

UC Irvine

UC Irvine Previously Published Works

Title

Partitioning sources of soil-respired CO₂ and their seasonal variation using a unique radiocarbon tracer

Permalink

<https://escholarship.org/uc/item/7x42q2wq>

Journal

Global Change Biology, 12(2)

ISSN

1354-1013

Authors

CISNEROS-DOZAL, LUZ MARIA
TRUMBORE, SUSAN
HANSON, PAUL J

Publication Date

2006-02-01

DOI

10.1111/j.1365-2486.2005.001061.x

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

Partitioning sources of soil-respired CO₂ and their seasonal variation using a unique radiocarbon tracer

LUZ MARIA CISNEROS-DOZAL*, SUSAN TRUMBORE* and PAUL J. HANSON†

*Department of Earth System Science, University of California, Irvine, California 92697-3100, USA, †Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831-6422, USA

Abstract

Soil respiration is derived from heterotrophic (decomposition of soil organic matter) and autotrophic (root/rhizosphere respiration) sources, but there is considerable uncertainty about what factors control variations in their relative contributions in space and time. We took advantage of a unique whole-ecosystem radiocarbon label in a temperate forest to partition soil respiration into three sources: (1) recently photosynthesized carbon (C), which dominates root and rhizosphere respiration; (2) leaf litter decomposition and (3) decomposition of root litter and soil organic matter >1–2 years old.

Heterotrophic sources and specifically leaf litter decomposition were large contributors to total soil respiration during the growing season. Relative contributions from leaf litter decomposition ranged from a low of $\sim 1 \pm 3\%$ of total soil respiration ($6 \pm 3 \text{ mg C m}^{-2} \text{ h}^{-1}$) when leaf litter was extremely dry, to a high of $42 \pm 16\%$ ($96 \pm 38 \text{ mg C m}^{-2} \text{ h}^{-1}$). Total soil respiration fluxes varied with the strength of the leaf litter decomposition source, indicating that moisture-dependent changes in litter decomposition drive variability in total soil respiration fluxes. In the surface mineral soil layer, decomposition of C fixed in the original labeling event (3–5 years earlier) dominated the isotopic signature of heterotrophic respiration.

Root/rhizosphere respiration accounted for $16 \pm 10\%$ to $64 \pm 22\%$ of total soil respiration, with highest relative contributions coinciding with low overall soil respiration fluxes. In contrast to leaf litter decomposition, root respiration fluxes did not exhibit marked temporal variation ranging from 34 ± 14 to $40 \pm 16 \text{ mg C m}^{-2} \text{ h}^{-1}$ at different times in the growing season with a single exception ($88 \pm 35 \text{ mg C m}^{-2} \text{ h}^{-1}$). Radiocarbon signatures of root respired CO₂ changed markedly between early and late spring (March vs. May), suggesting a switch from stored nonstructural carbohydrate sources to more recent photosynthetic products.

Key words: ¹⁴C, litter decomposition, radiocarbon, root respiration, soil respiration sources

Received: July 23, 2004; *accepted:* October 11, 2004

Introduction

A key area of uncertainty in the terrestrial C cycle concerns the processes, referred to collectively as respiration, that control the vast majority of C lost annually. Carbon is added to ecosystems through photosynthesis and lost either rapidly, by fueling plant metabolism and growth, or more slowly, through the decomposition of dead plant organic matter. The por-

tion of total ecosystem respiration that takes place below-ground, soil respiration, has been estimated in forests to be as much as 69% of the total ecosystem respiration (plant and soil) and 55% of the carbon assimilated through photosynthesis annually (Janssens *et al.*, 2001). Besides representing a large loss of carbon, soil respiration is also a major contributor to inter-annual variability in the net ecosystem balance (Goulden *et al.*, 1996; Valentini *et al.*, 2000).

Soil respiration is derived from autotrophic and heterotrophic sources. Autotrophic respiration includes CO₂ derived from root metabolism and the activity of microorganisms in the rhizosphere. Heterotrophic respiration includes CO₂ released during microbial

Correspondence: Luz Maria Cisneros-Dozal, 3200 Croul Hall, Department of Earth System Science, University of California, Irvine, CA 92697-3100, USA. Fax: (949) 824 3874, e-mail: cisnerol@uci.edu

decomposition of soil organic matter. While autotrophic respiration is linked to the supply of photosynthetic products from plants, heterotrophic respiration is derived from several different kinds of dead plant material (leaf litter, root detritus, soil organic matter) available to decomposers. Knowledge of the relative contributions of the two sources is relevant to studies of C cycling as each source returns C to the atmosphere on different time scales (years or less through root/rhizosphere respiration vs. decades to centuries through soil organic matter decomposition). Furthermore, we need to better understand all potential factors (environmental and/or phenological) controlling their seasonal variation to make accurate predictions of future C sequestration in forests. Important questions include: (1) how much of total soil respiration comes from autotrophic vs. heterotrophic sources? (2) what portion of heterotrophic respiration comes from decomposition of different substrate types? and (3) how do changes in phenology, soil moisture and temperature, affect each of the sources of soil respiration?

Different approaches have been used to find the relative contributions to soil respiration. Three widely applied methods include component separation, root removal and the use of isotopes, described in detail by Hanson *et al.* (2000). Component separation extrapolates measurements of CO₂ flux for components (e.g. incubations, excised roots) to the soil volume, but can be subject to artifacts associated with respiration rates measured under non-field conditions and ultimately limited by knowledge of factors like the quantity of roots in the soil. Root removal is commonly accomplished by trenching or girdling trees, and is limited in the short term by the time it takes for roots to die and in the long term by enhanced decomposition of severed or dead roots. Isotope studies offer the advantage that measurements are made in intact systems, but they tend to be relatively expensive and typically require lumping respiration sources that have similar isotopic signatures.

Estimates of the relative contribution of root respiration to soil respiration vary considerably. Kelting *et al.* (1998) estimated root respiration to account for 32% of soil respiration by trenching in a red oak stand (*Quercus rubra* L.). Also using trenching methods, Epron *et al.* (1999) estimated root respiration to contribute 60% of the annual total CO₂ from soils in a beech stand (*Fagus sylvatica* L.). A large scale girdling experiment in a boreal forest (*Pinus sylvestris* L.) was used to estimate that root respiration accounted for up to 56% of total soil respiration in the middle of the summer, but recognized that the contribution could be even higher given the longevity of starch reserves in recently-severed roots (Högberg *et al.*, 2001). Another trenching

experiment at a smaller scale in a Mediterranean forest (*Quercus* spp.), indicated an annual contribution of 23%, but other factors such as enhanced decomposition in trenched plots and recent logging of the site could have affected the results (Rey *et al.*, 2002). The reporting of root respiration as a percentage of total respiration rather than an absolute quantity may be masking differences in root inventory, allocation, and total soil respiration among sites.

In this paper, we report on the application of a unique ¹⁴C tracer to the characterization of soil respiration components. Large enrichments of the ¹⁴C signatures of plants, litter and soil resulted from a release of ¹⁴C in 1999 from one or more hazardous waste incinerators near our study site on the Oak Ridge Reservation, Oak Ridge, Tennessee. This isotope label was incorporated into living plants to varying degrees according to distance from the source (Trumbore *et al.*, 2002). We used this unique ¹⁴C-tracer to describe how respiration sources vary with season and plant activity.

Methods

Site description

The study site is located in the Oak Ridge Reservation (ORR) of the Oak Ridge National Laboratory in East Tennessee, USA (35°58 N and 84°17 W). Mean annual precipitation is 1358 mm and mean annual temperature is 14.1 °C. Vegetation is dominated by *Quercus* spp and *Acer* spp (Johnson & Van Hook, 1989).

While the large ¹⁴C release occurred in July/August 1999, leaves did not show a strong increase in radiocarbon content until the following spring. We observed a large gradient in radiocarbon content of this new leaf growth, with highest amounts of incorporated label in the western portion of the reserve (Trumbore *et al.*, 2002). In the fall of 2000, leaf litter from two areas in the western and eastern portions of the ORR was collected, mixed (to homogenize), dried and stored. Repeated measurements of aliquots of the stored leaf litter showed that leaves collected from the western portion of the reserve had ¹⁴C values ranging from +952‰ to +1055‰, while the ones collected from the eastern reserve had values from +215‰ to +230‰. Hereafter, these will be referred to as 'HL' (high labeled) and 'LL' (low labeled) litter, respectively.

The Enriched Background Isotope Study (EBIS) established a total of four sites, two on Inceptisols and two on Ultisols, along the gradient of ¹⁴C label from the western to the eastern side of the reserve. The sites provided a gradient of differential labeling of plants, including roots, with high levels of label on the western side and low levels on the eastern side, hereafter

referred to as 'HR' (high labeled root) and 'LR' (low labeled root) sites, respectively. After preliminary measurements showed no large differences in the isotopic signature of soil respiration with soil type, we limited our measurements to two sites: Tennessee Valley Authority, hereafter referred to as 'TVA' (HR site) and Walker Branch (LR site) both on the Ultisol soil type.

At each site, a total of eight 7 m × 7 m plots were established to manipulate the ¹⁴C content of leaf litter. Natural leaf litter was excluded by covering plots with landscape cloth from October to November, and ambient litter was replaced with HL or LL litter over three consecutive years. Litter exclusions occurred in the fall of 2000, 2001, and 2002, and leaf litter additions took place in May 2001, February 2002 and February 2003, with 4 plots at each site receiving HL vs. LL litter. We used this factorial design, with combinations of HR-HL, HR-LL, LR-HL, LR-LL, together with the large ¹⁴C differences in sources among plots and sites, to partition soil respiration into root/rhizosphere respiration and decomposition sources.

Moisture and temperature measurements

Litter-layer water content and soil water status were measured at long-term reference plots at each site. Continuous direct measurements of forest litter water content were based on the electrical resistance characteristics of wet vs. dry litter (Hanson *et al.*, 2003). Buried frequency domain reflectometer waveguides (CS615; Campbell Scientific, Inc., Logan, Utah) were installed in the A and B horizons (~30 cm). All data were logged as hourly means and stored on a data logger (CR10; Campbell Scientific, Inc., Logan, Utah). Additional soil temperature (between 0 and 5 cm depth into the mineral soil) and air temperature data were collected during field measurements.

Determination of ¹⁴C signatures

Total soil respiration

Soil respiration was measured concurrently with sampling for isotopes in the months of May, July and September of 2002, March, May, September and October of 2003 and March of 2004 at the two sites. The isotopic signatures measured in March 2004 at the TVA site were not used as they were confounded by an unexpected new and localized release of ¹⁴C on the date of our measurements. This new release event did not affect the isotopic signatures measured at the Walker Branch site. Measurements were carried out in 6 plots per site including 3 plots with HL and 3 with LL litter treatment.

Closed dynamic chambers were used to measure total soil respiration and to collect CO₂ for radiocarbon analysis as described by Gaudinski *et al.* (2000). Briefly, an infrared gas analyzer (LI-800, LiCor, Inc. Lincoln, Nebraska) was attached to a pumping system that circulated air to and from the chamber, and the CO₂ concentration increase was monitored for approximately 5 to 10 min. During this time, CO₂ concentrations rose from 380–400 ppm to 500–900 ppm depending on the respiration rates. The rate of increase was usually linear with a few exceptions due to either pump failure or to the effect of placing the chamber lid on the collar which sometimes caused an initial perturbation of the CO₂ flux that lasted for 40 seconds to 1 minute. Non-linear rates of CO₂ increase were not considered in the estimation of fluxes. Once the flux was measured, the air in the headspace of the chamber was scrubbed of CO₂ initially present (which has a different radiocarbon signature from that of soil respiration) by passing the air coming from the chamber through soda lime. The scrubbing was complete when 3 times the volume of the chamber passed through soda lime which took ~40 min at flow rates of ~0.8 L min⁻¹. Low flow rates (<1 L min⁻¹) were desirable for preventing disturbance of the CO₂ concentrations in the soil profile beneath the chamber. The concentration of CO₂ in the chamber reached a steady state value at the end of the scrubbing period that depended on the soil respiration and air circulation rates, but usually remained higher than ambient CO₂ concentrations (between 500 and 600 ppm). When scrubbing was complete, the flow was redirected to a U-shaped tube filled with molecular sieve (mesh size 13X; Advanced Speciality Gas Equipment, South Plainfield, NJ, USA) for trapping of the CO₂ emitted inside the chamber. A container filled with drierite (calcium sulfate) was placed before the trap to remove water that would decrease the efficiency of the molecular sieve (Gaudinski *et al.*, 2000). Activated molecular sieve traps CO₂ at ambient temperatures and releases it when heated at 475 °C (Bauer *et al.*, 1992). The trapping time depended on the respiration rates, with the goal of collecting sufficient carbon for ¹⁴C and ¹³C analysis (about 2–3 mg C total). CO₂ collection from all 6 plots at each site took place between 10 am and 5 pm.

Molecular sieve traps were taken to the University of California, Irvine (UCI) where CO₂ was released and the traps reactivated by baking at 610 °C. The released CO₂ was purified cryogenically and converted to graphite using the Zn reduction method (Vogel, 1992). An aliquot of each ¹⁴C sample was analyzed for ¹³C using continuous flow isotope ratio mass spectrometry at UCI. Approximately 0.1 micro liters of purified CO₂ were removed from the vacuum line with a syringe and injected into a He-flushed septum-capped vial. The

isotopic signature of the CO₂ was measured using a Gas bench II inlet to a Delta-plus stable isotope mass spectrometer.

Graphite targets were sent to Lawrence Livermore National Laboratory (LLNL) for ¹⁴C analysis by accelerator mass spectrometry. The results are expressed in Δ¹⁴C the deviation from a standard (oxalic acid) in per mil (‰):

$$\Delta^{14}\text{C} = \left[\frac{^{14}\text{C}/^{12}\text{C}]_{\text{sample}}}{^{14}\text{C}/^{12}\text{C}]_{\text{standard}}} - 1 \right] \times 1000,$$

where the ¹⁴C/¹²C ratio of the sample is corrected for mass-dependent isotope fractionation to a common value of -25‰ in δ¹³C (Stuiver & Polach, 1977). Overall accuracy of our Zn-reduced targets measured at LLNL, based on repeated measures of secondary standards (oxalic acid 2, ANU, Tiriwood), is ± 5 per mil for modern samples.

At each site, we obtained the ¹⁴C signature of total soil respiration (hereafter referred to as Δ¹⁴C_{total respiration}) from each of the 6 plots. The values per treatment reported here and used in the calculations are the means (± standard deviation) of three measurements. We tested for significance of differences of these means between treatments using one-way ANOVA.

Heterotrophic respiration

We collected leaf litter (Oi plus Oe/Oa horizons) and soil cores representing the top 5 cm of mineral soil from each of the six plots (3 plots with HL and 3 with LL litter treatment) at each site. We did not sample deeper soil layers because heterotrophic respiration sources decrease sharply with depth and only ~ 25% of total fluxes originated in the mineral soil comes from layers between 8 to 70 cm depth (E and B horizons) at this site (Gaudinski and Trumbore, 2003). Soil and leaf litter samples were incubated separately as to obtain independent radiocarbon signatures for each horizon. To calculate the isotopic signature of heterotrophic respiration (Δ¹⁴C_{heterotrophic}), we weighted the isotopic signature of litter and soil layers according to their contribution to the total decomposition flux (see below).

Leaf litter was sampled by collecting all material above the mineral soil in a 15 × 15 cm area. The sample was placed in an airtight plastic bag and refrigerated during transport and storage. Soil samples were collected using a core (4.7 cm diameter by 5 cm long) inserted vertically into the soil. The core was carefully removed and the soil extruded with a minimum of disturbance into a flask, which was capped and refrigerated for transport and storage. Samples of soil and leaf litter were collected on all sampling dates except in

July 2002. The isotopic signature of heterotrophic respiration used for July was calculated as the mean of the signatures obtained in May and September at each site. No data are available for October 2003 at both sites.

The leaf litter and soil samples were refrigerated for at least 1 week (up to a month) prior to further analysis. While this amount of time is dictated by the time of transport from the field, it also allows time for fine roots that can be present in the samples to die so that their autotrophic respiration no longer contributes significantly to the CO₂ evolved in the incubation. One liter jars (Mason) were used to separately incubate soil cores and leaf litter samples. Humidity inside the jar was maintained by pouring approximately 100 mL of water over glass beads placed in the bottom of the jar. The flasks containing the soil cores were uncapped, weighed (for gravimetric determination of moisture content after the incubation was finished) and placed inside the 1 L jars over the glass beads. Leaf litter samples were weighed and a sub-sample was placed in perforated aluminum foil packets before sealing in the Mason jar.

For approximately 1 week after sealing the jars, we measured the rate of accumulation of CO₂ in the jar headspace using an infrared gas analyzer (LI-6252, Inc. Lincoln, Nebraska). After CO₂ fluxes were determined, air inside the jars was scrubbed of CO₂, and CO₂ was allowed to re-accumulate until there was sufficient concentration (1–2% CO₂) for ¹³C and ¹⁴C analysis. CO₂ samples for isotope analyses were collected by connecting the jars to evacuated stainless steel canisters (of 0.5 liters in volume). The ¹⁴C and ¹³C signatures were determined as described above. At each site, we report soil and leaf litter decomposition signatures (Δ¹⁴C_{soil decomposition} and Δ¹⁴C_{leaf litter decomposition} respectively) as the mean (± standard deviation) of three plots with identical litter addition treatments.

Autotrophic respiration

To determine the radiocarbon signature of autotrophic respiration (Δ¹⁴C_{autotrophic}), we incubated live roots in the field. Since this ¹⁴C signature is dependent on photosynthetic carbon supplies, and is independent of leaf litter carbon inputs, roots for analysis were collected outside the experimental plots to avoid within-plot disturbance. At two or more randomly chosen locations, we excavated roots mostly in the upper 5 cm of mineral soil, though some may be from slightly deeper as root networks were pulled out without distinction of depth, size (most were < 5 mm diameter), or species.

After sufficient roots for three replicates were collected, they were shaken and washed free of soil,

divided into 3 sub-samples and placed immediately in airtight containers. Roots were normally collected at 5–6 pm local time. Isotopes were sampled using the same methodology described for total soil respiration, though we did not measure CO₂ flux rates as we assumed manipulation of the roots may have altered the fluxes (Rakoczay *et al.*, 1997). Air inside the containers was scrubbed of CO₂, CO₂ was allowed to re-accumulate, and was collected by trapping on molecular sieve. The accumulation of CO₂ for trapping started within an hour after roots had been removed from the soil and lasted for an amount of time from 10 min to an hour depending on the respiration rate of the sub-sample. Molecular sieve traps were transported to UCI and the ¹⁴C and ¹³C signatures of the CO₂ collected determined as described above. At each site we report the mean (± standard deviation) of three replicates.

Calculation of respiration components

Fraction of total soil respiration coming from leaf litter decomposition (FLD)

Contrasting the levels of ¹⁴C in CO₂ respired from HL and LL treatments at a given site allowed us to uniquely determine the contribution of leaf litter decomposition to total soil respiration using isotope mass balance:

$$\begin{aligned} \Delta^{14}\text{C}_{\text{total respiration}} &= \Delta^{14}\text{C}_{\text{autotrophic}} \times \text{FRR} \\ &+ \Delta^{14}\text{C}_{\text{leaf litter decomposition}} \times \text{FLD} \\ &+ \Delta^{14}\text{C}_{\text{soil decomposition}} \times \text{FSD}, \end{aligned} \quad (1)$$

where FRR, FLD and FSD are the fractional contributions of autotrophic respiration, leaf litter decomposition and soil decomposition, respectively, to total soil respiration. The $\Delta^{14}\text{C}_{\text{autotrophic}}$, $\Delta^{14}\text{C}_{\text{leaf litter decomposition}}$ and $\Delta^{14}\text{C}_{\text{soil decomposition}}$ values were derived from our field and laboratory incubations, as described above. Since we incubated not only the labeled leaf litter added to plots, but the entire litter layer (Oi + Oe/Oa), FLD as calculated above reflects the contribution from the decomposition of the entire O horizon. At a given site, $\Delta^{14}\text{C}_{\text{autotrophic}}$ and FRR should be the same for both treatments. The difference between HL and LL treatments in $\Delta^{14}\text{C}_{\text{soil decomposition}}$ was generally very small compared to the difference in $\Delta^{14}\text{C}_{\text{leaf litter decomposition}}$ (see the results section). Therefore, we could assume that the differences in ¹⁴C between HL and LL treatments were overwhelmingly caused by leaf litter decomposition. Applying Eqn (1) to each treatment and subtracting LL from HL we solved for FLD as follows:

$$\begin{aligned} \text{FLD} &= (\Delta^{14}\text{C}_{\text{total HL}} \\ &- \Delta^{14}\text{C}_{\text{total LL}}) / (\Delta^{14}\text{C}_{\text{leaf litter decomposition HL}} \\ &- \Delta^{14}\text{C}_{\text{leaf litter decomposition LL}}) \end{aligned} \quad (2)$$

We report one single value of FLD for each site (HR and LR) and sampling date. The associated uncertainty was estimated by error propagation.

Fraction of total soil respiration coming from autotrophic respiration (FRR)

To estimate the fraction of total respiration derived from autotrophic respiration using ¹⁴C, we applied the mass balance approach described in Trumbore *et al.*, (2002):

$$\begin{aligned} \Delta^{14}\text{C}_{\text{total respiration}} &= \Delta^{14}\text{C}_{\text{autotrophic}} \times \text{FRR} \\ &+ \Delta^{14}\text{C}_{\text{heterotrophic}} \times (1 - \text{FRR}) \end{aligned} \quad (3)$$

Here, FRR includes metabolic respiration from live roots as well as anything with a similar ¹⁴C signature, such as symbiotic mycorrhizal fungi and the activity of microorganisms decomposing recent photosynthetic products. While we will hereafter refer to FRR as the fraction coming from root respiration, it should be recognized that it refers to the sum of all of these processes.

The radiocarbon signature of heterotrophic respiration ($\Delta^{14}\text{C}_{\text{heterotrophic}}$) was determined as the weighted contribution of CO₂ derived from leaf litter and soil organic matter decomposition:

$$\begin{aligned} \Delta^{14}\text{C}_{\text{heterotrophic}} &= \Delta^{14}\text{C}_{\text{leaf litter decomposition}} \times L \\ &+ \Delta^{14}\text{C}_{\text{soil decomposition}} \times (1 - L) \end{aligned} \quad (4)$$

where *L* is the fractional contribution of leaf litter decomposition to total heterotrophic respiration. One way to determine *L* is to compare the relative rates of CO₂ evolution from our incubations of leaf litter and soil. However, the temperature and moisture conditions of our incubations differed from field conditions, and if we attempt to scale the rates of heterotrophic respiration derived from laboratory incubations to conditions in the field, we overestimate observed soil respiration by at least a factor of two. Hence, we could not use our laboratory incubations to derive weighting factors with any confidence.

A second method of determining *L* is obtained by assuming that FRR and *L* are the same for both HL and LL treatments for a given site. Since *L* and FLD represent the contribution of decomposing litter to heterotrophic and total respiration, respectively, they are

related by:

$$\text{FLD} = L \times (1 - \text{FRR}) \quad (5)$$

Substituting Eqns (4) and (5) into Eqn (3) results in an equation with two unknowns, FRR and FLD. Since we have calculated FLD using Eqn (2) above, we can solve this equation for FRR, with the requirement that FRR and FLD be identical for both HL and LL treatments within a given site. We only report values of FRR that satisfied this assumption within the error we calculated for FLD using Eqn (2). We report the uncertainty in FRR by treatment calculated using the standard deviations of total soil respiration, root and heterotrophic respiration according to Phillips & Gregg, (2001).

Results

Total soil respiration fluxes

Although there was high spatial variability in soil respiration fluxes, we saw no systematic differences in CO₂ fluxes between the two sites (Fig. 1; data for March 2004 at the TVA site were not reported). The lower fluxes measured at TVA in July 2002 and Walker Branch in September 2003 were most likely due to moisture differences between the sites. Since the two sites were sampled on different days and rainfall from isolated summer precipitation events is very unevenly distributed across the landscape, the two sites do not always replicate each other. Soil moisture varied from 20% to 35% volumetric water content (at approximately 10 cm depth) across sites during the three sampling periods in 2002, except in July, where moisture at TVA site fell to 11%. In September 2003, we have no data for soil moisture content, but leaf litter at Walker Branch was extremely dry and crunchy, while soil respiration measurements at TVA were carried out the day after a heavy rain event.

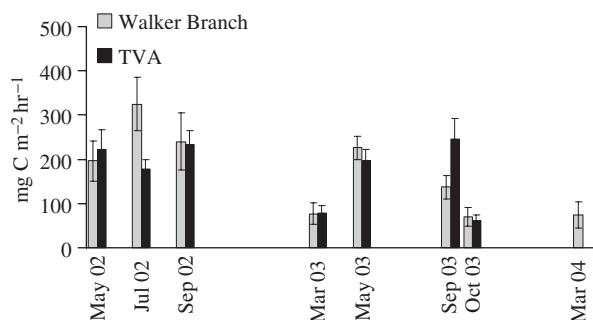


Fig. 1 Total soil respiration fluxes at the Walker Branch and TVA sites on each sampling date. At each site we report the mean (\pm standard deviation) of 6 chamber measurements including 3 per litter treatment. No data are available for March 2004 at the TVA site.

¹⁴C signatures of total soil respiration, root respiration and heterotrophic sources

We observed a marked difference in the radiocarbon signatures of leaf litter decomposition between HL and LL treatments at both TVA (HR) and Walker Branch (LR) sites (Fig. 2). The ¹⁴C-labeled litter addition imparted a higher radiocarbon signature to total soil respiration on all dates except those where litter was extremely dry (July 2002 and September 2003). The isotopic signature of decomposing soil organic matter increased slightly with time, though differences between HL and LL treatments remained small compared to those associated with leaf litter decomposition. Across sites and litter treatments, the radiocarbon signatures were overall higher at TVA (HR) than at Walker Branch (LR), reflecting the continued influence of ¹⁴C-enriched carbon acquired by plants during the 1999 labeling event.

The ¹⁴C signature of root respiration varied with time (Fig. 2). Temporal variations between sites followed very similar patterns, although values in the HR site remained $\sim 100\%$ higher than the LR site throughout 2002–2003. The very depleted values that we observed at the Walker Branch site in July 2002 (-0.28%) and May 2003 (2%) were attributable to a local release of fossil fuel derived CO₂, which has been verified by measurements of reduced radiocarbon content in ambient air sampled at this site since 2000.

Fraction of total soil respiration coming from leaf litter decomposition (FLD)

According to Eqn (2), the fraction of total soil respiration derived from leaf litter decomposition (FLD) was proportional to the difference in the ¹⁴C signature of total soil respiration between HL and LL treatments. FLD ranged from near zero in September 2003 to 0.42 ± 0.16 in May 2003 (Fig. 3). Lowest FLD values were found on July 2002 (TVA; HR) and September 2003 (WB; LR).

Fraction of total soil respiration coming from root respiration (FRR)

Our approach of solving for FRR by requiring FLD and FRR to be identical in HL and LL plots at a given site produced consistent results for the Walker Branch site on most dates (Table 1). We had greater problems with the TVA site and our constraints were met only on May 2003. When the isotopic differences among root respiration, heterotrophic respiration and total respiration were small compared to their respective standard deviations, HL and LL treatments did not yield consistent

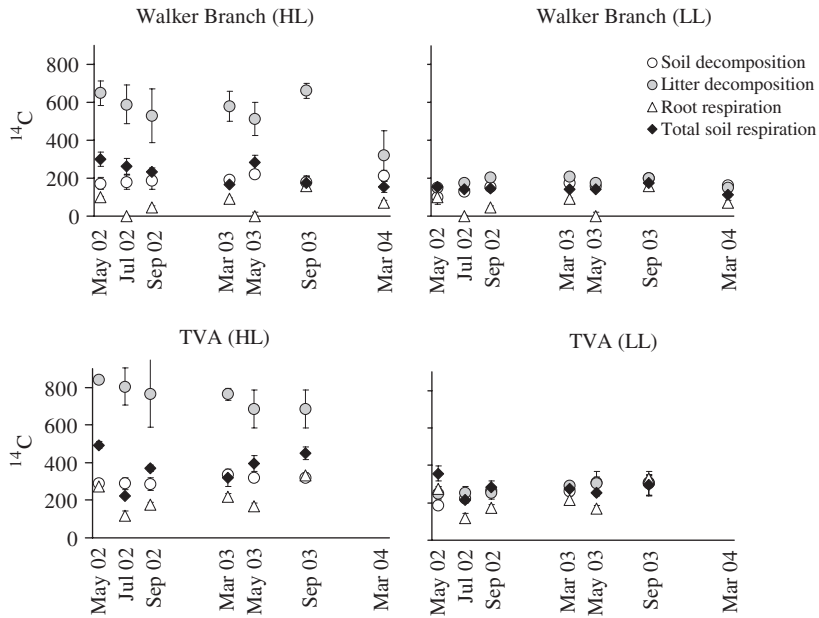


Fig. 2 ^{14}C signatures (in per mil, ‰) of total soil respiration, root respiration, soil decomposition and leaf litter decomposition by litter treatment, HL and LL (high and low ^{14}C labeled leaf litter respectively) for the Walker Branch and TVA sites. Each value represents the mean of 3 replicates (\pm standard deviation). The isotopic signatures of soil decomposition and leaf litter decomposition that we report for July 2002 at both sites are the means of the signatures measured in May and September 2002.

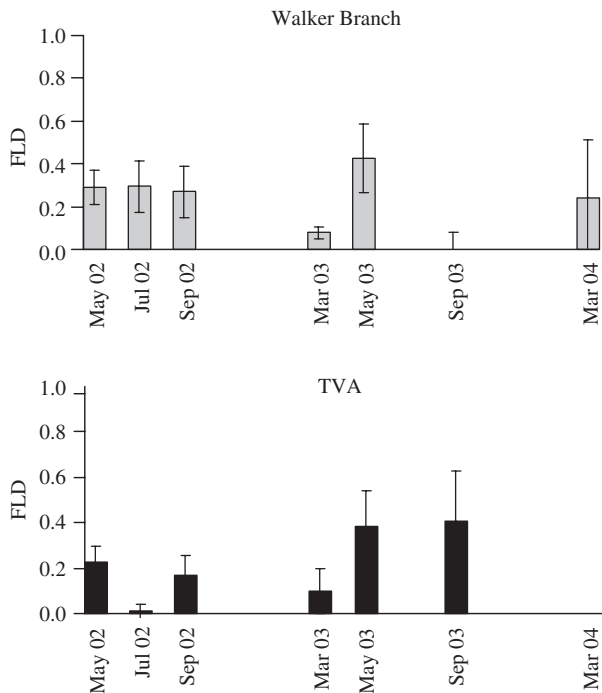


Fig. 3 Fraction of total soil respiration coming from leaf litter decomposition (FLD) at the Walker Branch and TVA sites by sampling date. The fractions were calculated using Eqn (2). No data are available for March 2004 at the TVA site.

results (blank entries in Table 1). On several occasions the $\Delta^{14}\text{C}$ of total soil respiration fell outside the range of the $\Delta^{14}\text{C}$ of its heterotrophic and autotrophic sources

and thus the mass balance approach could not be applied (dates marked 'na' in Table 1). Increased uncertainties in FRR calculated from the HL treatment at the Walker Branch site on September 2002 and 2003 resulted from small isotopic difference between autotrophic respiration and the estimated signature of heterotrophic respiration. For subsequent calculations, we have used the Walker Branch results, assuming the largest error among the two treatments, except for September 2002 and 2003, when we used the LL treatment values only.

Partitioning of total soil respiration

A summary comparing the absolute contributions (in $\text{mg C m}^{-2} \text{h}^{-1}$) of root respiration and leaf litter decomposition for the Walker Branch site is shown in Fig. 4. Fluxes labeled as 'other' were calculated from mass balance (total respiration *minus* root respiration *minus* leaf litter respiration), and have isotopic signatures close to those evolved in soil incubations (FSD; Eqn 1).

Heterotrophic respiration, especially leaf litter decomposition, constituted a large portion of the total CO_2 respired in the growing season (May and September), though it was also the most variable component in space and time, ranging from nearly zero in September 2003 and $\sim 6 \pm 3 \text{ mg C m}^{-2} \text{h}^{-1}$ in March 2003, to $96 \pm 38 \text{ mg C m}^{-2} \text{h}^{-1}$ in May 2003. Root respiration fluxes remained fairly constant ranging from 34 ± 14

Table 1 Fraction of total soil respiration coming from root respiration (FRR) for HL and LL (high and low ¹⁴C-labeled leaf litter) treatments at the Walker Branch (WB) and Tennessee Valley Authority (TVA) sites

Site	Treatment	2002			2003			2004
		May	July	September	March	May	September	March
WB	LL	na		0.17 (0.05)	0.45 (0.11)	0.16 (0.08)	0.64 (0.22)	0.53 (0.12)
	HL			0.16 (0.21)	0.45 (0.10)	0.16 (0.10)	0.63 (0.61)	0.53 (0.19)
TVA	LL	na		na		0.40 (0.18)	na	na
	HL					0.40 (0.10)		na

Numbers in brackets represent the standard error according to Phillips & Gregg, (2001).

Dates when the isotopic signatures did not satisfy the mass balance are indicated as "na"; blank entries indicate times when HL and LL treatments did not satisfy the assumptions upon which the calculations were based.

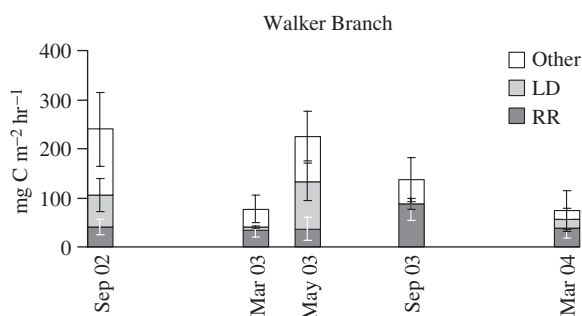


Fig. 4 Absolute contributions from leaf litter decomposition (LD), root/rhizosphere respiration (RR) and other heterotrophic sources (other) to total soil respiration for the Walker Branch site. Fluxes shown under "other" were calculated as the remaining difference and are assumed to have the isotopic signature of the incubated soil organic matter.

to $40 \pm 16 \text{ mg C m}^{-2} \text{ h}^{-1}$ on the different sampling dates with the exception of September 2003 ($88 \pm 35 \text{ mg C m}^{-2} \text{ h}^{-1}$). Heterotrophic respiration was larger than root respiration on the days we sampled during the peak of the growing season, but in early spring (March 2003 and 2004), root respiration and heterotrophic sources contributed equally to total soil respiration.

Discussion

Large differences in the radiocarbon signatures of leaf litter between treatments allowed us to successfully apply a ¹⁴C mass balance approach to separate sources of soil respiration into components derived from root/rhizosphere respiration, leaf litter decomposition, and other heterotrophic sources (Fig. 4). We were able to partition total soil respiration at different times in the growing season and identify the effect of moisture on heterotrophic sources. We achieved consistent results across sites in many cases, indicating that our methods

of determining isotopic signatures for respiration components do not have significant systematic errors (though our methods for determining fluxes, especially in incubations, do). The partitioning approach gave consistent results even when the isotopic signature of root respiration varied by over 100‰ from one sampling date to the next. Our method does have the problem that if the separation between isotopic signatures of respiration components is not larger than their respective standard deviations (Fig. 2), we cannot solve for FRR in any meaningful way.

Leaf litter decomposition

Estimates of the relative contribution of decomposing leaf litter to total soil respiration (FLD) showed good agreement across sites when moisture was not a limiting factor (Fig. 3). The greater standard deviations of $\Delta^{14}\text{C}_{\text{leaf litter decomposition}}$ characteristic of HL treatments (Fig. 2) translated into large uncertainties in the estimates of FLD on some dates. In March 2004, greater uncertainty for FLD was expected as isotopic differences in $\Delta^{14}\text{C}_{\text{leaf litter decomposition}}$ between treatments were reduced by dilution of the ¹⁴C label in leaf litter in the fall of 2003 (the first fall season that native litter fall was allowed into the plots after 3 years of ¹⁴C labeled litter additions).

The strong dependence of FLD on moisture conditions was evidenced by the marked difference between sites in July 2002 and September 2003. The link between leaf litter decomposition and moisture has been documented by Lee *et al.* (2004) who found a linear decrease in the relative contribution of the litter layer with lower litter water contents in a manipulated study in a mixed forest. In a tropical forest, an indication of the dependence of FLD on litter moisture content was observed by Goulden *et al.* (2004) when patterns of soil respiration rates varied in concert with the moisture content of the surface leaf litter.

In our study, times of lower total soil respiration fluxes (July 2002 at TVA and September 2003 at Walker Branch; Fig. 1) coincided with times of lower relative contributions from leaf litter decomposition (Fig. 3). As a result of the dependence on moisture conditions, and in contrast with root respiration, fluxes from leaf litter decomposition were highly variable in time. This suggests that litter decomposition fluxes can dictate the temporal variation in total soil respiration in response to changing moisture conditions, in agreement with findings from other studies (Hanson *et al.*, 2003; Borken *et al.*, 2005). Hanson *et al.* (2003) developed a model to predict total soil respiration validated against repeated measurements of litter fluxes and moisture. Model predictions were found to improve when the response of litter fluxes to variations in litter moisture contents were taken into account. In addition of being a considerable fraction of soil respiration (up to 42%), the response to moisture conditions emphasizes the relevance of this component of total soil respiration.

Other heterotrophic sources

Across sites and same litter treatments, the radiocarbon signature of soil decomposition was always higher at TVA (HR) than at Walker Branch (LR) (on average by $\sim 120\%$, Fig. 2). In contrast, the difference in the radiocarbon signature of soil decomposition between HL and LL treatments within a given site remained small ($\sim 40\%$). This indicates that a major source of decomposing material in the 0–5 cm soil layer is derived from carbon fixed in the original 1999 ^{14}C release, rather than the labeled litter treatment applied subsequently. Dead roots are the likely source since differences in soil organic matter in this layer remained small in 2002–2003 (J. Jastrow, 2004, personal communication). In theory, we should have been able to perform a similar analysis to that of FLD to calculate the fraction of heterotrophic respiration coming from labeled root decomposition from the difference between HR and LR sites in $\Delta^{14}\text{C}_{\text{soil decomposition}}$. However, since root respiration radiocarbon values differed between sites, we could not independently solve for the root decomposition end member as we could for FLD.

Root respiration sources

The accurate estimation of $\Delta^{14}\text{C}_{\text{heterotrophic}}$ is obviously important in the calculation of FRR (Eqn 3) and this in turn depends strongly on the value of 'L' (Eqn 4). Overestimation of 'L', as would have occurred with the use of our incubation data as weighting factors, would have led to an overestimation of $\Delta^{14}\text{C}_{\text{heterotrophic}}$,

especially in HL treatments (Fig. 2). This in turn would have translated into an overestimation of FRR. We are confident that the isotopic signatures measured in incubations ($\Delta^{14}\text{C}_{\text{leaf litter decomposition}}$ and $\Delta^{14}\text{C}_{\text{soil decomposition}}$) are unlikely to be a cause of error as radiocarbon values of CO_2 derived from incubated material have been shown to be independent of temperature or moisture variation even with very large changes in CO_2 evolution rate (Dioumaeva *et al.*, 2003; Cisneros-Dozal unpublished data).

The estimates of the fractional contribution of root respiration to total respiration (FRR) reported here are not easily compared to the annual means commonly reported in literature. We have not attempted to estimate annual root respiration contributions because of the limited nature of our sampling. However, FRR ranged from a low of 16% to a high of 64% in our study, within the range of published estimates. The annual mean contribution of root respiration to soil respiration has been estimated at $\sim 60\%$ from trenching experiments (Epron *et al.*, 1999; Högberg *et al.*, 2001). A lower estimate by Kelting *et al.* (1998) of 32% in early June was recognized to be a minimum since the sampling did not take place during the period of maximum root growth. In addition, the use of excised roots to measure rates of root respiration remains questionable, because their function has been observed to decrease rapidly after excision (Rakonczay *et al.*, 1997). An earlier study carried out in the ORR estimated that respiration from live roots accounted for 35% of total soil respiration annually for *Liriodendron tulipifera* L. spp. (Edwards & Harris, 1977). Rey *et al.* (2002) reported an annual contribution from root respiration of 23% although previous disturbance at the site were thought to be the cause of this low estimate. It is important to note that in our study, times of highest relative contributions (FRR) were not necessarily times of high root respiration fluxes and thus both quantities should be reported. For instance, FRR values in March 2003 and 2004 were higher in comparison to other dates (with the exception of September 2003, Table 1), however the absolute fluxes were not different (Fig. 4).

In contrast to other studies (Epron *et al.*, 2001; Högberg *et al.*, 2001; Rey *et al.*, 2002), we did not observe marked temporal variation in root respiration fluxes with the exception of the increase in September 2003 (Fig. 4). Several studies indicate a link between soil respiration and plant activity through the phenological control on root respiration fluxes (Högberg *et al.*, 2001; Janssens *et al.*, 2001; Reichstein *et al.*, 2003; Curiel *et al.*, 2004). For this forest, a correlation between root growth and leaf area expansion was observed by Joslin *et al.* (2001), with the highest root elongation intensity taking place after the completion of leaf area expansion. In

terms of root respiration fluxes however, we did not see an increase during the periods of maximum root growth observed in May, June and July for *Quercus* spp (Joslin & Wolfe, 2003). We did observe an increase from May to September in 2003; however an earlier increase from March to May would have been expected in accordance with the timing of root growth. More frequent measurements (which may obviate some of the differences in soil moisture and temperature conditions between widely spaced sampling intervals) may be required to capture the relationship between root growth and root respiration patterns.

The observed large differences in the ¹⁴C signature of root respiration between sampling dates (Fig. 2) was an unexpected result. These temporal changes were very similar at both sites, which would seem to rule out sampling or measurement errors as the cause. Other causes could be related to changes in the internal sources of C being utilized, or to fluctuations in the radiocarbon content of atmospheric CO₂ (i.e., perhaps further local releases). Trumbore *et al.* (2002) showed that nonstructural carbohydrate pools were labeled in the 1999 event. Depending on the carbohydrate turnover rate, the ¹⁴C content of this storage pool could be greater than that of more recent photosynthetic products. For example, leaf buds grown each spring have higher ¹⁴C values than atmospheric CO₂, and parasitic plants growing in early spring also have ¹⁴C signatures close to our root respiration ¹⁴C measurements for the same time period (Trumbore, unpublished data). The observed decline in the ¹⁴C of CO₂ respired by roots at both HR and LR sites from May to July in 2002 and March to May in 2003 indicates the use of stored C as an energy source prior to leaf-out, with a change to current photosynthetic C after the forest canopy matures and the root growth peaks in July. A further indication that roots use more recently fixed carbon during the summer is the very low ¹⁴C values for root respiration observed at Walker Branch in July 2002 and May 2003, (i.e., a site under the influence of recent local fossil fuel burning). However, root respiration values remain higher at the HR site (TVA) than the LR site (Walker Branch) throughout the summer, indicating that either some portion of root respiration is derived from storage pools or that differences in atmospheric ¹⁴CO₂ persist between sites. We have taken bi-weekly time averaged samples of air at 1 m height above-ground at both HR and LR sites to monitor for events like ¹⁴C releases, but it is difficult to link these to values we observe in root respiration measured on one day. Further analysis of radiocarbon in carbohydrates of root tissues is needed in order to better elucidate the source of carbon respired throughout the growing season.

Conclusions

We have used ¹⁴C labeled leaf litter and roots together with a mass balance approach to quantify heterotrophic and autotrophic (root and rhizosphere) respiration sources in a temperate forest. Heterotrophic sources, especially leaf litter decomposition, account for a large fraction of total soil respiration in this temperate deciduous forest. Heterotrophic respiration in the surface mineral soil layer (0–5 cm) was dominated by the decomposition of C fixed 3–5 years earlier (during the 1999 labeling event). Leaf litter decomposition is a major contributor to overall heterotrophic CO₂ fluxes, but it is also the most variable in space and time in response to changing moisture conditions. The instantaneous relative contributions from leaf litter decomposition ranged from $\sim 1 \pm 3\%$ to $42 \pm 16\%$, corresponding to absolute fluxes of 6 ± 3 to $96 \pm 38 \text{ mg C m}^{-2} \text{ h}^{-1}$, and decreased to near zero when leaf litter was extremely dry. As a result of its dependence on moisture conditions, leaf litter decomposition is apparently the main source of temporal variation in total soil respiration in this forest.

In contrast, root/rhizosphere respiration fluxes did not exhibit marked temporal variation, ranging from 34 ± 14 to $40 \pm 16 \text{ mg C m}^{-2} \text{ h}^{-1}$ with a single exception of increased fluxes ($88 \pm 35 \text{ mg C m}^{-2} \text{ h}^{-1}$) in September 2003. The relative contributions from root/rhizosphere respiration ranged from 16 ± 10 to $64 \pm 22\%$. Times of highest relative contributions did not necessarily coincide with times of highest absolute fluxes, underscoring the importance of reporting quantities. The radiocarbon signatures of root respiration provided preliminary information on the source of C respired by roots and indicated that sources of C respired may shift from stored C pools in early spring to recent photosynthetic products as the summer progresses.

The 1999 ¹⁴C-pulse being studied in the EBIS project provided a unique opportunity for rapid evaluation of the components of soil respiration. The same isotope mass balance approaches can presumably be applied in other ecosystems where the sources of soil respiration differ substantially in age (such as in boreal forests). While similar studies can not be done for all established forest ecosystems, other situations where anthropogenic activities may have perturbed the background isotopic ¹⁴C status may exist and should be investigated.

Acknowledgments

Funding for the EBIS project was provided by the U.S. Department of Energy (DOE), Office of Science, Biological and Environmental Research (BER), as a part of the Terrestrial Carbon Processes (TCP) Program. EBIS project participants appreciate access and use of Tennessee Valley Authority (TVA) land on

Chestnut Ridge near the Oak Ridge Reservation allowed under Contract No. 105906 between TVA and the Oak Ridge National Laboratory. Additional support came from a NASA Earth System Science Graduate Student Fellowship and from NSF EAR-0223514 for carbon cycle research. We thank T. Perez and X. Xu for their helpful comments.

References

- Bauer J, Williams PM, Druffel ER. (1992) Recovery of sub-milligram quantities of carbon dioxide from gas streams by molecular sieve for subsequent determination of isotopic natural abundance. *Analytical Chemistry*, **64**, 824–827.
- Borken W, Savage K, Davidson EA, Trumbore SE (2005) Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. *Global Change Biology*. doi: 10.1111/j.1365-2486.2005.01058.x
- Curiel Yuste J, Janssens IA, Carrara A, Ceulemans R. (2004) Annual Q_{10} of soil respiration reflects plant phenological patterns as well as temperature sensitivity. *Global Change Biology*, **10**, 161–169.
- Dioumaeva I, Trumbore S, 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.
- Edwards NT, Harris WF (1977) Carbon cycling in a mixed deciduous forest floor. *Ecology*, **58**, 431–437.
- Epron D, Dantec VL, Dufrêne E *Et al.* (2001) Seasonal dynamics of soil carbon dioxide efflux and simulated rhizosphere respiration in a beech forest. *Tree Physiology*, **21**, 145–152.
- Epron D, Farque L, Lucot E *Et al.* (1999) Soil CO_2 efflux in a beech forest: the contribution of root respiration. *Annals of Forest Science*, **56**, 289–295.
- Gaudinski JB, Trumbore SE (2003) Soil Carbon Turnover. In: *North American Temperate Deciduous Forest Responses to Changing Precipitation Regimes*, Vol. 166 (eds, Hanson PJ, Wullschleger SD), Springer, New York pp. 190–209.
- 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.
- Goulden ML, Miller SD, Rocha HR *Et al.* (2004) Diel and seasonal patterns of tropical forest CO_2 exchange. *Ecological Applications*, **14** (Suppl), S42–S54.
- Goulden ML, Munger JW, Fan SM *Et al.* (1996) Exchange of carbon dioxide by a deciduous forest – response to interannual climate variability. *Science*, **271**, 1576–1578.
- Hanson PJ, Edwards NT, Garten CT *Et al.* (2000) Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry*, **48**, 115–146.
- Hanson PJ, O'Neill EG, Chambers MLS, Riggs JS, Joslin JD, Wolfe MH (2003) Soil Respiration and Litter decomposition. In *North American Temperate Deciduous Forest Responses to Changing Precipitation Regimes*, Vol. 166 (eds, Hanson PJ, Wullschleger SD), Springer, New York pp. 163–189.
- Högberg P, Nordgren A, Buchmann N *et al.* (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, **411**, 789–792.
- Janssens IA, Lankreijer H, Matteucci G *et al.* (2001) Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. *Global Change Biology*, **7**, 269–278.
- Johnson DW, Hook RI, (eds) (1989) *Analysis of biogeochemical cycling processes in Walker Branch Watershed*. Springer-Verlag, New York pp. 14.
- Joslin JD, Wolfe MH (2003) Fine Root Growth Response. In *North American Temperate Deciduous Forest Responses to Changing Precipitation Regimes* (Hanson PJ, Wullschleger SD), Eds., Springer, New York pp. 274–302.
- Joslin JD, Wolfe MH, Hanson PJ *et al.* (2001) Factors controlling the timing of root elongation intensity in a mature upland oak stand. *Plant and Soil*, **228**, 201–212.
- Kelting DL, Burger JA, Edwards GS *et al.* (1998) Estimating root respiration, microbial respiration in the rhizosphere, and root-free soil respiration in forest soils. *Soil Biology and Biochemistry*, **30**, 961–968.
- Lee X, Wu H-J, Sigler J *et al.* (2004) Rapid and transient response of soil respiration to rain. *Global Change Biology*, **10**, 1017–1026.
- Phillips DL, Gregg JW (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia*, **127**, 171–179.
- Rakonczay Z, John R S, Kelting DL *et al.* (1997) Carbon efflux rates of fine roots of three tree species decline shortly after excision. *Environmental and Experimental Botany*, **38**, 243–249.
- Reichstein M, Rey A, Freibauer A *et al.* (2003) Modeling temporal and large-scale spatial variability of soil respiration from soil water availability, temperature and vegetation productivity indices. *Global Biogeochemical Cycles*, **17**, 15.1–15.15.
- Rey A, Pegoraro E, Tedeschi V *Et al.* (2002) Annual variation in soil respiration and its components in a coppice oak forest in Central Italy. *Global Change Biology*, **8**, 851–866.
- Stuiver M, Polach HA (1977) Reporting of ^{14}C data. *Radiocarbon*, **19**, 355–363.
- Trumbore SE, Gaudinski JB, Hanson PJ *Et al.* (2002) Quantifying ecosystem-atmosphere carbon exchange with a ^{14}C label. *EOS, Transactions, American Geophysical Union*, **83**, 265, 267–268.
- 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 JS (1992) A rapid method for preparation of biomedical targets for AMS. *Radiocarbon*, **34**, 344–350.