because of perturbations to the ecosystem. This can occur either as the dominant vegetation shifts from having a C_3 photosynthetic pathway to C_4 pathway (or *vice versa*) (Robinson & Scrimgeour, 1995; Rochette & Flanagan, 1997; Rochette *et al*., 1999), or if the ecosystem is exposed to elevated CO_2 levels either enriched or depleted in $\delta^{13}C$ (Hungate *et al*., 1997; Andrews *et al*., 1999; Pataki *et al*., 2003; Pendall *et al*., 2003). Even more recently, this approach has begun to be used in ecosystems that are not subject to these disturbances (Dawson *et al*., 2002). For natural abundance $\delta^{13}C$, there are a number of biological processes that occur within the ecosystem in both plants (Park & Epstein, 1961; Leavitt & Long, 1982, 1986; Francey *et al*., 1985; Benner *et al*., 1987; Martinelli *et al*., 1998; Pate & Arthur, 1998) and soil (Nadelhoffer & Fry, 1988; Ehleringer *et al*., 2000; Garten *et al*., 2000) that discriminate for and against the heavy isotope of C. In addition, changes in the atmospheric $\delta^{13}C$ value over the past 150 years have altered the isotopic value of C entering ecosystems (Park & Epstein, 1961; Enting *et al*., 1995; Trolier *et al*., 1996; Fung *et al*., 1997). In combination, these processes can either reinforce or cancel any isotopic differences between autotrophic and heterotrophic respiration depending on a host of factors specific to each ecosystem.

In this paper, we use a similar isotopic approach to partitioning respiration, but instead make use of radiocarbon ($\Delta^{14}C$) measurements of soil respiration and the components to that flux. Here, we attempted to separate the contribution of autotrophic and heterotrophic respiration to total soil respiration in mature black spruce

forests as part of a broader study on the impact of disturbance. In contrast to natural variations in $\delta^{13}C$ within ecosystems, radiocarbon values of component C pools and respiration fluxes vary because of a single factor: the residence time of C within the ecosystem (Trumbore 2000; Schuur *et al*., 2003). This is because radiocarbon measurements are corrected for all the mass-dependent fractionations that affect $\delta^{13}C$, leaving time-dependent changes in the atmospheric $\Delta^{14}C$ and radioactive decay as the processes that control variation in $\Delta^{14}C$.

Radiocarbon is a naturally occurring isotope, although its atmospheric abundance was nearly doubled in the early 1960s by atmospheric testing of thermonuclear weapons. Over recent decades, the northern hemisphere atmospheric $\Delta^{14}CO_2$ value continues to decline from its peak in 1963 as atmospheric C exchanges with biosphere and ocean C reservoirs, and fossil fuel burning adds more ^{14}C-free CO_2. The rate of decline over the last two decades has averaged $\sim$5–8‰ per year (Levin & Hesshaimer, 2000), roughly twice the accuracy of the ^{14}C measurement by accelerator mass spectrometry. Autotrophic respiration is largely dominated by C that has recently entered the ecosystem, and therefore should have $\Delta^{14}C$ values close to contemporary atmospheric CO_2. In contrast, the CO_2 respired by heterotrophs reflects the age of the substrates they consume, which may range from days to years and longer (Gaudinski *et al*., 2000). Respired C that was fixed since 1956 will have the extra ^{14}C derived from weapons testing and will have higher $\Delta^{14}C$ values than contemporary CO_2; C derived from substrates fixed prior to 1956 will have $\Delta^{14}C$ values <0‰ (Trumbore, 2000). Given that much of the C in boreal forest litter and soil organic matter has residence times of years to decades (Trumbore, 2000), we hypothesized that $\Delta^{14}C$ values of heterotrophically respired CO_2 would be higher than the current atmospheric CO_2, allowing us to partition the soil respiration isotope value observed in field measurements into component fluxes.

Because of the nature of respiration partitioning approaches, the definition of autotrophic and heterotrophic respiration is dependent in part on the methodology. This is true for all experimental approaches aimed at separating these two sources (Hanson *et al*., 2000). In reality, there is a range of C substrates utilized by plants and microbes and evolved as CO_2 at different rates. In terms of isotope measurements, the isotopic value of CO_2 respired by plants is likely to be quite similar to the isotopic value of CO_2 respired by rhizosphere microbes metabolizing root exudates. Using radiocarbon, we are functionally separating the contribution of fast cycling C (with short ecosystem residence time, such as root and rhizosphere respiration) from slower cycling C (with longer ecosystem residence time) to total soil respiration flux. In practice, we obtained component isotope measurements from DI-rinsed roots (with any adhered microbes) and microbially derived CO_2 from incubation of organic horizon and mineral soil to represent the end-member isotope signatures. We estimated the contribution of living mosses to autotrophic respiration and assumed that rhizosphere respiration was included in our estimate of heterotrophic respiration based on soil incubations.

Materials and methods

Site description

We conducted the respiration partitioning experiment in three mature (>80 years) black spruce (*Picea mariana* (Mill. B.S.P.)) forests located in Interior Alaska that differed in tree density, understory plant species composition, and soil C storage (Table 1, Mack *et al*., unpublished data). The Caribou Poker Creek Research Watershed (CPCRW) is an experimental watershed located near Fairbanks, Alaska at 65°10′N latitude and 147°30′W longitude. Our site was a ridge top forest at approximately 610 m elevation with a feathermoss understory, predominantly *Pleurozium schreberi* and *Hylocomium splendens*. The soil was a rocky silt loam Entisol and was not underlain by permafrost. The other two sites (termed here the Tower site and the Twelve Mile site) were located near Delta Junction, Alaska at

Table 1 Stand and soil characteristics from three mature black spruce forests located in Interior Alaska (Mack *et al*., unpublished data)

Site	Tree density number (ha^{-1}) ($\pm$ SE)	Basal area ($m^2\,ha^{-1}$) ($\pm$ SE)	Organic soil C mass ($g\,m^{-2}$) ($\pm$ SE)	Mineral soil C mass (10 cm) ($g\,m^{-2}$) ($\pm$ SE)
Tower	3744 (462)	8.06 (1.00)	1790 (220)	3660 (360)
Twelve Mile	4933 (415)	10.17 (0.95)	4890 (1050)	3540 (170)
CPCRW	12 800 (1850)	14.86 (1.76)	2160 (280)	2710 (220)

CPCRW, Caribou Poker Creek Research Watershed.

64°03′N latitude and 145°42′W longitude (Table 1). The Tower site was at approximately 490 m elevation and had a lower spruce density with an understory composed of lichens (predominantly *Cladonia* spp., and *Cladina* spp.) and a lower abundance of feathermoss species similar to those found at CPCRW. This soil was also a rocky silt loam and not underlain by permafrost. The Twelve Mile site was located less than 10 km from the Tower site at approximately 520 m elevation, but appears to be underlain by discontinuous permafrost because of a locally thicker silt cap overlying the rocks. This site had a higher tree density compared with the Tower site, and a wider range of understory moss species including the presence of *Sphagnum* spp., more typical of sites with poor soil drainage. Because of cold soil temperatures in boreal forests, much of the plant and microbial activity is restricted to the surface organic horizon. There is typically a thin A horizon (<5 cm) in the mineral soil with mostly unweathered silt parent material below that depth. Stand-replacing fires are the dominant disturbance that shapes the structure and function of these boreal black spruce forests. The sites we investigated represent the endpoint in forest succession that develops in between fire events over approximately 100-year cycles, with the time interval in any one location depending on both stochastic and site specific factors.

Soil respiration $^{14}CO_2$

Soil respiration $^{14}C/^{12}C$ and $^{13}C/^{12}C$ isotopic ratios were measured using a modified dynamic flow chamber system analogous to systems used for measuring soil CO_2 fluxes (Norman *et al*., 1997; Gaudinski *et al*., 2000). At each site, permanent collars (25.4 cm diameter) remained inserted about 5 cm in the soil/moss surface for the duration of the study. At one or two intervals during the growing season of 2001 (June–August), depending on site, 10L dark chambers were mounted on the collars during mid-day (10:00–14:00 hours) and real-time CO_2 fluxes were measured over two minute intervals. Next, air was drawn from the chambers through an infrared gas analyzer (IRGA) to determine the CO_2 concentration, and then either through a soda lime CO_2 scrubber or through a molecular sieve before returning to the chamber. To collect respired CO_2 alone, the airstream from the chamber system was first scrubbed with soda lime to remove background atmospheric CO_2 that was present when the chamber top was fit to the collar. Carbon dioxide fluxes were determined by monitoring the rise in CO_2 concentration in the closed chamber–sampler system, and the airflow (thus scrubbing rate) was adjusted such that level of CO_2 in the system remained at or slightly above ambient conditions at the soil surface. Basically, CO_2 was scrubbed from the system at a rate similar to the rate of CO_2 efflux from the soil. Scrubbing was maintained until the equivalent of three-chamber volumes of air ($\sim$30 L) had passed through the scrubber. At that point, CO_2 remaining in the chamber–sampler system was derived largely from soil respired CO_2. The airstream was then diverted through a molecular sieve (Alltech 13 X) that quantitatively traps CO_2 until 1.0–1.5 mg of CO_2-C was adsorbed. In the laboratory, the molecular sieve traps were heated to 625 °C, which desorbs CO_2 (Bauer *et al*., 1992). Carbon dioxide was then cryogenically purified on a vacuum line, subsampled for $\delta^{13}C$ analysis, and reduced to graphite coating a cobalt catalyst using zinc powder and titanium hydride as reductants (Vogel, 1992). Samples were sent to the Lawrence Livermore AMS facility for radiocarbon analysis. Subsamples of the purified CO_2 were analyzed for $\delta^{13}C$ at the University of California, Irvine mass spectrometry facility. Radiocarbon data are reported as $\Delta^{14}C$, the deviation of the $^{14}C/^{12}C$ ratio of the sample from that of an absolute standard (0.95 times oxalic acid I, at −19‰ $\delta^{13}C$ and decay corrected to 1950; Stuiver & Polach, 1977). Data reported in $\Delta^{14}C$ notation are reported at a common $\delta^{13}C$ value to correct for mass-dependent fractionation effects.

In addition to using $^{13}C/^{12}C$ isotopic ratios to correct the $^{14}C/^{12}C$ ratios for discrimination by photosynthesis (Stuiver & Polach, 1977), we also used them to correct for incomplete scrubbing or leaks in the chamber–sampler system (Gaudinski *et al*., 2000). Incomplete scrubbing was quantified by comparing the $\delta^{13}C$ of the trapped CO_2 to the atmospheric value ($\sim$−8.5‰) and the expected value of the soil respiration based on limited chamber-based Keeling plot observations ($\sim$−27‰). Carbon dioxide samples from field respiration at all sites had an average $\delta^{13}C$ value of −23.6‰, ranging from −21.2‰ to −25.9‰. This value was higher than the expected respiration $\delta^{13}C$ value because of the presence of atmospheric air that increased the $\delta^{13}C$ value. The fraction of air in the sample (F) was estimated as

$$F = (\delta^{13}C_{measured} - \delta^{13}C_{soil\,resp})/(\delta^{13}C_{atm} - \delta^{13}C_{soil\,resp}).$$

The fraction of air ranged from a low of 6% to a high of 30% of the measured sample, and averaged 18% across all samples. We then calculated the true $\Delta^{14}C$ value of soil respiration by removing the signature of $\Delta^{14}C$ signature of the atmosphere using a mass balance

$$\Delta^{14}C_{soil\,resp} = \Delta^{14}C_{measured} - (F \times \Delta^{14}C_{air}))/(1 - F).$$

Removing the effect of atmospheric air increased our estimate of the soil respiration $\Delta^{14}C$ value by an average

of 7‰; in comparison, the overall accuracy of the AMS $\Delta^{14}C$ measurement was ± 5‰.

Soil incubations

We collected three replicate soil samples per site in mid-August of 2001 using a 10 cm × 10 cm quadrat to volumetrically collect the surface organic soil down to the mineral soil, and a 5.4 cm diameter corer to collect the top 10 cm of mineral soil. All cores were split, and the halves kept intact to preserve the soil structure. Soil core splits were incubated in separate glass jars at 5 °C and 15 °C to determine the temperature sensitivity (expressed as a Q_{10} value) for respiration from these soils. Intact soil incubations can be especially important when measuring the isotopic value of CO_2 from mineral soils where aggregate structure can be disrupted by homogenization (W. Baisden, unpublished data). Rates of CO_2 production were measured daily using an IRGA to monitor the change in CO_2 concentration in the incubation jar headspace over time. Jars were kept sealed and were flushed with CO_2-free air whenever CO_2 concentrations exceeded 1%. Soils were incubated in the laboratory for 6 days. After that period, the jars were completely scrubbed with CO_2-free air, and evolved $^{13}CO_2$ and $^{14}CO_2$ was allowed to accumulate. Field experimentation has shown that soil respiration fluxes dropped by $\sim 50\%$ within 3–5 days after girdling of trees in a high-latitude forest in Sweden (Högberg *et al*., 2001). We used that as an estimate of the length of time where roots plus mycorrhizae in the soil cores would have depleted a large proportion of their labile C reserves and would then contribute relatively little to the soil incubation CO_2 flux. Carbon dioxide evolved from the incubation after this initial period was then assumed to be dominated by the heterotrophic decomposition of soil organic matter. Carbon dioxide in the jar headspace was then collected from the 15 °C temperature incubation into evacuated 0.5-L glass flask for $\delta^{13}C$ and $\Delta^{14}C$ analysis as described above. Previous research has shown that the $\Delta^{14}C$ isotopic composition of evolved CO_2 is not affected by the temperature of incubation (Dioumaeva *et al*., 2003).

To calculate the $\Delta^{14}C$ value to use as the heterotrophic end member for respiration partitioning, we combined the isotopic signatures obtained from the separate incubations of organic and mineral horizons, weighted each layers' contribution by: (1) the relative CO_2 flux on a per gram dry soil basis, (2) the relative amount of soil mass in the two horizons, and (3) by the average field temperature for the horizons. The weighting procedures were done within each replicate core from each site to give one heterotrophic isotope flux from each core; the replicates were then averaged to give the overall mean for each site.

Root incubations

We also measured the radiocarbon value of CO_2 emitted from root respiration in 2002 by conducting short laboratory incubations of fine roots. Our initial hypothesis for the partitioning experiment was that autotrophic respiration would have similar radiocarbon values to the present atmosphere because of the short residence time of most photosynthate in plants. However, our calculated estimates of heterotrophic respiration using this assumption were very high, and we learned from concurrent observations that roots respiration $\Delta^{14}C$ values may differ from that of the current atmosphere (Czimczick & Trumbore, 2003; Cisneros-Dozal *et al*., 2005). Therefore, we tested this assumption by directly measuring the isotopic value of CO_2 respired by roots to determine an autotrophic end member for respiration partitioning. At the Tower site in August of 2002, we excavated fine roots (all <2 mm diameter) from the surface soil. While we attempted to keep the root branches and fine root hairs plus mycorrhizae as intact as possible, difficulties in separating very fine (<0.5 mm diameter) live roots intact from soil may mean we undersampled this category and biased our results toward fluxes from larger diameter (0.5–2 mm) roots. Roots were removed from the soil, gently rinsed with DI water to remove soil particles, and placed into half pint canning jars ($n = 2$) that were then sealed. Each jar contained approximately 10 g of fresh roots. The air in the jar was scrubbed with CO_2-free air for 5 min to remove atmospheric CO_2, and then were allowed to incubate at 15 °C in the dark for 18 h. At the end of that period, accumulated CO_2 was collected for isotopic analysis as described for the soil incubation.

To calculate the $\Delta^{14}C$ value to use as the autotrophic end member for respiration partitioning, we combined the direct measurements of root respiration with estimates of moss respiration, as chambers in the field included living moss in addition to roots. We did not measure the $\Delta^{14}C$ value of moss respiration directly, but instead assumed it contributed CO_2 with a $\Delta^{14}C$ value similar to the current atmosphere. To calculate the $\Delta^{14}C$ value of total autotrophic respiration, we weighted contributions from moss and fine roots in proportion to their relative biomass. The average ratio at the CPCRW and Tower sites was 6 : 1, fine root biomass : green moss biomass. This yielded a $\Delta^{14}C$ value for autotrophic respiration that was 14‰ higher than contemporary atmospheric values, which we used for all three of our sites (Fig. 2). Use of a single value assumes that the root respiration ^{14}C signature and

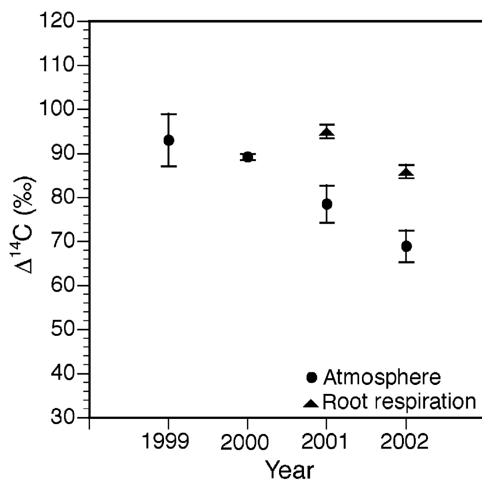

Fig. 1 Radiocarbon values for atmospheric CO_2 (●) and for root respiration (▲) measured in Interior Alaska (± SE). Atmospheric measurements were made each summer. The annual decline reflects the global pattern of fossil fuel dilution of radiocarbon in the atmosphere, along with biospheric and stratospheric CO_2 exchange. The root respiration value was directly measured in 2002, and estimated for 2001 based on a dominant contribution from a recent photosynthate pool that reflects the atmospheric values, plus a minor contribution from a storage pool of C in black spruce that appears to contribute to root respiration.

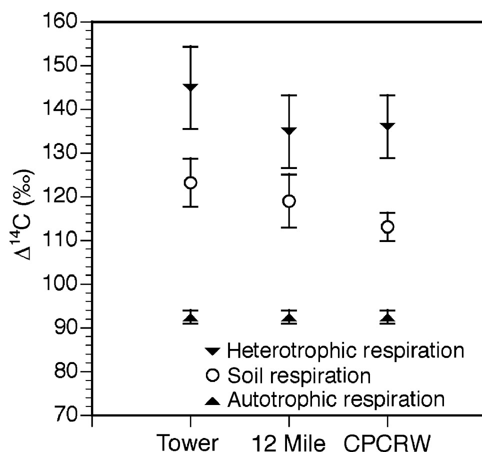

Fig. 2 Radiocarbon values for heterotrophic respiration (▼), total soil respiration (○), and autotrophic respiration (▲) (± SE).

the proportional contribution of moss and roots to autotrophic respiration are the same across our three mature forest sites. The sensitivity of our results to these assumptions is reviewed in the Discussion section.

Analyses

The soil respiration isotope value is a combination of CO_2 derived from autotrophic and heterotrophic respiration. Therefore, we used the isotope value of autotrophic and heterotrophic respiration as end members in a two pool mixing model. From that, we calculated the proportional contribution of heterotrophic respiration to total soil respiration. For the respiration partitioning error determination, we used the method of Phillips & Gregg (2001) to account for variation in both the isotopic value of the soil respiration, and the variation of the isotopic composition of the end members. This method uses the standard deviation of the isotopic observations in combination with the number of replicates in order to calculate an error term for the partitioning calculation.

For most other statistical analyses, we used *t*-tests and ANOVA to distinguish differences among groups, and correlation to look at covariance between variables (SPSS 11.0).

Results

End-member determination

Root respiration. We found that the root respiration $\Delta^{14}C$ in 2002 was significantly higher than local atmospheric $\Delta^{14}C$ measurements from the same year ($P = 0.035$) (Fig. 1). These measurements suggested that the assumption that the $\Delta^{14}C$ isotope value for autotrophic respiration would be identical to that of atmospheric CO_2 was not valid in this study. Instead, it appeared that at least some fraction of the root respiration derived from a storage pool reflecting atmospheric $\Delta^{14}C$ from previous years.

Because the global (Levin & Hesshaimer, 2000) and local (Fig. 1) atmospheric $\Delta^{14}C$ is decreasing each year because of fossil fuel burning and biospheric exchange of CO_2, the root respiration $\Delta^{14}C$ in 2001 at the time of the field measurements and soil incubations is expected to be higher than what we directly measured in our incubations in 2002. We estimated the 2001 root respiration (+ 95‰) assuming the difference in ^{14}C between root respiration and atmospheric CO_2 in 2001 was the same as in 2002; in other words, that most of the C respired by roots was still dominated by recent photosynthate and that this component would have declined at the same rate as atmospheric CO_2.

Heterotrophic respiration. To determine the heterotrophic end member for the partitioning experiment, we used the measured $\Delta^{14}C$ value from the organic and mineral soil incubations and calculated a weighted $\Delta^{14}C$ value that would be expected to be emitted under field conditions (Table 2). The $\Delta^{14}C$ signature of respired CO_2 from all sites and horizons was dominated by C fixed as atmospheric nuclear weapons testing, even

Table 2 Fluxes and isotopes of carbon dioxide from 15 °C laboratory incubations of organic and mineral soil horizons

Site	Soil horizon	Carbon flux (μg C g dw^{-1} day^{-1}) (± SE)	Temperature dependency Q_{10} (± SE)	δ^{13}C (‰) (± SE)	Δ^{14}C (‰) (± SE)
Tower	Organic	327.2 (103.4)	3.8 (0.5)	−27.22 (0.17)	+ 137 (15)
	Mineral	9.4 (1.6)	2.9 (0.4)	−26.86 (0.16)	+ 180 (23)
Twelve Mile	Organic	434.1 (70.8)	3.0 (0.2)	−27.65 (0.14)	+ 142 (2)
	Mineral	12.6 (3.5)	3.5 (0.2)	−27.01 (0.68)	+ 68 (26)
CPCRW	Organic	448.3 (81.2)	2.8 (0.3)	−28.50 (0.07)	+ 143 (2)
	Mineral	46.4 (23.9)	3.3 (1.0)	−27.49 (0.15)	+ 135 (15)

Temperature dependency was calculated by comparing flux rate at 15 °C to flux rate of paired incubations at 5 °C.
CPCRW, Caribou Poker Creek Research Watershed.

though the organic matter in these soils has been accumulating for more or less the past century. Positive Δ^{14}C values respired from the mineral horizon suggests that either root growth or downward C transport has transferred younger C into mineral soils, where in general the soil organic matter is comprised of much older C (Gaudinski *et al*., 2000). Elevated values relative to the 2001 atmospheric isotope value (+ 78‰) is a result of the decomposition of organic matter that had resided in the ecosystem for some years to decades, thus it reflected the higher atmospheric Δ^{14}C values of the recent past for most soils. The mineral soil at one site had a respiration Δ^{14}C value below the current atmosphere indicating a relatively longer turnover time compared with the others. The Δ^{14}C values of respired CO_2 are consistent with an average 'age' of ~ 9 years for the organic horizons and ~ 72 years for the mineral horizons, although these 'ages' should be interpreted with caution as there are a number of potential contributing pools to that respiration flux such as roots, dead moss, wood, and humus (Dioumaeva *et al*., 2003). The increased residence time of C in the mineral horizons is reflected in enriched δ^{13}C of respired CO_2 that could be a result of fractionation during decomposition, or other processes affecting soil organic matter (Nadelhoffer & Fry, 1988; Ehleringer *et al*., 2000).

To determine the heterotrophic respiration end member under field conditions, we combined: (1) differences in organic horizon and mineral horizon C mass with, (2) differences in respiration rate per unit mass, and with (3) the temperature dependency of C flux to account for differences in thermal regime between horizons (average summer temperatures at 2 cm was ~ 10 °C and at 11 cm was ~ 5 °C). Across all sites and all cores, the organic horizon respired 5–90 times the amount of CO_2 per gram soil compared with the mineral soil (Table 2). This difference was in part offset by the larger mass of soil in the mineral horizon, which ranged between three and eight times greater mass than that contained in the organic horizon (Table 1). The temperature sensitivity in CO_2 evolution rate is reported as a Q_{10} equivalent value; these ranged from 2.8 to 3.8 (Table 2). The combination of these three effects weighted the organic soil flux (and Δ^{14}C values) from 4.6 times greater at the CPCRW site and 5.5 times greater at the Tower site, to 31.5 times greater at the Twelve Mile site compared with the mineral soil flux. The effect of this difference in fluxes was that the organic horizon played the dominant role in determining the total isotope flux, while the mineral soil only had a minor effect in modifying the net isotopic value estimated for the heterotropic end member. In sum, the end-member isotope value for heterotrophic respiration was estimated to range from + 135‰ to + 145‰ across all sites, and was not significantly different among sites (Fig. 2).

Soil respiration and partitioning. Soil respiration Δ^{14}C values corrected for residual air had a small range across sites from + 109‰ to 123‰, but there were no significant differences between dates at any of the sites (Table 3). Respiration rates showed more variability, ranging from ~ 75 to 150 mg C m^{-2} hr^{-1} for fluxes measured in June and August 2001. While temporal coverage was sparse, lower fluxes were observed in June and higher fluxes in August, although this difference was larger at CPCRW and smaller at the Tower site. These flux measurements made in conjunction with the isotope sampling were consistent with more extensive soil respiration measurements that were made at the sites (unpublished data). Because there were no differences at a site between sampling dates, we calculated a single, flux-weighted mean for each site to compare with autotrophic and heterotrophic end-member isotope sources (Fig. 2). These values ranged from + 113‰, to + 123‰, but again were not

Table 3 Soil respiration fluxes and isotopes from three black spruce forests in Alaska

Site	Date	Carbon flux ($mg\,C\,m^{-2}\,hr^{-1}$) (± SE)	$\Delta^{14}C$ (‰) (± SE)	*N*
Tower	June 13	75.6 (32.3)	123 (10)	3
	August 27	88.5 (11.2)	123 (7)	3
Twelve Mile	–	–	–	–
	August 28	90.9 (10.8)	119 (7)	3
CPCRW	June 15	68.9 (9.2)	109 (1)	2
	August 1	152.8 (46.8)	115 (6)	2

CPCRW, Caribou Poker Creek Research Watershed.

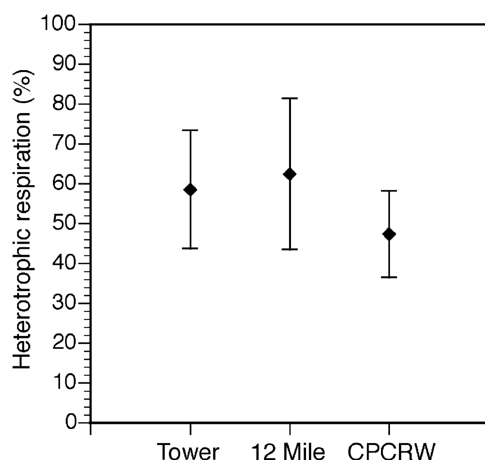

Fig. 3 The proportion of heterotrophic respiration calculated using an end-member mixing model. The standard error bars include the variability in both the source pool and the mixed pool observations.

significantly different from one another (Fig. 2). In general, the Tower site with the highest heterotrophic $\Delta^{14}C$ value also had the highest soil respiration $\Delta^{14}C$ value while the CPCRW site had the lowest $\Delta^{14}C$ value for both.

For all sites, the soil respiration $\Delta^{14}C$ was intermediate in value and significantly different ($P < 0.05$) from both the heterotrophic and autotrophic end members at all sites. We used the isotope value of autotrophic and heterotrophic respiration end members to calculate the proportional contribution of heterotrophic respiration to total soil respiration (Fig. 3). All three sites ranged from 47% to 63% heterotrophic respiration, with the Twelve Mile site having the highest proportion of soil respiration derived from heterotrophic respiration, while the CPCRW site had the lowest proportion. However, using the full error term based on the variability of both the end-member pools (root and soil incubations) and the observed mixture (soil respiration), the three sites were not significantly different from one another (Phillips & Gregg, 2001).

Discussion

Combining radiocarbon measurements from soil respiration with soil and root incubations proved to be a useful approach for separating the proportional contribution of fast-cycling C, largely derived from autotrophs, and slower-cycling C respired by heterotrophs, to total soil respiration. Root respiration $\Delta^{14}C$ was elevated relative to the atmosphere (Fig. 1) suggesting that there was a storage pool of C that had some minor, but measurable, contribution to current respiration by the roots. In comparison, heterotrophic respiration $\Delta^{14}C$, consistent with a mean turnover time of about 8–9 years, was even more elevated relative to the current atmosphere. While the heterotrophic respiration isotopic signature is the net value derived from the decomposition of a wide array of substrates with different turnover times, the elevated $\Delta^{14}C$ values suggest a large contribution from C that had resided in the ecosystem for the better part of a decade (Fig. 2), both as living plant tissue and as dead organic matter. Field measurements of the soil respiration $\Delta^{14}C$ values were intermediate between our estimates of the heterotrophic and autotrophic end-member isotopic values reflecting the contribution of both of these sources to total soil respiration (Fig. 2). This large difference in the end-member values allowed the source partitioning calculation to be successful, reflected in the similar result obtained in three different forests.

The CPCRW site had a somewhat lower mean contribution from heterotrophic respiration to total soil respiration, while the Twelve Mile site had the highest mean contribution. While no single-site factor appeared to explain this difference, the proportion of heterotrophic respiration across sites was correlated with the soil flux (organic soil mass (Table 1) multiplied by organic soil respiration per unit mass (Table 2)) divided by the stand density (Pearson's $r = 0.839$), but of course the number of sites is low. This ratio is related to changes in both the amount and respiration rate of soil, and indirectly to the density of fine roots. However, given the within-site variability in the isotopic measurements and error propagation, sites did not differ from one another using standard ANOVA. In some ways, these can be viewed as replicate mature black spruce forests for this partitioning experiment even though they differed somewhat in stand density and soil characteristics. Somewhat unexpectedly, we saw no differences in early vs. late season in the $\Delta^{14}C$ value of soil respiration

and thus no seasonal change in the respiration partitioning (Table 3). This could be in part because of limited sampling, or potentially to compensating seasonal shifts in the $\Delta^{14}C$ value of respiration sources. For example, roots may use more or less storage C to fuel metabolism and growth depending on photosynthetic rates. Or, changes in soil or moss temperature and moisture conditions may influence their proportional contribution. These factors remain to be tested experimentally, but sensitivity analyses can help determine which may be important.

The calculation of the relative contribution of autotrophic and heterotrophic respiration to total soil respiration is dependent on the isotopic values of the end members. Differences in how the experiment was performed could potentially have an effect on the partitioning of respiration. For example, extending the time period before sampling the soil incubation CO_2 could perhaps increase the isotope value of the heterotrophic end member as a more labile C pool, with lower $\Delta^{14}C$ content (closer to the current atmosphere) would presumably be depleted from the sample. A 5‰ increase in the $\Delta^{14}C$ value would have the effect of about a 10% decrease in the estimated proportion of heterotrophic respiration to 39% at CPCRW, 51% at Tower, and 53% at Twelve Mile. A hypothetical 5‰ increase represents the depletion of a labile pool with an isotopic signature similar to root respiration that comprised 10% of the soil incubation flux. However, very little data exists that supports the idea that the radiocarbon signature could change in such a short time frame. The trend of changing isotopic signature over time (months) from incubations of soil was not observed in a longer incubation of peat material from Canada (Dioumaeva *et al*., 2003), but repeated measurements of radiocarbon at shorter time intervals (weeks) have not been made.

Variation in the autotrophic end-member isotope value can have a similar, although opposite, effect on the respiration partitioning calculation. The high $\Delta^{14}C$ values we observed for root respiration (Fig. 1) were not expected, and further research efforts are required to understand their cause. High $\Delta^{14}C$ values could potentially have been an artifact of the incubation time in this experiment (long incubation times may favor contributions from larger diameter roots that can store more C), or biases in the sampling of roots themselves. However, other recent studies using different experimental methods and shorter incubations times have found similar results. Root respiration isotope values in a ^{14}C-labeled temperate forest again reflected the influence of C storage pools in shorter term (1–2 h) incubations (Cisneros-Dozal *et al*., 2005). Other short-term root incubations in Canadian black spruce forests have revealed similar elevated patterns compared with the atmosphere, at least during some times of the year (Czimczik & Trumbore, 2003).

Given that the $\Delta^{14}C$ of atmospheric CO_2 drops by 5–8‰ per year, a 16‰ enrichment of root respiration over contemporary CO_2 values means that, on average, the C being respired was fixed $\sim$ 3 years ago. However, it is clear from the rapid drop observed in soil respiration in girdling experiments that a substantial fraction of root respiration must be derived from recent photosynthetic products (Högberg *et al*., 2001). If we assume 50% of the CO_2 respired by roots is fixed within the current growing season, then the remaining 50% must be enriched by 28‰, or reflect C that was fixed on average 4–5 years ago. Similarly, assuming that 90% of the CO_2 is fixed in the current growing season requires that the remaining stored C source that contributes the remaining 10% of root respiration has $\Delta^{14}C$ values of 140‰ and an average age of $\sim$ 25 years. Distinguishing among these possibilities requires that we identify and determine the $\Delta^{14}C$ values of the stored C that feeds root respiration, likely nonstructural carbohydrates. Future studies should attempt to measure the $\Delta^{14}C$ values of nonstructural carbohydrates directly, and/or conduct longer incubations of roots with multiple sampling points to see if the isotopic signature of respired C changes with time.

If the isotopic value of root respiration were exactly the same as the atmospheric value this would have the effect of a 17–30% increase in the estimated proportion of heterotrophic respiration to 60% at CPCRW, 67% at Tower, and 72% at Twelve Mile. This represents the upper end of the range of possible autotrophic end-member values, and moves the partitioning estimate in the same direction as would an increase in the proportional contribution of moss respiration (assuming an isotopic value the same as the atmosphere) to total soil respiration. As mosses, unlike trees, lack mechanisms for storing C long term, this appears to be a reasonable assumption. However, if mosses fix a significant quantity of soil respired CO_2, the $\Delta^{14}C$ value of moss respired CO_2 would reflect the fact that soil respiration has a higher $\Delta^{14}C$ value than contemporary atmospheric CO_2. In these forests, there is very little to no daytime gradient of CO_2 because of the open nature of the black spruce canopy. In other systems with even more closed tree canopies, measurements of tissue that was identified to have grown in the current year did not differ from local atmospheric measurements, indicating that recycling was not detectable in the $\Delta^{14}C$ measurement (Gaudinski *et al*., 2001). In any case, recycling (photosynthetic fixation of respired CO_2) is more of a concern for $\delta^{13}C$ compared with $\Delta^{14}C$ because of the larger proportional difference between respiration and the atmosphere isotope values for $\delta^{13}C$.

While these sensitivity analyses demonstrate the effect of changing isotopic values of the autotrophic and heterotrophic end members, this partitioning experiment clearly shows that heterotrophic respiration comprises at least half, and in several cases the majority of total soil respiration, up to 63%. This agrees with other measurements in black spruce forest in boreal Canada where heterotrophic respiration contributed around 50% of total summertime soil respiration (O'Connell *et al*., 2003). Also, our data correspond well with the results of a recent, comprehensive review of respiration partitioning experiments, where mature forests with relatively low total soil respiration, similar to these forests ($\sim 500\,g\,m^{-2}\,yr^{-1}$), generally had heterotrophic respiration contributing $>50\%$ of total soil respiration (Bond-Lamberty *et al*., 2004). In contrast, several other studies in mature Alaskan boreal black spruce forest using trenching experiments or other physical methods of separating roots and soil in the field have found that autotrophic respiration comprises the majority of total soil respiration (Vogel *et al*., in press). This apparent discrepancy with our results may be explained, in part, by the nature of what is estimated as heterotrophic respiration. As described earlier, a longer measurement period for heterotrophic respiration in isolation from roots leads to more depletion of the labile soil organic matter pool in the rhizosphere. Trenching experiments that remove sources of root exudates would presumably remove this fraction of labile C decomposed by heterotrophs (Hanson *et al*., 2000). In those experiments, this labile fraction would be ascribed to the estimate of autotrophic respiration, as it is calculated as the difference between intact control and trenched plot measurements. In contrast, our relatively short incubations probably still included a large fraction of the labile C pools decomposed by microbes as part of the heterotrophic contribution, thus leading us to our higher estimation of that component. The partitioning review found no significant difference among experimental techniques that might relate to artifacts such as these, but isotope studies generally appeared too few in number to be evaluated effectively (Bond-Lamberty *et al*., 2004).

In conclusion, source partitioning using isotopes (radiocarbon in particular) is a good, independent, approach for separating respiration sources. Additionally, because radiocarbon values indicate age, these isotope measurements can provide important new insight into plant and ecosystem C cycling not available with other methods. Here, the data suggest that stored C in these long-lived trees may play a role in the annual cycle of C in these forests. For all partitioning approaches it is important to recognize the assumptions of the method, especially when comparing with other methods. When the assumptions are different, it is particularly instructive to compare different methodologies for source partitioning within the same ecosystem. One advantage of isotope partitioning is that this approach allows the exploration of finer time scales than is possible with trenching or girdling. Future experimentation with this technique includes multiple measurements of soil respiration coupled with repeated root and soil incubations to determine the end-member isotopic values and changes in respiration partitioning over the course of the growing season (see Cisneros-Dozal *et al*., 2005). In addition, radiocarbon partitioning could be coupled with stable C isotope partitioning. This dual C isotope approach will help further resolve component pools and their contributions to soil and ecosystem respiration (Schuur *et al*., 2003).

Acknowledgements

We would like to thank Terry Chapin and the BNZ-LTER for access to field sites, and the use of equipment and laboratory space. We also thank Grace Crummer for a constructive critique of this manuscript. This research is based upon work supported by an NSF Postdoctoral Research Fellowship in Biological Informatics to EAGS; a NSF EAR Grant #0223193 to EAGS and SET; a NASA New Investigator Award to EAGS; a A. W. Mellon Foundation grant to EAGS; and an A. W. Mellon Foundation grant to SET.

References

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.

Bauer J, Williams PM, Druffel ERM (1992) Recovery of submilligram quantities of carbon dioxide from gas streams by molecular sieve for subsequent determination of isotopic natural abundance. *Analytical Chemistry*, **64**, 824–827.

Benner R, Fogel ML, Sprague EK *et al*. (1987) Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. *Nature*, **329**, 708–710.

Bond-Lamberty B, Wang C, Gower ST (2004) A global relationship between the heterotrophic and autotrophic components of soil respiration? *Global Change Biology*, **10**, 1756–1766.

Bowden RD, Nadelhoffer KJ, Boone RD *et al*. (1993) Contributions of aboveground litter, belowground litter, and root respiration to total soil respiration in a temperate mixed hardwood forest. *Canadian Journal of Forest Research*, **23**, 1402–1407.

Burton AJ, Pretziger KS, Zogg GP, Zak DR *et al*. (1998) Drought reduces root respiration in sugar maple forests. *Ecological Applications*, **3**, 771–778.

Burton AJ, Zogg GP, Pretziger KS *et al*. (1997) Effect of measurement CO_2 concentration on sugar maple root respiration. *Tree Physiology*, **17**, 421–427.

Cisneros-Dozal LM, Trumbore SE, Hanson PJ (2005) Partitioning sources of soil-respired CO_2 and their seasonal variation using a unique radiocarbon tracer. *Global Change Biology*, doi: 10.1111/j.1365–2486.2005.01061.x

Czimczik CI, Trumbore SE (2003) Changing sources of respired CO_2 with time since fire in a boreal forests. *Eos Transactions on AGU*, **84** (Fall Meet. Suppl.) (Abstract B22B-03) www.agu.org

Davidson EA, Verchot LV, Henrique Cattanio J *et al*. (2000) Effects of soil water content on soil respiration in forests and cattle pastures of eastern Amazonia. *Biogeochemistry*, **48**, 53–69.

Davidson EA, Richardson AD, Savage KE *et al*. (2005) A distinct seasonal pattern of the ratio of soil respiration to total ecosystem respiration in a spruce-dominated forest. *Global Change Biology*, doi:10.1111/j.1365–2486.2005.01062.x.

Dawson TE, Mambelli S, Plamboeck AH *et al*. (2002) Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics*, **33**, 507–559.

Dioumaeva I, Trumbore SE, Schuur EAG *et al*. (2003) Decomposition of peat from upland boreal forest: temperature dependence and sources of respired carbon. *Journal of Geophysical Research*, **108**, 8222.

Ehleringer JR, Buchmann N, Flanagan LB (2000) Carbon isotope ratios in belowground carbon cycle processes. *Ecological Applications*, **10**, 412–422.

Enting IG, Trudinger CM, Francey RJ (1995) A synthesis inversion of the concentration and delta^{13}C of atmospheric CO_2. *Tellus*, **47B**, 35–52.

Ewel KC, Cropper WPJ, Gholz HL (1987) Soil CO_2 evolution in Florida slash pine plantations. II. Importance of root respiration. *Canadian Journal of Forest Research*, **17**, 330–333.

Francey RJ, Gifford RM, Sharkey TD *et al*. (1985) Physiological influences on carbon isotope discrimination in huon pine (*Lagarostrobus franklinii*). *Oecologia*, **66**, 469–481.

Fung IY, Field CB, Berry JA *et al*. (1997) Carbon-13 exchanges between the atmosphere and biosphere. *Global Biogeochemical Cycles*, **11**, 507–533.

Gardenas AI (2000) Soil respiration fluxes measured along a hydrological gradient in a norway spruce stand in soiuth Sweden (Skogaby). *Plant and Soil*, **221**, 273–280.

Garten CT, Cooper LW, Post WM *et al*. (2000) Climate controls on forest soil C isotope ratios in the Southern Appalachian Mountains. *Ecology*, **81**, 1108–1119.

Gaudinski JB, Trumbore SE, Davidson EA *et al*. (2000) Soil carbon cycling in a temperate forest: radiocarbon-based estimates of residence times, sequestration rates and partitioning of fluxes. *Biogeochemistry*, **51**, 33–69.

Gaudinski JB, Trumbore SE, Davidson EA *et al*. (2001) The age of fine-root carbon in three forests of the Eastren United States measured by radiocarbon. *Oecologia*, **129**, 420–429.

Hanson PJ, Edwards NT, Garten CTJ *et al*. (2000) Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry*, **48**, 115–146.

Högberg P, Nordgren A, Buchmann N *et al*. (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, **411**, 789–792.

Hungate BA, Holland EA, Jackson RB *et al*. (1997) The fate of carbon in grasslands under carbon dioxide enrichment. *Nature*, **388**, 576–579.

Janssens IA, Lankreijer H, Matteucc G *et al*. (2001) Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. *Global Change Biology*, **7**, 269–278.

Kelting DL, Burger JA, Edwards GS (1997) Estimating root respiration, microbial respiration in the rhizosphere, and root-free soil respiration in forest soils. *Soil Biology and Biochemistry*, **30**, 961–968.

Leavitt SW, Long A (1982) Evidence for $^{13}C/^{12}C$ fractionation between tree leaves and wood. *Nature*, **298**, 742–744.

Leavitt SW, Long A (1986) Stable carbon isotope variability in tree foliage and wood. *Ecology*, **67**, 1002–1010.

Levin I, Hesshaimer V (2000) Radiocarbon – a unique tracer of global carbon cycle dynamics. *Radiocarbon*, **42**, 69–80.

Luo Y, Wan S, Hui D (2001) Acclimatization of soil respiration to warming in a tall grass prairie. *Nature*, **413**, 622–625.

Martinelli LA, Almeida S, Brown IF *et al*. (1998) Stable carbon isotope ratio of tree leaves, boles and fine litter in a tropical forest in Rondonia, Brazil. *Oecologia*, **114**, 170–179.

Nadelhoffer KJ, Fry B (1988) Controls of natural ^{15}N and ^{13}C abundances in forest soil organic matter. *Soil Science Society of America Journal*, **52**.

Norman JM, Kucharik CJ, Gower ST (1997) A comparison of six methods for measuring soil-surface carbon dioxide fluxes. *Journal of Geophysical Research*, **102**, 28771–28777.

O'Connell KEB, Gower ST, Norman JM (2003) Net ecosystem production of two contrasting boreal black spruce forest communities. *Ecosystems*, **6**, 248–260.

Palta JA, Nobel P (1989) Influence of soil O_2 and CO_2 on root respiration for *Agave deserti*. *Physiological Planta*, **76**, 187–192.

Park R, Epstein S (1961) Metabolic fractionation of ^{13}C and ^{12}C in plants. *Plant Physiology*, **36**, 133–138.

Pataki DE, Ellsworth DS, Evans RD *et al*. (2003) Tracing changes in ecosystem function under elevated carbon dioxide conditions. *Bioscience*, **53**, 805–818.

Pate J, Arthur D (1998) Delta ^{13}C analysis of phloem sap carbon: novel means of evaluating seasonal water stress and interpreting carbon isotope signatures of foliage and trunk wood of Eucalyptus globulus. *Oecologia*, **117**, 301–311.

Pendall E, Del Grosso S, King JY *et al*. (2003) Elevated atmospheric CO_2 effects and soil water feedbacks on soil respiration components in a Colorado grassland. *Global Biogeochemical Cycles*, **17**, 1046.

Phillips DL, Gregg JW (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia*, **127**, 171–179.

Qi J, Marshall JD, Mattson KG (1994) High soil carbon dioxide concentrations inhibit root respiration of Douglas fir. *New Phytologist*, **128**, 435–442.

Raich JW, Schlesinger WH (1992) The global carbon dioxide flux in soil respiration and its relationship to climate. *Tellus*, **44B**, 81–99.

Randerson JT, Chapin FSI, Harden JW *et al*. (2002) Net ecosystem production: a comprehensive measure of net carbon accumulation by ecosystems. *Ecological Applications*, **12**, 937–947.

Robinson D, Scrimgeour CM (1995) The contribution of plant C to soil CO_2 measured using delta ^{13}C. *Soil Biology and Biochemistry*, **27**, 1653–1656.

Rochette P, Angers DA, Flanagan LB. (1999) Maize residue decomposition measurement using soil surface carbon dioxide fluxes and natural abundance of carbon-13. *Soil Science Society of America Journal*, **63**, 1385–1396.

Rochette P, Flanagan LB (1997) Quantifying rhizosphere respiration in a corn crop under field conditions. *Soil Science Society of America Journal*, **61**, 466–474.

Schuur EAG, Trumbore SE, Mack MC *et al*. (2003) Isotopic composition of carbon dioxide from a boreal forest fire: inferring carbon loss from measurements and modeling. *Global Biogeochemical Cycles*, **17**, 1001.

Scott-Denton LE, Rosenstiel TN, Monson RK (2005) Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Global Change Biology*, doi: 10.1111/j.1365–2486.2005.01064.x

Stuiver M, Polach H. *et al*. (1977) Reporting of ^{14}C data. *Radiocarbon*, **19**, 355–363.

Trolier M, White JWC, Tans PP *et al*. (1996) Monitoring the isotopic composition of atmospheric CO_2: measurements from the NOAA global air sampling network. *Journal of Geophysical Research*, **101**, 25897–25916.

Trumbore SE (2000) Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. *Ecological Applications*, **10**, 399–411.

Valentini R, Matteucci G, Dolman AJ *et al*. (2000) Respiration as the main determinant of carbon balance in European forests. *Nature*, **404**, 861–865.

Vogel JG, Valentine D, Ruess R Soil and root respiration in mature Alaskan black spruce forests that vary in soil organic matter decomposition rates. *Canadian Journal of Forest Research*, **35**, 161–174.

Vogel JS (1992) A rapid method for preparation of biomedical targets for AMS. *Radiocarbon*, **34**, 344–350.