Plant, Cell & Environment

Plant, Cell and Environment (2010) **33**, 1804–1819 doi: 10.1111/j.1365-3040.2010.02185.x

Diffusive fractionation complicates isotopic partitioning of autotrophic and heterotrophic sources of soil respiration

ANDREW B. MOYES[1]*, SARAH J. GAINES[1], ROLF T. W. SIEGWOLF[2] & DAVID R. BOWLING[1]

[1]*University of Utah, Department of Biology, 257 South, 1400 East, Salt Lake City, UT 84112, USA and* [2]*Paul Scherrer Institut, 5232 Villigen PSI, Switzerland*

ABSTRACT

Carbon isotope ratios ($\delta^{13}C$) of heterotrophic and rhizospheric sources of soil respiration under deciduous trees were evaluated over two growing seasons. Fluxes and $\delta^{13}C$ of soil respiratory CO_2 on trenched and untrenched plots were calculated from closed chambers, profiles of soil CO_2 mole fraction and $\delta^{13}C$ and continuous open chambers. $\delta^{13}C$ of respired CO_2 and bulk carbon were measured from excised leaves and roots and sieved soil cores. Large diel variations (>5‰) in $\delta^{13}C$ of soil respiration were observed when diel flux variability was large relative to average daily fluxes, independent of trenching. Soil gas transport modelling supported the conclusion that diel surface flux $\delta^{13}C$ variation was driven by non-steady state gas transport effects. Active roots were associated with high summertime soil respiration rates and around 1‰ enrichment in the daily average $\delta^{13}C$ of the soil surface CO_2 flux. Seasonal $\delta^{13}C$ variability of about 4‰ (most enriched in summer) was observed on all plots and attributed to the heterotrophic CO_2 source.

Key-words: *Acer negundo*; carbon isotope; rhizosphere; roots; soil respiration; trenching.

INTRODUCTION

Soil respiration remains one of the largest sources of uncertainty about carbon cycling within ecosystems because soil biological communities and processes are complex, relatively inaccessible and highly sensitive to disturbance. Two broad categories of soil organisms can be distinguished by their carbon sources: (1) the bulk soil heterotrophic component feeding on soil organic matter; and (2) the rhizosphere component, which in the present study is taken to include roots, mycorrhizal fungi and rhizomicrobial heterotrophs feeding on carbon supplied by roots. Simple partitioning of soil respiration into these two components has been achieved by interruption of photosynthate transport belowground to intact soils by methods such as trenching (Hanson *et al*. 2000) and stem girdling (Högberg *et al*. 2001). Recent attempts to combine stable carbon isotope ratio ($\delta^{13}C$) measurements with these approaches have yielded additional information about soil respiration and its components.

The $\delta^{13}C$ of phloem sugars transported to roots initially depends on photosynthetic discrimination in leaves (Δ). Because root respiration in temperate forests typically represents a large fraction of total soil respiration (Högberg *et al*. 2001; Subke, Inglima & Cotrufo 2006), environmental variables that drive changes in Δ by affecting assimilation rate or stomatal conductance to CO_2 may be correlated with variability in $\delta^{13}C$ of soil respiration, possibly with a source-to-sink transport time lag (Ekblad & Högberg 2001; Ekblad *et al*. 2005). The $\delta^{13}C$ of CO_2 respired by roots and other rhizosphere components may also be affected by utilization of fast or slow turnover carbon pools (Schnyder *et al*. 2003) or allocation between growth vs. maintenance (Ocheltree & Marshall 2004).

The $\delta^{13}C$ of CO_2 respired by heterotrophic soil microorganisms depends on the substrates within soil organic matter utilized for decomposition. Total soil organic matter is generally enriched in ^{13}C relative to leaf litter, and becomes progressively more enriched with depth (Ehleringer, Buchmann & Flanagan 2000). Carbon dioxide produced during decomposition can be depleted (Mary, Mariotti & Morel 1992; Fernandez, Mahieu & Cadisch 2003) or enriched (Andrews *et al*. 2000; Böstrom, Comstedt & Ekblad 2007) in ^{13}C relative to bulk soil organic matter.

Total soil respiration tends to be a few‰ enriched in ^{13}C relative to site-specific bulk leaf $\delta^{13}C$ (Bowling, Pataki & Randerson 2008). However, root respiration has been found to be ^{13}C-depleted relative to leaf and shoot tissues in laboratory studies with herbaceous species (Badeck *et al*. 2005; Klumpp *et al*. 2005; Schnyder & Lattanzi 2005). If this relationship extends to woody plants under field conditions, there would be an unknown, putative ^{13}C-enriched soil CO_2 source necessary to account for soil respiration being generally enriched relative to leaf tissues (Bowling *et al*. 2008). If consistent isotopic differences exist between a ^{13}C-depleted root source and a ^{13}C-enriched heterotroph source, this would be useful for non-disruptive soil respiration partitioning. However, reports from forest trees have shown ^{13}C-enriched respiration from roots (Gessler *et al*. 2007) and trunks (Brandes *et al*. 2006) relative to substrates

Correspondence: A. B. Moyes. Fax: +801 581 2174; e-mail: amoyes@ucmerced.edu

*Current address: School of Natural Sciences, UC Merced, 5200 North Lake Drive, Merced, CA, USA.

such as water soluble phloem exudates. Studies comparing $\delta^{13}C$ of root and soil respiration are necessary to identify and define these relationships. Further, application of isotopes to understand the importance of phloem transport to soil respiration and its component sources requires measurements that extend from isolated roots to include the entire rhizosphere, and a clearer understanding of the processes and conditions that influence the carbon isotope content of belowground respiration.

The present study was conducted to determine the natural abundance $^{13}C/^{12}C$ ratio and variability of individual heterotroph (bulk soil) and rhizosphere sources of soil respiration under deciduous boxelder (*Acer negundo*) trees to understand how utilization of these individual carbon sources might vary with phenology and environmental variables. Measurements of rates and $\delta^{13}C$ of soil respiration were collected on replicated trenched and untrenched plots (without and with active roots) using multiple independent methods. Data from the snow-free periods of two consecutive years are presented, including one entire season (bud burst through leaf senescence) when all methods were applied simultaneously. Comparisons were made between $\delta^{13}C$ of soil respiration on untrenched and trenched plots; respired CO_2 from sieved soil cores (soils alone), roots and leaves; and bulk C from soils and root and leaf tissues.

Our continuous open chamber data and experimental treatments provided a unique opportunity to examine the possible causes of diel fluctuations in $\delta^{13}C$ of the soil surface CO_2 flux. Diel variability in $\delta^{13}C$ of soil respiration has been observed in some recent studies with high-frequency isotopic flux data (Kodama *et al*. 2008; Bahn *et al*. 2009; Marron *et al*. 2009). In these studies diel $\delta^{13}C$ variability was generally interpreted to represent variability in source $\delta^{13}C$ (by implicit assumption of steady-state gas transport). In the current study, we test the alternative hypothesis that diel variability in the carbon isotope content of the soil respiration surface flux can be driven by non-steady states of diffusion within the soil profile.

Transient diffusive fractionations occur whenever boundary conditions, production rates, or soil diffusivities change and a system begins to develop towards a new steady state (Amundson *et al*. 1998; Risk & Kellman 2008; Nickerson & Risk 2009b). Diel variation in surface fluxes is produced when a lighter isotopologue ($^{12}CO_2$) and a heavier isotopologue ($^{13}CO_2$) are released from points of respiration simultaneously in a time-varying manner (e.g. with respiratory production driven by changes in soil temperature). Because of the small differences in diffusivities of $^{12}CO_2$ and $^{13}CO_2$ in air, soils are likely to approach isotopic steady state more slowly than net flux steady state. Thus, daily varying production rates have the potential to perpetuate a transient diffusive state for the isotope ratio of CO_2 exiting the soil, though the net surface CO_2 flux may be near constant equilibrium with production and $\delta^{13}C$ of respiration may be constant. To further investigate this possibility, an isotopic gas transport model treating production and transport of $^{12}CO_2$ and $^{13}CO_2$ independently was run with variable rates and depths of CO_2 production, while maintaining $\delta^{13}C$ of CO_2 production at a constant value. Model results were compared to continuous chamber data from this and previously published studies.

METHODS

Experimental design

This project made use of an experimental garden on the University of Utah campus (40°45′39.3″N, 111°49′48.8″W, 1481 m) established for intensive physiological monitoring of boxelder (*Acer negundo*) trees (Hultine *et al*. 2008). The 100 m by 40 m site was graded and covered with topsoil from a nearby location in 2001, and then 36 trees grown from locally collected cuttings were planted along a six tree by six tree grid. By the time of the present study, the trees were mature and had been setting seed for several years. A barrier was installed in 2005 to bisect the study area into two replicate halves by burying 6.35-mm-thick polyvinyl chloride (PVC) sheets vertically to 2 m depth. Artificial streams were then created in each side by pumping water from a nearby natural stream through perforated tubing within excavated, gravel-lined streambeds that meandered between the trees. Soils were kept at high moisture content throughout each subsequent year by flowing these streams continuously from just after snow melt in April until rain and snow appeared again in November, when leaves were senescent. For additional site-related details, see Hultine *et al*. (2008).

For the present study, the central, 2-m-deep barrier was used to isolate trenched and untrenched (control) plot pairs under individual boxelder trees (Fig. 1). Six trees were growing close enough to this barrier to have canopies that extended above it from one side to the other. In March of 2007 '+Roots' (normal, control plots that contained roots and rhizosphere) and '-Roots' (treatment plots with roots severed by trenching at the start of the study) plot pairs

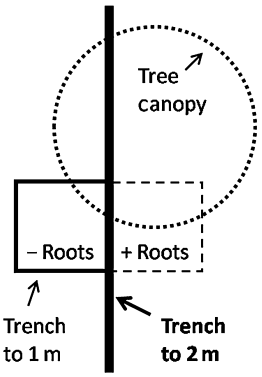

Figure 1. Overhead view of plot setup showing one of six replicate plot pairs under individual boxelder trees. A 2–m deep trenched root barrier runs through the centre of the site with trees (canopy shown by dotted circle) positioned on alternating sides. On the opposite side of this trench a 1 m-deep trenched barrier excluded understory roots from trenched (–Roots) plots. The dashed line on the +Roots side indicates an untrenched plot boundary, with no associated soil disturbance.

were established under each of these trees. One area under each canopy on the same side of the main barrier as the trunk was designated as a '+Roots plot'. An adjacent, approximately 1.5 m^2 '-Roots' plot was created on the opposite side by trenching on three additional sides to 1 m depth and lining with 1-mm-thick polyethylene sheeting. The edges where the two sides of this plot met the main 2-m-deep barrier were sealed with a silicone sealant before backfilling. This study coincided with a nitrogen fertilization experiment at the site, in which half of the study area received a nitrogen addition to the stream water. The arrangement of the trenched/untrenched plots was such that half of each trenching treatment group (three plots each) was within each nitrogen treatment, allowing for detection of any effects of fertilization on our results.

Understory vegetation within and immediately surrounding all plots was removed weekly throughout the study. Any live roots present within the trenched plots would have been severed by trenching and surface clearing in March of 2007 and represented a potential substrate source for decomposition during the following two growing seasons of the study. However, given that the 2 m root barriers were already isolating these areas from roots of nearby trees, the majority of live roots in these plots would have been from herbaceous understory vegetation (mostly C_3 grasses and forbs), which had only recently begun to germinate at the time of plot installation and clearing.

Meteorological measurements

Air temperature and relative humidity probe measurements (HMP 45 AC, Vaisala, Woburn, MA, USA) were collected every 30 s and stored as 10 min averages during the entire study period by an on-site micrometeorological station described by Hultine *et al*. (2008). Soil moisture and temperature were measured within a subset of plots to identify any differences associated with the trenching treatment. Soil temperature was measured with thermocouples (type T) inserted to 5 cm depth in two plot pairs and soil moisture was recorded with reflectometry probes (CS615, Campbell Scientific, Logan, UT, USA) placed at 15 cm in one plot pair. These were measured at 10 s intervals and stored as 10 min averages by a data logger (CR10X, Campbell Scientific), beginning in April 2008.

Soil CO_2 and $\delta^{13}C$ profile measurements

Gas wells were installed in each of the 12 plots in early April, 2007. The gas wells consisted of individual lengths of stainless steel tubing (6.35 mm OD) with open, buried ends at 1, 2, 4, 7, 10 and 35 cm below the surface and fittings containing septa (Microsep F-138, Alltech, Deerfield, IL, USA) on the ends protruding above the soil surface. To install the upper five wells a small, 10-cm-deep hole was excavated near one corner of each plot. Then a 20 cm length of tubing was inserted horizontally through the pit wall at each measurement depth in randomly fanning directions, but generally towards the centre of the plot. A metal rod was temporarily placed inside the tube during insertion to prevent clogging. A second piece of tubing with a 90° bend was then attached to each horizontal tube, a septum fitting was placed on the aboveground end, and the hole was backfilled. The 35 cm wells consisted of a single length of tubing with a septum fitting and were installed vertically towards the centre of each plot, with a metal rod used during installation to prevent clogging.

Gas samples were collected from each gas well in evacuated 12 mL vials (Exetainer, Labco, High Wycombe, Buckinghamshire, UK) using a two-ended needle. Plots were visited for gas well sampling roughly biweekly during the snow-free periods of 2007 and 2008 (March/April–November), which included the entire period from budburst to leaf senescence each year. Mole fraction of CO_2 was measured from each vial by injecting a 0.5 mL sample into a CO_2-free air stream, through a port just upstream of an infrared gas analyser (IRGA, Li-7000, Li-Cor, Lincoln, NE, USA) and integrating the CO_2 peak (Davidson & Trumbore 1995). Peak areas measured from prepared CO_2 standard gases were used to calculate sample CO_2 mole fractions. A second gas sample was then injected into a tunable diode laser absorption spectrometer (TGA 100A, Campbell Scientific) for measurement of $\delta^{13}C$ of CO_2 as described in detail by Moyes *et al*. (2010). For this measurement the volume of sample injected depended on CO_2 mole fraction, and samples were calibrated using injections from three prepared $\delta^{13}C$ standard cylinders. Measurement uncertainties were 5% of reading for mole fractions and 0.25‰ for $\delta^{13}C$.

Closed chamber soil respiration rate measurements

Ten-cm diameter PVC collars were inserted to 4 cm depth in each plot for closed chamber measurements. Soil respiration rates were measured manually using a portable gas exchange system and a closed chamber (Li-6400-09, Li-Cor) on the same days that soil gas wells were sampled.

Determination of rhizosphere and heterotroph respiration rates and $\delta^{13}C$

Respiration fluxes on trenched plots were assumed to represent the contribution of heterotrophic soil organisms (soil organic matter-driven) to total soil respiration. This amount was subtracted from the flux measured on untrenched plots to give the contribution of rhizosphere (photosynthate-driven) respiration to total soil respiration. Mole fraction and $\delta^{13}C$ data from soil gas well profiles were used to calculate $\delta^{13}C$ of respired CO_2 for each sampling date, using either data from individual profiles or composite data from all +Roots or –Roots replicate plots, via the two-end member Keeling plot approach (Keeling 1958). For this analysis it was necessary to assume that CO_2 in gas samples from the entire soil profile would reflect a mixture of only two sources (atmospheric and respired CO_2), with full

equilibration between production and diffusive transport of CO_2. Comparison of the gas well approach to chamber measurements (described below) provided a test of this assumption. Intercepts of lines fit to $\delta^{13}C$ vs. 1/mole fraction of soil CO_2 were used, and a steady state, 4.4‰ diffusive enrichment correction was subtracted from each intercept to calculate the $\delta^{13}C$ of respired CO_2 from each plot or treatment (Cerling *et al*. 1991; Davidson 1995). The calculated $\delta^{13}C$ of the soil CO_2 source from trenched plots was taken to represent the $\delta^{13}C$ of CO_2 respired from soil heterotrophs (δ_{Het}). This source and the $\delta^{13}C$ of respired CO_2 from the rhizosphere (δ_{Rhiz}) were assumed to combine to produce the $\delta^{13}C$ of CO_2 respired in untrenched plots (δ_{Tot}). δ_{Rhiz} was calculated as:

$$\delta_{Rhiz} = \frac{(F_{tot} * \delta_{tot}) - (F_{Het} * \delta_{Het})}{F_{tot} - F_{Het}} \quad (1)$$

where F_{tot} and F_{Het} are the closed chamber flux rate measurements and δ_{tot} and δ_{Het} are the $\delta^{13}C$ calculated for respiration sources from untrenched and trenched plots, respectively.

Open chamber determination of rates and $\delta^{13}C$ of rhizosphere and heterotroph respiration

Four permanent, 30.5-cm-diameter PVC collars were inserted 5 cm into the ground in two +Root/–Root plot pairs in early April, 2008. Two flow-through open chamber lids modelled after Rayment & Jarvis (1997) were used to measure continuous flux rates and $\delta^{13}C$ of soil respiration with a tunable diode laser absorption spectrometer as described by Moyes *et al*. (2010). Equipment availability limited measurements to two chambers during a given time (one +Root, one –Root). Chamber lids were moved between pairs of collars approximately every two weeks and immediately following rain events. Lids were sealed to the collars using putty (Terostat VII, Henkel Technologies, Dusseldorf, Germany) and left in place until they were moved to the other collar pair (lids did not open). Soil respiration flux rates were calculated as:

$$Flux = \frac{(C_o - C_i) * Flow}{A} \quad (2)$$

where C_o and C_i are the mole fractions of CO_2 in the dry inlet and outlet flows from the chambers, 'Flow' is the number of moles of dry air passing through the chamber per second and A is the soil surface area enclosed by the chamber. The isotope composition of the soil respiration flux ($\delta^{13}C_{SR}$) was calculated as:

$$\delta^{13}C_{SR} = \frac{(C_o * \delta_o) - (C_i * \delta_i)}{C_o - C_i} \quad (3)$$

where δ_o and δ_i are the $\delta^{13}C$ of the CO_2 in the inlet and outlet flows in‰. Flow through each chamber was periodically adjusted between 1 and 4.5 L min^{-1} to maintain a roughly 50–100 μmol mol^{-1} difference in CO_2 between inlet and outlet flows. This range represented a trade-off optimum, as smaller gradients limit isotope precision and larger gradients would lead to flux underestimation (Davidson *et al*. 2002). Prior to field deployment, chambers were tested for differential pressure effects over a range of chamber flow rates with the chamber bottom sealed to a bench top in the laboratory. Flow rates of up to 4.5 L min^{-1} produced differential pressures smaller than –0.2 Pa (lower within the chamber). Use of a sealed bench top in place of a porous soil medium identified the maximum pressure perturbation associated with each flow rate (Xu *et al*. 2006). Longdoz, Yernaux & Aubinet (2000) reported that a pressure difference of this magnitude across a chamber placed in soil increased fluxes by less than 10%, and the chosen maximum flow rate of 4.5 L min^{-1} was below limits reported to produce minimal effects on CO_2 flux measurements with similar chambers (Rayment & Jarvis 1997; Fang & Moncrieff 1998).

Chamber measurements were made every 10 min, and data are reported as 3 h and daily averages to reduce noise. The flux and $\delta^{13}C$ of respired CO_2 from trenched plots was assumed to reflect the heterotrophic contribution to soil respiration, and the rhizosphere-respired CO_2 flux and $\delta^{13}C$ were calculated from untrenched and trenched CO_2 fluxes and $\delta^{13}C$ as described above.

$\delta^{13}C$ of leaves, roots, soil and respired CO_2 from each

Examination of the diel pattern of bulk $\delta^{13}C$ of ecosystem components (sun leaf, shade leaf, root, untrenched plot soil and trenched plot soil), and the $\delta^{13}C$ of respired CO_2 from each was conducted 29–30 July 2008. Four sets of samples were taken from three trees and their associated '+Roots/–Roots' plots every 6 h beginning at 0900 h. At each sampling time, three individual fully expanded leaves, containing three leaflets, from the top (sun) and bottom (shade) of each canopy were cut and stored in dark conditions for 10 min before respiration measurements. This consistent delay was chosen to allow leaves to dark-acclimate and avoid transient isotope effects upon darkening (Barbour *et al*. 2007). At each sampling time, a 5-cm-diameter core was taken to a depth of 20 cm from each plot using a bucket auger. Roots, when present, were manually picked from these cores, rinsed with distilled water and patted dry. The soil was then sieved to remove particles larger than 2 mm and the remaining fraction was subsampled. A gas exchange system composed of a closed loop with an IRGA (Li-820, Li-Cor), a pump (UNMP830 KVDC-B, KNF, Freiburg, Germany), a glass sample cuvette and two 100 mL glass flasks in parallel was used to collect samples for analysis of CO_2 and $\delta^{13}C$. The system was connected to a cylinder containing 400 μmol mol^{-1} (–9.45‰) CO_2 in air and flushed before each measurement. Next a leaf, root, or soil sample was placed in the chamber, held in place with glass wool and the system was flushed from the tank again. The gas cylinder was then disconnected and the pump turned on to circulate the air in the system in a closed

loop. Once mixing was adequate, which was apparent in the stability of IRGA measurements and took about 5–10 s, the pump was stopped and the stopcocks on one of the flasks were immediately closed. The pump was started again and CO_2 was allowed to accumulate until the mole fraction had risen by ~50 μmol mol^{-1}, when the pump was stopped and the second flask was sealed. Mole fraction and $\delta^{13}C$ of CO_2 in the flasks were measured on a continuous flow isotope ratio mass spectrometer (IRMS, Delta Plus, ThermoFinnigan, Bremen, Germany). $\delta^{13}C$ of respired CO_2 from the sample was calculated similarly to Eqn 3 (initial and final flasks treated as inlet and outlet). Solid organic samples were immediately placed in drying ovens at 60 °C after respiration measurements. Soil samples were acid washed to remove carbonates. Dried samples were milled and measured via continuous flow IRMS coupled with an elemental analyser (EA 1108, Carlo Erba, Rodano, Italy).

Isotopic diffusion model

To examine the extent to which diel variability in $\delta^{13}C$ of soil respiration may be produced by diffusive fractionation effects, a model was developed in which $\delta^{13}C$ of soil CO_2 production was held constant and production and diffusion of $^{12}CO_2$ and $^{13}CO_2$ in the soil were treated independently under varying physical conditions. Model parameters were selected to encompass observed values for those variables that were measured in the current study, and to include realistic values for those that were not. The aim was to include enough variability in model parameters to identify sensitivity of the diel range of modelled $\delta^{13}C$ of the surface CO_2 flux to variability in each parameter. A total of 320 different simulations were conducted by varying the following parameters in a factorial manner: the shape of the CO_2 production function with depth, the maximum depth of CO_2 production (0.1, 0.2, 0.4, or 0.8 m), the rate of CO_2 production at the surface at 10 °C (0.5, 1, 2, 10, or 20 μmol m^{-3} s^{-1}), Q_{10} of production of CO_2 (1, 2, 3 or 4), and the volumetric water content profile (0.05, 0.10, 0.15, 0.20, 0.20 m^3 m^{-3} ('dry') or 0.15, 0.30, 0.35, 0.35, 0.40 m^3 m^{-3} ('wet') at 0, 0.10, 0.20, 0.45 and 1 m depth nodes, respectively). In each simulation, four days were run at one time. Within each 4 d set, the maximum $\delta^{13}C$ of the modelled surface CO_2 flux from the fourth day was compared to the maximum from the first day. Each simulation would continue until these two values were within 0.05‰ of one another. At that point, the rates and $\delta^{13}C$ of the modelled surface CO_2 flux from the final day were recorded and a new simulation would start with the next parameter set, using the profiles of $^{12}CO_2$ and $^{13}CO_2$ from the last time step of the previous model run as initial conditions.

Within the model, a soil column of unit area and a soil depth of 1 m was divided into layers of 2 cm depth increments. Model time steps were 0.002 h (7.2 s). These depth and time increments were found to produce consistent model stability. Total porosity was set to 0.5 m^3 m^{-3} throughout the soil profile and volumetric water content (θ, m^3 m^{-3}) was linearly interpolated between 'dry' or 'wet' node values. Air-filled porosity was calculated for each depth by subtracting θ from total porosity.

CO_2 production at 10 °C was either input as a decreasing function of depth after Kirkham & Powers (1972):

$$R_{10}(z) = R_{10,z=0}\left(1-\left(\frac{z}{z_{R=0}}\right)^{1/4}\right) \quad (4)$$

where $R_{10,z=0}$ is the CO_2 production rate at 10 °C at the surface in μmol m^{-3} s^{-1} and $z_{R=0}$ is the depth where production goes to zero; or represented by a constant value over a depth interval:

$$R_{10}(z) = \begin{cases} R_{10,z=0} & z < z_{R=0} \\ 0 & z \geq z_{R=0} \end{cases} \quad (5)$$

The CO_2 production profile was then adjusted for changing soil temperature with depth and time. Soil temperature was modelled after Campbell & Norman (1998) with surface temperature set to vary between 10 and 25 °C:

$$T(z,t) = T_{ave} + A_o * \exp(-z/d) * \sin[\omega(t-8) - z/d] \quad (6)$$

where T_{ave} is the average surface temperature, A_o is half of the peak-to-peak diel variability of surface temperature, d is a damping depth and ω is π/12 and sets the period to 24 h. Damping depth was set to 0.05 for dry, and 0.1 for wet soil conditions (Campbell & Norman 1998). CO_2 production in each layer and time step was adjusted according to temperature at each depth following the Q_{10} equation (Curiel Yuste, Janssens & Ceulemans 2005):

$$R(z,t) = R_{10}(z) * Q_{10}^{((T(z,t)-10)/10)} \quad (7)$$

where Q_{10} is a coefficient defining the temperature sensitivity of CO_2 production. Individual production rates for $^{12}CO_2$ and $^{13}CO_2$ were then calculated to reflect a constant $\delta^{13}C$ of total production of −25‰. The number of moles of CO_2 produced within a given layer over each time step was calculated as:

$$R_{i,j-1} = R(z,t) * \Delta z * \Delta t \quad (8)$$

where subscripts i and j reflect vertical layers and model time steps, respectively, Δz is the difference in depth (m) between successive layers and Δt is the length of each time step (s).

Diffusion coefficients of CO_2 were calculated for each soil layer and time step following:

$$D(z,t) = D_o(z,t) * \xi(z) \quad (9)$$

with $D_o(z,t)$ being the diffusivity of CO_2 in air, given by:

$$D_o(z,t) = D_{ao} * \left(\frac{T(z,t)}{293.15}\right)^{1.75} * \left(\frac{101.3}{P}\right) \quad (10)$$

where P is 85 kPa (local atmospheric pressure for Salt Lake City) and D_{ao} is 15.7 mm^2 s^{-1}, the reference value for CO_2 diffusivity in air at 293.15 K and 101.3 kPa (Campbell &

Norman 1998). $\xi(z)$ is a tortuosity factor, which was calculated based on air-filled (ε) and total (φ) porosities following Millington (1959):

$$\xi(z) = \frac{\varepsilon(z)^{\frac{10}{3}}}{\phi^2} \tag{11}$$

The diffusion coefficients for $^{12}CO_2$ and $^{13}CO_2$ for each layer and time were then calculated from the corresponding total CO_2 value to maintain a ratio (D_{12CO2}/D_{13CO2}) of 1.0044 (Cerling *et al*. 1991).

Vertical fluxes of $^{12}CO_2$ and $^{13}CO_2$ between layers were calculated as:

$$F_{ij} = \left(\frac{C_{i,j-1} - C_{i-1,j-1}}{\Delta z}\right) * \left(\frac{D_{i,j-1} + D_{i-1,j-1}}{2}\right) * \Delta t \tag{12}$$

where C is the isotopologue molar density in μmol m^{-3}. The new molar density of CO_2 in each layer after each model time step ($C_{i,j}$) was then calculated as the sum of the molar density in the previous time step ($C_{i,j-1}$), the flux out through the upper boundary (F_{out}), the flux in through the lower boundary (F_{in}) and the amount produced within the layer ($R_{i,j-1}$) following Nickerson & Risk (2009b):

$$C_{i,j} = \frac{C_{i,j-1} * \varepsilon * \Delta z - F_{out} + F_{in} + R_{i,j-1}}{\varepsilon * \Delta z} \tag{13}$$

To maintain a constant surface boundary condition and calculate surface fluxes of $^{12}CO_2$ (F_{12CO2}) and $^{13}CO_2$ (F_{13CO2}), the uppermost 'soil' layer was maintained at CO_2 mole fraction of 385 μmol mol^{-1} and $\delta^{13}C$ of –8.5‰. Calculated fluxes of $^{12}CO_2$ and $^{13}CO_2$ across the upper boundary of the uppermost layer were summed to produce the total surface CO_2 flux and used to calculate the surface flux $\delta^{13}C$ ($\delta^{13}C_F$) following

$$\delta^{13}C_F = \left(\frac{F_{13CO2}/F_{12CO2}}{R_{std}} - 1\right) * 1000 \tag{14}$$

where R_{std} is the $^{13}C/^{12}C$ ratio of the Vienna PDB scale (0.01124) (Craig 1957).

RESULTS

Soil respiration fluxes in plots with roots followed the seasonal pattern of air and soil temperature, being highest in midsummer when leaves were on the trees, and lowest in winter while trees were dormant (Fig. 2). Seasonal variability in soil respiration on plots without roots was much smaller, leading to a calculated relative contribution of rhizosphere respiration of up to ~75% of the total CO_2 flux on plots with roots in the summer. $\delta^{13}C$ of soil respiration from individual open chambers (Fig. 2f) and soil gas profiles grouped by treatment (Keeling plots constructed with all measurements from a particular treatment and sampling date, Fig. 2c) were enriched in ^{13}C in summer by about 4‰ relative to winter on all plots, independent of trenching. During peak flux rates in midsummer, $\delta^{13}C$ of soil respiration calculated from soil gas Keeling plots (Fig. 2c) and from daily averages of open chamber data (Fig. 2f) was more enriched in plots with live roots (~–25.5‰) than in trenched plots (~–26.5‰). Because the majority of soil respiration on plots with roots during summer was associated with rhizosphere respiration (Fig. 2b.), the calculated rhizosphere-respired $\delta^{13}C$ endmember was only slightly more enriched (<1‰) in $^{13}CO_2$ than the total soil flux on these plots (Fig. 2c).

Respiration $\delta^{13}C$ data from open chambers indicated similar seasonal and treatment effects to those produced from gas well profiles from the same individual plots (Fig. 3), with both methods showing consistent seasonal patterns of summertime enrichment in $\delta^{13}C$ of soil respiration, and isotopically heavier respired CO_2 from plots with roots. These patterns were apparent in a comparison of flux $\delta^{13}C$ vs. flux rates for the two method combinations (Fig. 4a,b), where high summer fluxes associated with the +Roots treatment were generally more enriched in $^{13}CO_2$ compared to low cold season fluxes from both treatments.

No strong diel patterns of $\delta^{13}C$ of respiration were observed in the overnight gas exchange measurements from leaves, roots, or soils and so averages from all replicates and sampling times are presented (Fig. 4c). Only the bulk samples from the 0300 h sampling are presented. $\delta^{13}C$ of respiration from sieved soil samples was more enriched from plots with roots than without roots, consistent with chamber and profile measurements of intact soil (Fig. 4a,b). This contrasted with the difference between $\delta^{13}C$ of bulk soil carbon between treatments, which was most enriched in samples from plots without roots. Measurements of $\delta^{13}C$ of respiration from root samples were more enriched than all other measured respiration sources and plant tissues. Sun leaf biomass and respiration were enriched in ^{13}C relative to shade leaves, and leaf respiration was enriched relative to leaf biomass for both sun and shade leaves.

Large diel variation was observed in open chamber measurements of $\delta^{13}C$ of the soil CO_2 surface flux during some periods from some plots (Figs 3 & 5). In Fig. 3, data with large peak-to-peak variability appearing as random noise were in fact regular, diel fluctuations, as seen in Fig. 5c. When observed, this variation was generally in phase with 5 cm soil temperatures (Fig. 6), being most enriched in the afternoon and most depleted in the early morning (Figs 5 & 6). The magnitude of diel variation in respiration $\delta^{13}C$ was highest when surface flux rates were low (Figs 6 & 7a). Diel variability in $\delta^{13}C$ of soil respiration was positively correlated with the coefficient of variation (CV) of the respiration flux (standard deviation of diel flux/average diel flux), but not the total magnitude of flux variability (Figs 6 & 7). This distinction is highlighted in data from a ten-day period from a +Roots and –Roots plot pair presented in Fig. 6: although the amplitude of flux variability in the +Roots plot was greater (panel c), the CV and the diel variability in $\delta^{13}C$ of soil respiration (panel b) were larger in the –Roots plot. These trends were consistent throughout the season regardless of the presence or absence of active roots (Fig. 7).

Model results supported the relationship presented in Fig. 7a, as the diel range of $\delta^{13}C$ exiting the surface layer

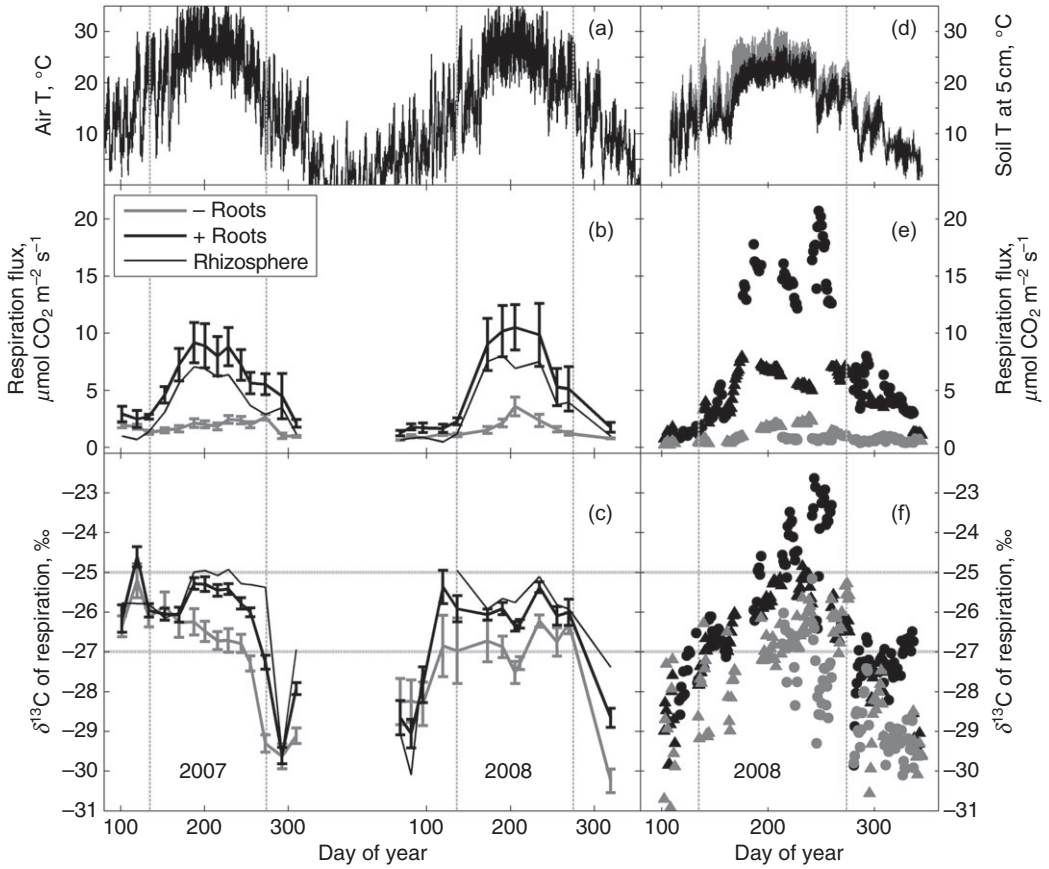

Figure 2. (a) Air temperature for the 2007 and 2008 study periods. (b) Average soil respiration fluxes by treatment measured with the closed soil chamber and the calculated average rhizosphere contribution to soil respiration rates. Error bars are one standard error of the mean. (c) $\delta^{13}C$ of respiration from Keeling plots generated from composite soil gas profile data by treatment and the calculated $\delta^{13}C$ of rhizosphere-respired CO_2. Error bars are one standard error of the intercept. (d) Average soil temperatures at 5 cm depth from two trenched (gray) and two untrenched (black) plots in 2008. (e) Average daily soil respiration fluxes measured with the open chambers from two trenched (gray) and two untrenched (black) plots (plot 1: circles, plot 2: triangles). (f) Average daily $\delta^{13}C$ of respiration measured with the open soil chambers from two trenched (gray) and two untrenched (black) plots (plot 1: circles, plot 2: triangles). Dotted vertical lines in all plots show the approximate dates of bud burst (May 15) and leaf senescence (October 1) of trees for the two growing seasons. Horizontal lines in the bottom panel highlight $\delta^{13}C$ values of –25 and –27‰ for comparison to Figs 3–5.

was largest when fluxes were small (Fig. 8a). Modelled variability in surface flux $\delta^{13}C$ was not as directly associated with flux variability (coefficient of variation, Fig. 8b) as was measured in the current study (Fig. 7b). Model simulations consistently produced maximum variability in $\delta^{13}C$ of the surface flux when CO_2 production was concentrated near the soil surface, such as within the top 10 cm (Fig. 8c). The diel phase of $\delta^{13}C$ of the surface flux produced by the model varied slightly, depending on input parameters, but generally modelled flux $\delta^{13}C$ peaked just before midday.

Soils at 5 cm depth reached higher afternoon temperatures by a few degrees during summer in the two measured trenched plots than in the two untrenched plots (Fig. 2d). Water content at 15 cm in the instrumented trenched plot remained relatively constant throughout the measured period in the absence of transpiration (data not shown). While a seasonal pattern was apparent in the 15 cm water content of the irrigated untrenched plot, minimum water content remained fairly high (>20%) and similar to the water content measured in the trenched plot during summer. During coring for soil samples on July 30, 2008, tree roots were found to have grown through a seam in the plastic sheeting and into one trenched plot. No data from this plot were used for '+ Roots/–Roots' treatment comparisons, but chamber data from this plot were plotted in Fig. 7 as '+ Roots'. The open soil chamber collar was moved to another trenched (–Roots) plot where measurements resumed. Effects of the trenching treatment overshadowed any effects of the coincident nitrogen addition treatment at the site on the soil respiration fluxes and $\delta^{13}C$ of CO_2, so we pooled data according to trenching only.

DISCUSSION

Measurements of soil respiration $\delta^{13}C$

This study examined the $\delta^{13}C$ of soil respiration using soil gas Keeling plots and open soil chambers, including an

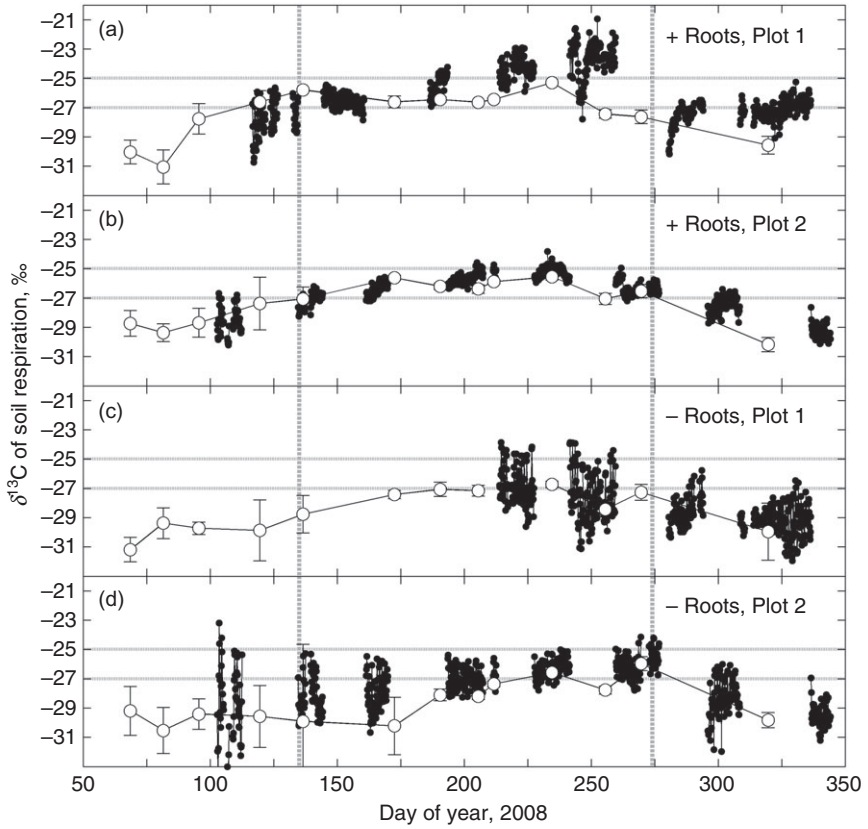

Figure 3. Carbon isotope ratio of soil-respired CO_2 derived from soil gas profiles (○) and three-hour means from open chambers (●) on individual plots with (top two panels) and without (bottom two panels) roots during 2008. Dotted vertical lines show approximate dates of bud burst (May 15) and leaf senescence (October 1) of trees. Horizontal lines highlight $\delta^{13}C$ values of –25 and –27‰. Error bars are 1 standard error of the intercept. Periods with high variability in open chamber measurements of flux $\delta^{13}C$ are due to regular, diel patterns (see Fig. 5).

entire season of concurrent measurements. While laboratory experiments have demonstrated comparability and accuracy of these two methods with measurement of a controlled CO_2 source (Moyes *et al*. 2010), this level of agreement between the two methods in a field study (Figs 2, 3 & 4a,b) was encouraging. It is worth stressing that all current methods to measure $\delta^{13}C$ of soil respiration are wrought with methodological challenges because of the requirement for minute diffusive gradients to remain undisturbed (Nickerson & Risk 2009a). This is why we applied two independent approaches to measure soil flux $\delta^{13}C$, and sought to evaluate our results with a diffusive transport model. Open soil chambers were chosen because they induce minimal lateral diffusion (Nickerson & Risk 2009a) and remain in place long after diffusive re-equilibration of chamber artefacts should occur. Soil gas profiles were selected for comparison with the expectation that gas wells would equilibrate more slowly with changes in soil gas conditions, and thus be less sensitive to short-term disturbances and provide measurements representing flux variability over slightly longer time scales.

Diel flux $\delta^{13}C$ variability was observed with both open soil chambers in a manner similar to other published studies, and which agreed with model simulations (Fig. 8). Further, maximum $\delta^{13}C$ variability was measured while flow through the chamber (and thus any induced pressure gradient) was lowest to maintain a minimum mole fraction difference between inlet and outlet flows during low flux periods. Two sets of overnight measurements of gas wells were conducted and results (data not shown) suggested that Keeling intercepts followed the diel cycle observed with chambers, but this variability was dampened. This difference would be expected because changes in soil gas measurements require equilibration of the soil gas profile and gas well volume. For analysis of seasonal and trenching treatment effects, average daily values of soil respiration $\delta^{13}C$ from the soil chambers were compared to afternoon gas well Keeling plot values. From these data some strong biotic effects were evident. While the application of consistent methods should have rendered the relative seasonal and trenching effects largely neutral to any measurement artefacts, confidence in the absolute values of these effects comes from the similarity of results obtained with both methods.

Trenching treatment effects

The trenching treatment produced one set of plots with an entirely heterotrophic CO_2 source, which we compared to adjacent plots with a seasonal shift from a heterotrophic winter source to a primarily autotrophic (photosynthate-driven) summer CO_2 source. Trenching reduced summer soil respiration rates by about 75%, which provides an estimate of the seasonal maximum contribution from the rhizosphere to soil respiration at this site (Fig. 2b). This value is larger than the 31–65% reductions observed after girdling in North American (Scott-Denton, Rosenstiel & Monson 2006) and European (Högberg *et al*. 2001; Bhupinderpal-Singh *et al*. 2003; Subke *et al*. 2004) coniferous forest stands,

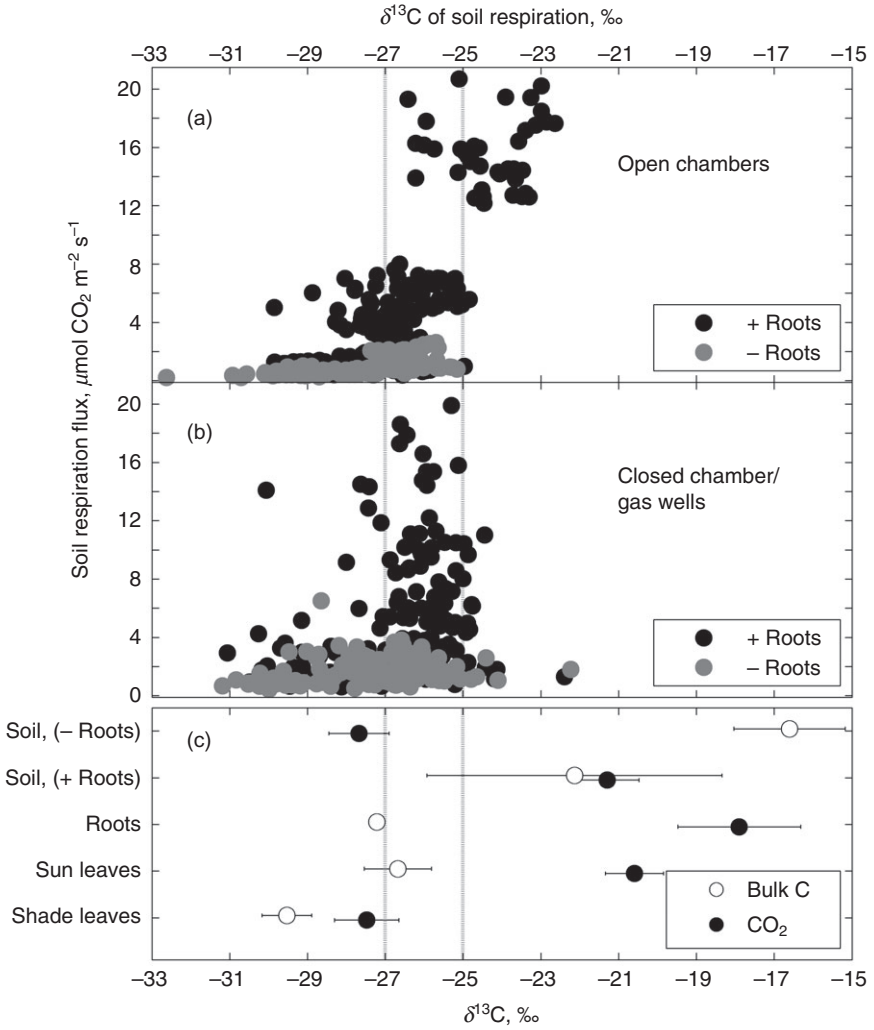

Figure 4. Comparison of fluxes and $\delta^{13}C$ of soil-respired CO_2 using two method combinations: afternoon closed chamber flux measurements vs. $\delta^{13}C$ of soil respired CO_2 from gas profile-derived Keeling plots (a), and daily average fluxes vs. $\delta^{13}C$ of soil-respired CO_2 from open chambers (b). (c) Bulk $\delta^{13}C$ values from sieved soils (soil with roots and rock pieces removed, from the +Roots or the –Roots plots) and plant tissues (open symbols) and the $\delta^{13}C$ of their respired CO_2 (closed symbols). Diel variation was not observed in $\delta^{13}C$ of respired CO_2, so measurements from all sampling times were averaged. Error bars are 1 SEM. Vertical lines highlight –25 and –27‰.

and similar to the 71% maximum summertime reduction of soil respiration seen in trenched plots in a Japanese mixed deciduous forest (Lee *et al*. 2003). Calculated rhizosphere respiration approached zero during the cold seasons when leaves were absent from the trees. Seasonal variation in soil respiration on trenched plots was small and decoupled from patterns on adjacent plots with roots. This is evidence that the trenching treatments in the current study were deep enough to exclude lateral diffusion of CO_2 beneath trench walls and root in-growth, which can lead to underestimation of rhizosphere respiration (Jassal & Black 2006). Additional factors that were not accounted for in our rhizosphere respiration estimates were the possible flux of CO_2 in the xylem stream (Aubrey & Teskey 2009) and priming of decomposition of soil organic matter.

During the growing season, soil respiration on plots with roots was predominantly more enriched in $^{13}CO_2$ than respiration from trenched plots (Figs 2–5). This difference of about 1‰ was attributed to enriched respiration from the rhizosphere, which represents a flux-weighted mean of root and mycorrhizal respiration and consumption of root exudates or root tissues by microorganisms. Root-stimulated mineralization of soil organic matter was assumed to produce a $\delta^{13}C$ of respiration matching that on root-free plots. Enrichment of rhizosphere respiration is supported by the enriched $\delta^{13}C$ of respiration measured directly from roots relative to soil sampled from trenched or untrenched plots (Fig. 4c). The large difference observed between $\delta^{13}C$ of root tissue and root-respired CO_2 is higher in magnitude than has been previously reported. Accumulation of carbon dioxide during our root respiration gas exchange measurements was slow, potentially indicating low or altered metabolic activity within the excised and washed roots sampled, and/or enhancing the possibility for measurement errors. While the magnitude of enrichment of root respiration observed in the current study is unprecedented, this result is qualitatively consistent with our soil flux $\delta^{13}C$ measurements. The same directional influence of roots on soil respiration $\delta^{13}C$ was also found in a recent evaluation of soil CO_2 sources in a *Fagus sylvatica* forest (Marron *et al*. 2009). Those authors found that $\delta^{13}C$ from root respiration was more enriched than CO_2 respired in soil or litter incubations. Studies involving *Eucalyptus delegatensis* (Gessler *et al*. 2007), *Fagus sylvatica* (Damesin & Lelarge 2003),

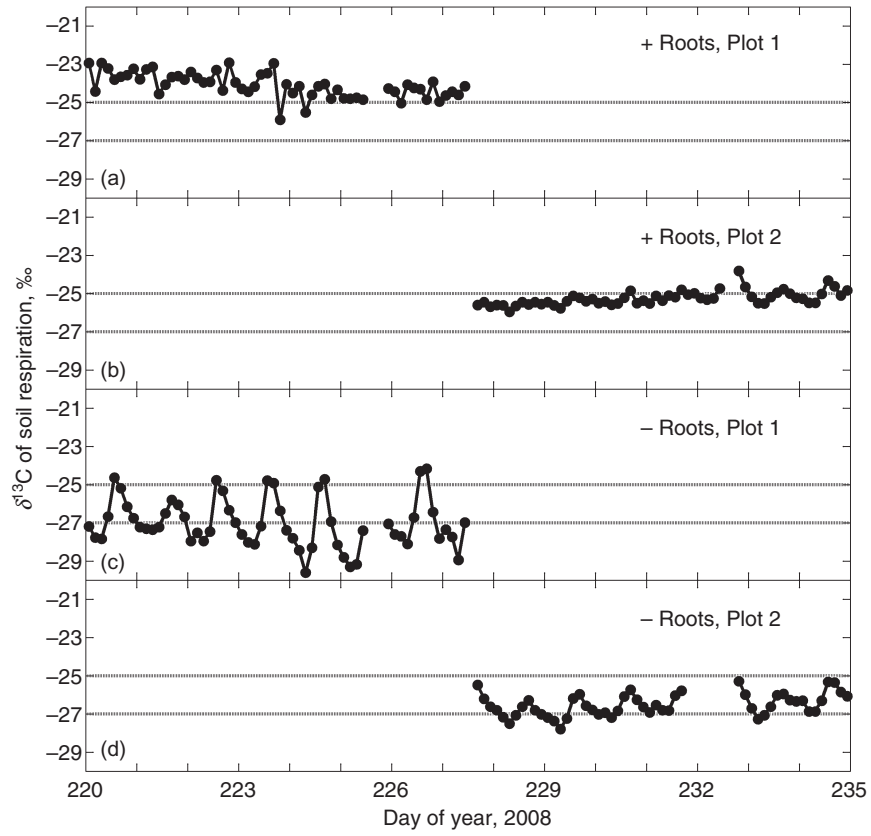

Figure 5. $\delta^{13}C$ of soil respiration from open chamber measurements from each of the four collars during days 220–235 of 2008, showing differences in diel $\delta^{13}C$ variability. Dotted lines highlight –25 and –27‰.

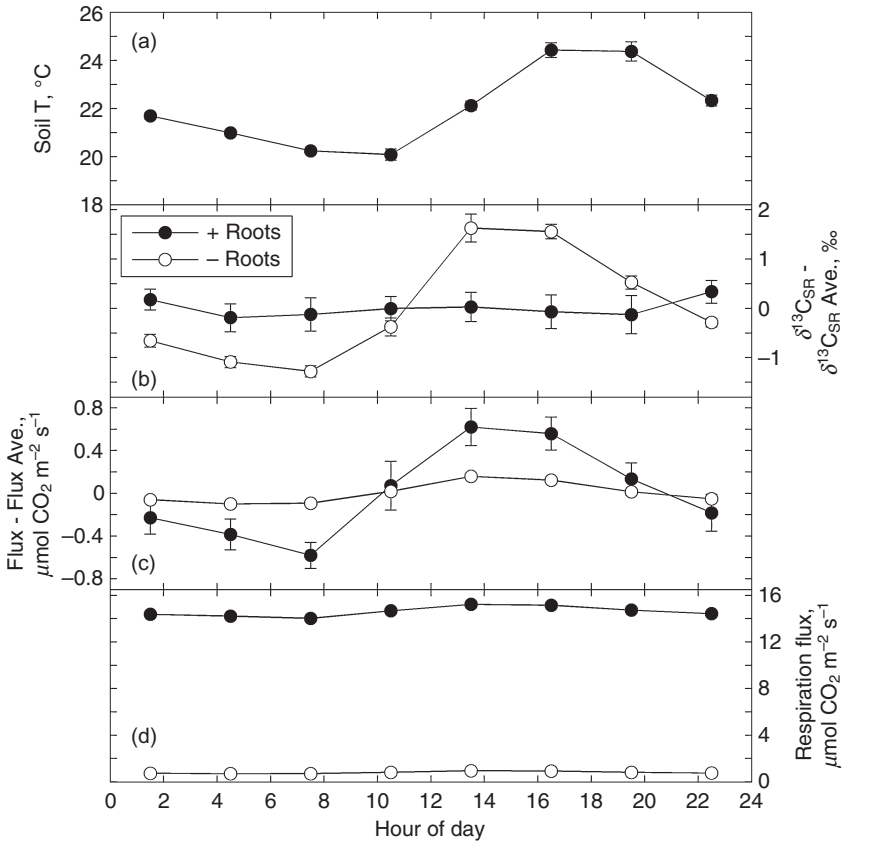

Figure 6. Open chamber data from the plot 1 pair of untrenched (+Roots) and trenched (–Roots) treatments, averaged from days 215–224, 2008. Error bars are 1 SEM and are smaller than symbols where not visible. (a) Average 3 hourly soil temperatures at 5 cm. (b) Diel variation in $\delta^{13}C$ of soil respiration ($\delta^{13}C_{SR}$) from the daily mean. (c) Diel variation from the mean soil respiration flux. (d) Diel flux magnitudes during the averaged period.

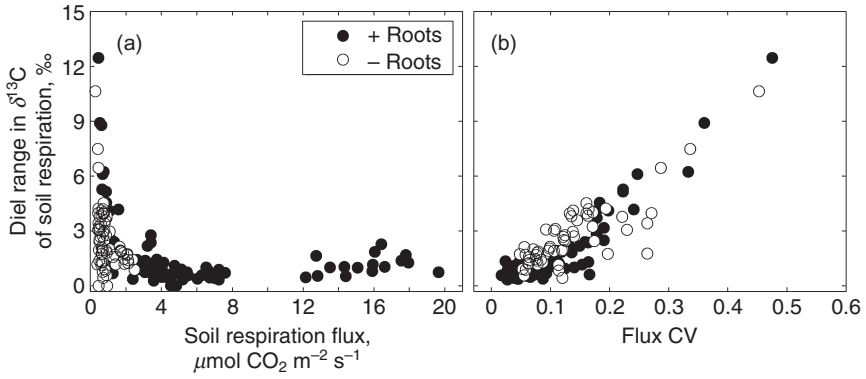

Figure 7. Diel variation in $\delta^{13}C$ of soil respiration plotted against soil respiration flux (a) and the coefficient of variation of the respiration flux (b) *see Fig. 6*. Each data point was calculated from an average of 3 consecutive days of open chamber data from the entire 2008 study period.

Quercus petraea (Maunoury *et al*. 2007) and *Pinus sylvestris* (Brandes *et al*. 2006) trees have additionally found CO_2 respired from trunks and/or roots to be enriched in ^{13}C relative to phloem carbon or bulk stem tissue**.**

Our observations from soil profiles and open soil chambers of a ^{13}C-depletion effect of root exclusion by trenching contrast with a girdling study in a Swedish boreal *Picea abies* forest, which showed no effect of girdling on the $\delta^{13}C$ of soil respiration (Betson *et al*. 2007). However, our observations are consistent with results reported by Subke *et al*. (2004) showing consistently ^{13}C-depleted CO_2 respired in girdled plots relative to controls in a German stand of the same boreal species. Prevost-Boure *et al*. (2009) found mixed isotopic results from trenching treatments in three separate broadleaf forests, but with occasionally significant differences pointing to ^{13}C depletion with trenching.

The observed treatment effect of ^{13}C-depleted respiration from trenched plots was also apparent in the midsummer measurements of respired CO_2 from sieved soil core samples with visible roots removed (Fig. 4c). This suggests that carbon from roots was likely distributed to the soil surrounding roots in untrenched plots as a substrate for microbial respiration, such as in the form of exudates or mycorrhizal fungal biomass. This carbon transfer might also explain the low respiration rates observed from root tissues despite high soil respiration rates on untrenched plots (Figs 2 & 4a,b), and the difference in bulk soil carbon $\delta^{13}C$ between treatments (Fig. 4c). Bulk soil organic carbon $\delta^{13}C$, particularly from soil in trenched plots, was more enriched than expected for a primarily C_3-vegetated area. Because the site was developed from transported local topsoil without complete records of vegetation composition or history of the source area, we cannot exclude the possibility of a mixed C_3/C_4 history affecting the isotope content of soil organic matter at the site. Additionally, though soil samples were tested for complete acidification, the enriched bulk soil

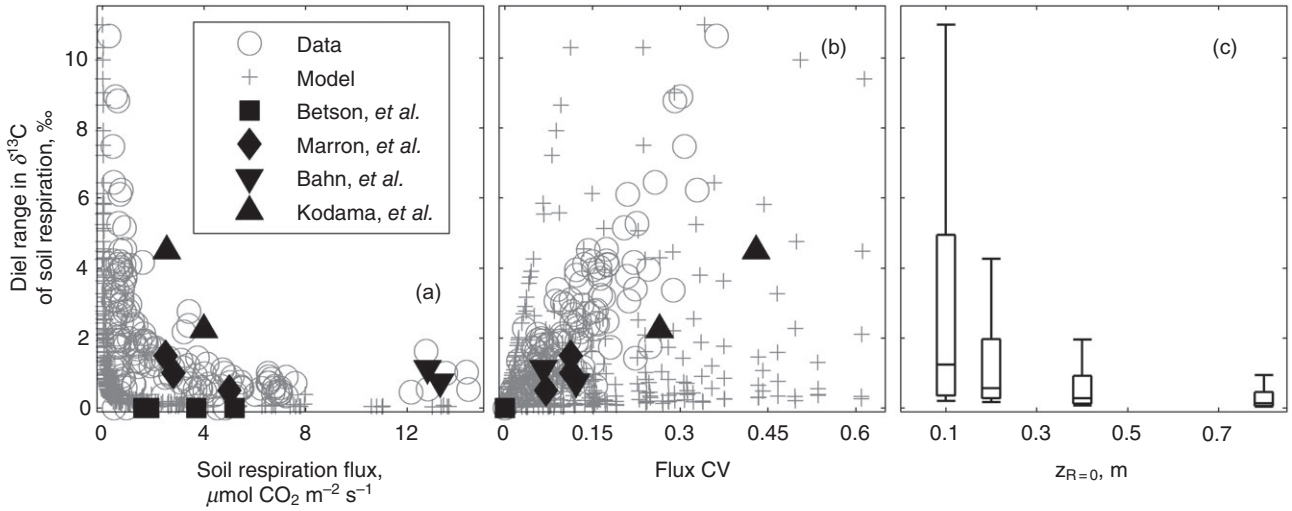

Figure 8. (a,b) Repeat of Figures 7a, b with additions of model output and results from four published studies for comparison. For flux CV calculation in (b), average fluxes were taken as the centre of the flux range, and the coefficient of variation for each flux was calculated by fitting a sine function to the flux average and diel amplitude and calculating its CV. (c), box and whisker plots from 320 model simulations (including data beyond the axes limits of (a) and (b) showing diel variability in soil flux $\delta^{13}C$ produced by the model for different input values of depth of zero production ($z_{R=0}$). Boxes depict quartiles above and below the median and contain 50 percent of observations centred on the median, and whiskers show 75 percent of observations centred on the median for each parameter category. For all model simulations $\delta^{13}C$ of CO_2 production was constant with depth and time at –25‰.

values could be explained by the presence of residual soil carbonates in the samples.

Seasonal variation in $\delta^{13}C$ of soil respiration

The seasonal $\delta^{13}C$ variability of soil respiration in the absence of active roots in the current study (Figs 2c, f & 3c, d) supports the conclusion that heterotrophic processes were responsible for seasonal variability in $\delta^{13}C$ of soil respiration. A similar pattern of enrichment between spring and summer $\delta^{13}C$ of decomposition substrates was seen in both girdled and ungirdled plots in a *Picea abies* forest (Ekberg, Buchmann & Gleixner 2007). This seasonal change was attributed to decomposition of more recalcitrant, ^{13}C-enriched compounds in summer, possibly due to priming in ungirdled plots and increased substrate supply of dying roots and symbionts in girdled plots. Marron *et al.* (2009) argued that summer ^{13}C-enrichment of soil respiration in a *Fagus sylvatica* stand was likely a combined effect of the seasonal contribution of enriched root respiration and seasonal variability in litter respiration $\delta^{13}C$. Alternatively, a seasonal change towards an enriched winter respiration source was observed in root exclusion plots in a Japanese larch forest (Takahashi *et al.* 2008). In the current study involving deciduous trees, a winter-depleted seasonal pattern was observed in plots with and without active roots and low fluxes on trenched plots provided no evidence of increased decomposition of root litter associated with trenching.

Heterotrophically driven variability in soil respiration $\delta^{13}C$ is in contrast to a general emphasis on the importance of weather conditions on photosynthetic discrimination (Δ) as a driver of regional and temporal variability of $\delta^{13}C$ of soil (Ekblad & Högberg 2001; Ekblad *et al.* 2005) and ecosystem respiration (Bowling *et al.* 2002; Scartazza *et al.* 2004; McDowell *et al.* 2004a; Knohl *et al.* 2005; Chen & Chen 2007). For example, a largely seasonal shift towards ^{13}C – depleted soil respiration in cold seasons was observed in a pine and spruce dominated forest in Sweden (Ekblad & Högberg 2001), which was attributed to seasonal changes in evaporative demand and consequent stomatal limitation to Δ. This connection between environmental variables affecting Δ and $\delta^{13}C$ of soil respiration assumes that sugars transported to the soil via the phloem provide a continuous link between above- and belowground $\delta^{13}C$ variability. This connection has been supported by demonstrating a dependence of the $\delta^{13}C$ of phloem sugars on stomatal conductance (Keitel *et al.* 2003; Gessler, Rennenberg & Keitel 2004). Given that a large proportion of forest soil respiration appears to be derived from recent assimilation (Högberg *et al.* 2001), some degree of coupling of Δ and $\delta^{13}C$ of ecosystem respiration is expected. While some field measurements have supported strong correlations between $\delta^{13}C$ of assimilation and respiration on the ecosystem scale (Bowling *et al.* 2002; Scartazza *et al.* 2004; Knohl & Buchmann 2005), others have shown a more nuanced or contingent relationship (McDowell *et al.* 2004b; Barbour *et al.* 2005). Though summers during the present study were relatively warm and dry, the irrigated boxelder trees were maintained in continuously moist soil and leaf tissue $\delta^{13}C$ did not reflect a strong stomatal limitation to photosynthesis (Fig. 4c). Relationships between seasonal or synoptic VPD variations and $\delta^{13}C$ of rhizosphere respiration were not strongly apparent in this data set, with the possible exception of a single storm event in late August, 2008 (Fig. 3a, days ~245, VPD data not shown).

The explanation for the changes in heterotrophic substrate utilization and possibly microbial community composition responsible for the consistent seasonal pattern observed in $\delta^{13}C$ of respired CO_2 in the current study in 2007 and 2008 is unknown. Although annual turnover of root litter was limited to untrenched plots, leaf litter fell onto all plots in each fall and was not removed, representing a seasonal pulse of new carbon for decomposition. Soil microbial communities, and the activity of their associated extracellular decomposing enzymes, have been found to alternate between cold and warm season assemblages where soil temperature varies strongly over the year (Schadt *et al.* 2003; Monson *et al.* 2006; Lipson 2007; Weintraub *et al.* 2007; Wallenstein, McMahon & Schimel 2009). Incubating soils at different temperatures has been shown to induce changes in $\delta^{13}C$ of respired CO_2 along with community composition (Andrews *et al.* 2000). Dry summer conditions may restrict heterotrophic activity to deeper soil layers retaining more moisture and where soil organic matter tends to be enriched in ^{13}C, producing a seasonal pattern (Steinmann *et al.* 2004; Theis *et al.* 2007). Thus, in addition to the effects of weather conditions on Δ, many seasonally-dependent environmental variables have the potential to cause or coincide with variability in heterotrophic respiration sources independently, highlighting the importance of considering these sources individually.

Diel variation in $\delta^{13}C$ of soil respiration

The largest diel variations in the $\delta^{13}C$ of soil respiration (>5‰) were observed on plots with and without roots during the low flux period immediately prior to the growing season, when soils were cooler than midsummer on average, but with strong diel fluctuations in soil temperature. These are the largest diel $\delta^{13}C$ ranges of soil respiration reported to date. Throughout the growing season, smaller daily cycles in the $\delta^{13}C$ of soil respiration were occasionally apparent (e.g. Fig. 5c,d) with amplitudes similar to those reported by Kodama *et al.* (2008), Marron *et al.* (2009), and Bahn *et al.* (2009), or were absent (e.g. Fig. 5a,b) as seen by Betson *et al.* (2007). The surface flux $\delta^{13}C$ has generally been assumed to reflect that of respiratory CO_2 production, even when flux $\delta^{13}C$ has been found to vary on a diel basis. Such fluctuations have been previously attributed to variability in $\delta^{13}C$ of phloem sugars supplied to roots or changing proportions of autotrophic and heterotrophic sources throughout the day. However, within the current study diel variability in flux $\delta^{13}C$ was observed on plots with and without active roots and thus could not have been due to these differences in carbon sources. Substrate $\delta^{13}C$ variability would only explain the

observed flux $\delta^{13}C$ variability if large apparent fractionations occurred during oxidation of soil organic matter with a strong soil temperature dependence.

Throughout the current study, the magnitude of diel variability in flux $\delta^{13}C$ was consistently correlated with the coefficient of variation of the flux, a measure of flux variability relative to average flux magnitude (Figs 6 & 7b). The independence of this relationship from potential source variations (e.g. seasonal substrate pulses, roots vs. heterotrophs) and its dependence on changing flux rates point to soil gas transport-related diffusive isotope effects as a likely cause of observed diel variability in flux $\delta^{13}C$. Measurements from the current study fit within the variability of model results, suggesting that all observed diel variability in surface flux $\delta^{13}C$ could be explained by diffusive transient effects in soil gas transport with a *constant* $\delta^{13}C$ of respiratory production. Model support for this conclusion was particularly strong if CO_2 production was low and concentrated near the surface (Fig. 8a,c), which is likely to reflect the activity and distribution of microbial communities during the early and late seasons when measured fluxes were smallest and isotopic variability was highest. While isotopic measurements of low respiration rates from sources localized near the surface might be especially susceptible to chamber influences on diffusive mole fraction gradients, the convergence of chamber data and model predictions (Fig. 8) does not highlight any measurement errors. On the contrary, if diel flux $\delta^{13}C$ variability reflects diffusive transient effects rather than changes in source substrate, as suggested here, this variability complicates the application of $\delta^{13}C$ of soil respiration to understanding soil respiratory source dynamics.

Data from the current study were compared with other reports of diel variations of $\delta^{13}C$ of soil respiration. Average soil respiration fluxes and diel amplitudes of fluxes and their isotope ratio were estimated visually from figures published in Betson *et al*. (2007), Kodama *et al*. (2008), Marron *et al*. (2009), and Bahn *et al*. (2009). Flux means and amplitudes were used to generate sine function curves from which the coefficient of variation was calculated for one day. For a more direct comparison, data from the current study were treated in the same way, using a sine curve fitted to an average daily flux pattern made from each consecutive three-day period to calculate a flux CV. Data from the four studies above were consistent with the observation of decreasing $\delta^{13}C$ variability with increasing fluxes seen in the current study and produced by the model (Fig. 8a). In addition, data from these four studies showed a similar correlation between the diel range of $\delta^{13}C$ and the CV of the soil CO_2 flux (Fig. 8b). Differences in the relationship between flux CV and isotopic variability across this study and those cited (especially Kodama *et al*. (2008) might have been due to a uniquely shallow depth of production at our study site (Fig. 8c), methodological differences between chamber measurement techniques, or differences in sampling frequency. The consistency of patterns across the studies evaluated in Fig. 8a, b with the current study and results from our constant source model suggests that, contrary to diel variations in $\delta^{13}C$ of respiration substrates, diel flux $\delta^{13}C$ variability could have been caused by physical processes alone.

Recent work by Bathellier *et al*. (2009) has suggested that $\delta^{13}C$ of root respiration may be less variable diurnally than $\delta^{13}C$ of leaf-respiration. Those authors found a constant $\delta^{13}C$ of root respiration during starvation-induced decrease in respiratory quotient (RQ), in contrast to the pattern of positive correlation between RQ and $\delta^{13}C$ of leaf respiration shown for the same species (*Phaseolus vulgaris*) by Tcherkez *et al*. (2003). The RQ-associated mechanism entails a shift in the proportion of pyruvate decarboxylation and Krebs cycle decarboxylation, which have opposing effects on $\delta^{13}C$ of respired CO_2. This mechanism was suggested by Hymus *et al*. (2005) to account for large observed diurnal variation in oak leaf-respired $\delta^{13}C$, which corresponded with daily cumulative assimilation rather than variability in $\delta^{13}C$ of leaf sugars. The disconnection between $\delta^{13}C$ of root respiration and substrate availability to roots observed by Bathellier *et al*. (2009) would support the interpretation that diel variability in $\delta^{13}C$ of soil respiration is more likely driven by transient diffusive transport effects than $\delta^{13}C$ of root-respired CO_2. In the current study, the observations of (1) identical relationships between variability in soil respiration rate and $\delta^{13}C$ regardless of presence or absence of roots (Fig. 7) (2) absence of diel $\delta^{13}C$ variability in soil respiration when rhizosphere respiration was highest; and (3) no diel variability in the $\delta^{13}C$ of respired CO_2 from soils or roots measured separately point to this same conclusion.

SUMMARY

In an analysis of $\delta^{13}C$ of soil-respired CO_2 in trenched and untrenched plots under deciduous trees, we found short-term (diel) variability, which appeared to be associated with abiotic processes, and longer-term (seasonal) differences associated with biotic processes. Diel variability in $\delta^{13}C$ of soil respiration ranged from 0–12‰, and was related to flux variability and average magnitude (small, variable fluxes produced maximum $\delta^{13}C$ variability). A diffusive transport model with a constant respiratory source $\delta^{13}C$ supported the conclusion that diel flux $\delta^{13}C$ variability was due to transient diffusive fractionations. Seasonal and treatment effects were analysed from soil chamber data averaged for each day to remove diel fluctuations, and slower-equilibrating soil gas profiles. Both methods showed that trenching reduced summertime soil respiration rates by 75% and $\delta^{13}C$ of soil respiration by ~1‰. A seasonal pattern of ~4‰ ^{13}C-enrichment in summer vs. spring and fall soil respiration was observed on all plots and attributed to seasonal variability of heterotrophic processes. This conclusion points to the need to consider heterotrophic processes in addition to photosynthetic discrimination as a potentially dominant driver of soil respiration $\delta^{13}C$.

ACKNOWLEDGMENTS

This project was supported by the University of Utah's Research Instrumentation Fund and other University of

Utah sources. ABM is grateful for generous support from the A. Herbert and Marian W. Gold Scholarship. Thanks to Thure Cerling and D. Kip Solomon for valuable insight into soil gas transport; Jim Ehleringer, Todd Dawson, Joy Ward and Kevin Hultine for establishing the experimental garden and providing meteorological data; Sean Schaeffer for help with planning and methodological details; and Timothy Jackson for site setup and logistical assistance. This manuscript was improved by many helpful comments from the editor and anonymous reviewers.

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Received 11 December 2009; received in revised form 26 March 2010; accepted for publication 17 May 2010