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Characterizing the impact of diffusive and advective soil gas transport on the measurement and interpretation of the isotopic signal of soil respiration

Zachary E. Kayler [a,*], Elizabeth W. Sulzman [b], William D. Rugh [c], Alan C. Mix [c], Barbara J. Bond [a]

[a] Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331-5752, USA
[b] Department of Crop and Soil Science, Oregon State University, OR 97331-5752, USA
[c] College of Oceanic and Atmospheric Sciences, Oregon State University, OR 97331, USA

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ABSTRACT

By measuring the isotopic signature of soil respiration, we seek to learn the isotopic composition of the carbon respired in the soil ($\delta^{13}C_{R\text{-}s}$) so that we may draw inferences about ecosystem processes. Requisite to this goal is the need to understand how $\delta^{13}C_{R\text{-}s}$ is affected by both contributions of multiple carbon sources to respiration and fractionation due to soil gas transport. In this study, we measured potential isotopic sources to determine their contributions to $\delta^{13}C_{R\text{-}s}$ and we performed a series of experiments to investigate the impact of soil gas transport on $\delta^{13}C_{R\text{-}s}$ estimates. The objectives of these experiments were to: i) compare estimates of $\delta^{13}C_{R\text{-}s}$ derived from aboveground and belowground techniques, ii) evaluate the roles of diffusion and advection in a forest soil on the estimates of $\delta^{13}C_{R\text{-}s}$, and iii) determine the contribution of new and old carbon sources to $\delta^{13}C_{R\text{-}s}$ for a Douglas-fir stand in the Pacific Northwest during our measurement period. We found a maximum difference of $-2.36‰$ between estimates of $\delta^{13}C_{R\text{-}s}$ based on aboveground vs. belowground measurements; the aboveground estimate was enriched relative to the belowground estimate. Soil gas transport during the experiment was primarily by diffusion and the average belowground estimate of $\delta^{13}C_{R\text{-}s}$ was enriched by 3.8–4.0‰ with respect to the source estimates from steady-state transport models. The affect of natural fluctuations in advective soil gas transport was little to non-existent; however, an advection–diffusion model was more accurate than a model based solely on diffusion in predicting the isotopic samples near the soil surface. Thus, estimates made from belowground gas samples will improve with an increase in samples near the soil surface. We measured a $-1‰$ difference in $\delta^{13}C_{R\text{-}s}$ as a result of an experiment where advection was induced, a value which may represent an upper limit in fractionation due to advective gas transport in forest ecosystems. We found that aboveground measurements of $\delta^{13}C_{R\text{-}s}$ may be particularly susceptible to atmospheric incursion, which may produce estimates that are enriched in ^{13}C. The partitioning results attributed 69–98% of soil respiration to a source with a highly depleted isotopic signature similar to that of water-soluble carbon from foliage measured at our site.

1. Introduction

Soil respiration is the second largest carbon flux of terrestrial ecosystems (Schimel, 1995) and it is crucial that we develop a thorough understanding of the physical and biological controls of the evolution and egress of soil CO_2. The isotopic signal of soil respiration ($\delta^{13}C_{R\text{-}s}$) is an integrative measure of the impact of recent environmental conditions on the oxidation of multiple carbon sources belowground, thus, making it a useful tool for carbon cycle research. Studies using $\delta^{13}C_{R\text{-}s}$ have provided valuable insight into plant–soil carbon metabolism, and respiratory carbon sources at various spatial scales (Crow et al., 2006; Steinmann et al., 2004). Yet, requisite to the interpretation of $\delta^{13}C_{R\text{-}s}$ is the need to validate the assumptions behind soil $\delta^{13}CO_2$ and its measurement.

Current methods to estimate $\delta^{13}C_{R\text{-}s}$ can be categorized into those made aboveground via closed or open top chambers (Ekblad and Högberg, 2000; Ohlsson et al., 2005; Takahashi et al., 2008) and those made belowground that use air samples extracted from the soil CO_2 concentration profile (Kayler et al., 2008; Steinmann et al., 2004). Both methods make two key assumptions concerning soil

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* Corresponding author. Current address: Institute for Landscape Matter Dynamics, Leibniz-Center for Agricultural Landscape Research (ZALF) e.V., Müncheberg D-15374, DE, Germany. Tel.: +49 33432 82240x485; fax: +49 33432 82343.
E-mail address: zachary.kayler@zalf.de (Z.E. Kayler).

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respiration, i) CO_2 transport through the soil is only by diffusion, and ii) soil CO_2 flux is at steady-state (Cerling et al., 1991; Amundson et al., 1998). Violations of these assumptions can have a large impact on the certainty in $\delta^{13}C_{R\text{-}s}$ estimates and subsequent source partitioning, and they require a thorough assessment.

The assumption that transport is solely by diffusion is critical to estimates of $\delta^{13}C_{R\text{-}s}$ because diffusion leads to a kinetic fractionation of ^{13}C and ^{12}C whereas advection or mass flow of CO_2 does not cause fractionation. The mass of ^{13}C is larger than that of ^{12}C and diffuses through the soil at a slower rate. For estimates of $\delta^{13}C_{R\text{-}s}$ calculated from gas samples withdrawn from the soil profile a correction of 4.4‰ is applied to account for this fractionation (Amundson et al., 1998). However, if gas transport is not only by diffusion but also by advection, then the correction due to diffusion becomes uncertain, and a correction less than 4.4‰ may apply. Advection as a gas transport mechanism has been suggested in many studies of different ecosystem types (Takle et al., 2004; Risk et al., 2002; Flechard et al., 2007) and similar observations have led to advection–diffusion transport models that have been verified where geologic sources predominate soil CO_2 flux (Camarda et al., 2007; Lewicki et al., 2003). However, only a few studies have addressed the influence of advection on the $\delta^{13}C_{R\text{-}s}$; indeed, most reports apply a correction that assumes gas transport is solely diffusive (Steinmann et al., 2004; Mortazavi et al., 2004).

The second assumption concerning the measurement of $\delta^{13}C_{R\text{-}s}$ is soil CO_2 flux is at isotopic steady-state, i.e. the isotopic signal leaving the soil surface is equal to the isotopic source (Amundson et al., 1998). This means that for measurements made at the soil surface, such as with a chamber, a correction for fractionation due to diffusion is unnecessary. If respiration is not at isotopic steady-state then there will be a disequilibrium between the source isotopic signature and the CO_2 emitted through the profile and to the surface. Such a phenomenon might occur with a shift in the dominant carbon substrate of respiration, for instance.

Ultimately, $\delta^{13}C$ analyses allows for the identification of carbon contributions to soil CO_2 efflux as well as the relative contribution of soil carbon pools to overall ecosystem respiration (Ehleringer et al., 2000; Bowling et al., 2008; Tu and Dawson, 2005; Chemidlin Prévost-Bouré et al., 2009). In the context of partitioning carbon sources, a large isotopic range between potential respiration sources is generally required for partitioning with natural abundance ^{13}C, which explains why isotopic labeling is often used. However, significant differences in the isotopic composition of carbon pools also occur in nature. For example, there is a potential 5‰ difference in the soluble carbon extracts of foliage and the bulk isotopic signature of SOM. This isotopic range is reflected in natural abundance measures of $\delta^{13}C_{R\text{-}s}$ which typically varies by 1–4‰ in magnitude over a growing season (Ekblad and Högberg, 2001; Ekblad et al., 2005; Takahashi et al., 2008; Kodama et al., 2008; Chemidlin Prévost-Bouré et al., 2009). The potentially small difference in the seasonal variability of $\delta^{13}C_{R\text{-}s}$ and the relatively narrow range in isotopic sources accentuate the importance of verifying measurement assumptions and accurately measuring $\delta^{13}C_{R\text{-}s}$ for partitioning carbon sources.

During a single day in the early growing season of 2006, we performed a series of field experiments designed to evaluate the impact of soil gas transport on estimates of $\delta^{13}C_{R\text{-}s}$ and the subsequent analysis of new and old carbon contributions. The objectives of this study were:

i. Compare estimates of $\delta^{13}C_{R\text{-}s}$ derived from aboveground and belowground measurement techniques. We hypothesized that there would not be a difference between the two estimates when the estimate from belowground samples was corrected for kinetic fractionation due to diffusion.
ii. Evaluate the roles of diffusion and advection in a forest soil on the estimates of $\delta^{13}C_{R\text{-}s}$. Our strategy to accomplish this included: a) employing both diffusion and advection–diffusion models that predict $^{13}CO_2$ concentrations belowground in the soil profile, and b) experimentally test the impact of advection by inducing a large negative pressure gradient (−4 kPa) at the soil surface to observe changes in $\delta^{13}C_{R\text{-}s}$ measured aboveground.
iii. Determine the contribution of new and old carbon sources to $\delta^{13}C_{R\text{-}s}$ for a Douglas-fir stand in the Pacific Northwest during our measurement period. We used an isotope mixing model to quantify the contribution of the isotopic signature of carbon in soluble extracts from leaves and phloem as well as the isotopic signature of bulk soil organic matter (SOM) to our estimates of $\delta^{13}C_{R\text{-}s}$.

2. Materials and methods

2.1. Site description

The experiment was conducted within a 96 ha watershed, located in the H J Andrews Experimental Forest in the western Cascades of central Oregon, USA (44.2°N, 122.2°W) (see Pypker et al., 2007 for a detailed description). We chose a subplot near the base of the watershed on the south facing slope. The soil has Andic properties and a loamy to silt loam texture. The organic layer is just 2 cm thick and is composed of primarily recognizable litter fragments with almost no discoloring and no signs of amorphous Oa materials. The A horizon extends to a depth of 9 cm where a diffuse AB transition occurs and extends to 30 cm; beyond this the B horizon extends to a depth of 42 cm.

2.2. Experimental design

Over two consecutive 45 min periods on May 2, 2006 we determined soil respiration and $\delta^{13}C_{R\text{-}s}$. We compared $\delta^{13}C_{R\text{-}s}$ estimates made from belowground (soil probe) and aboveground (mini-tower) techniques, described in Sections 2.3 and 2.4 respectively. We used 45 min intervals to accommodate any disturbance to the CO_2 profile caused by withdrawing gas samples from the soil probe. Three soil probes were installed one week prior to the experiment to minimize disturbance. A 45 min period began with the placement of the mini-tower in between the soil probes and on the litter surface. During the 45 min interval, CO_2 diffused through the mini-tower followed by sampling CO_2 from the tower and then from the soil probes. Following the sampling during diffusive transport we induced advection in the mini-tower and re-sampled the mini-tower for CO_2. We compared mini-tower estimates of $\delta^{13}C_{R\text{-}s}$ made during diffusive and the experimentally induced advective gas transport to observe the effects of advection on aboveground estimates of $\delta^{13}C_{R\text{-}s}$.

We used the CO_2 samples collected from soil probes in two soil profile models: one based on diffusion (Amundson et al., 1998) and one based on diffusion and advection (Camarda et al., 2007). These models, based solely on the soil probe samples, were used to test for i) isotopic steady-state and ii) advection due to background variation in pressure. Thus, for the entire study we sampled the soil probes a total of six times (none of which occurred during the advection experiment), and we sampled the mini-tower two times under diffusive transport and two times under advective transport. During the experiment, we also collected samples of foliage,

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phloem and soil organic matter for isotopic analysis which we describe in detail below (2.7. Carbon pool sampling).

2.3. Soil probe: belowground sampling of CO_2

This device facilitates sampling gas for isotopic composition from different depths in the soil. The soil probe contains three isolated wells made from PVC (poly-vinyl chloride). These wells are held at a fixed distance (5, 15 and 30 cm) by PVC tubing. The soil probe is further described in Kayler et al. (2008).

To sample gas from each depth of the soil probe, we used a gas-tight, 3-way ball valve (Whitey, Swagelok, USA) that was fitted with a hand vacuum pump (Mityvac, Lincoln Indust. Corp., USA) and two double-ended needles. One double-ended needle was inserted into a pre-filled N_2 exetainer (Labco Ltd., UK) and, when the 3-way valve was turned toward the pump, we could draw a vacuum (-27 kPa) within the exetainer. Then, with the exetainer under vacuum and still attached to the 3-way valve, we inserted the second double-ended needle into a septum of the soil probe and turned the valve in the other direction to allow the flux of soil gas from the probe into the exetainer. We waited 30 s to allow for equilibration then detached the exetainer and sealed the puncture of the exetainer septum with silicone sealant. The samples were then transported back to the laboratory and analyzed within 24 h. A standard gas was sampled in the field in the same manner to account for fractionation that may have occurred during sampling, transport or storage.

The gas samples collected from each soil probe were used in a two end-member isotopic mixing model to identify the isotopic signature of the source gas. We used the Miller–Tans mixing model (Miller and Tans, 2003) which describes a sample of the air in a system as a mixture of two sources of $^{13}CO_2$: the background atmosphere and the source of respiration. The Miller–Tans mixing model used with geometric mean regression has been found to give the most accurate and precise estimate of large CO_2 concentration regimes similar to soil respiration (Kayler et al., in press). In this case, $\delta^{13}C_{R\text{-}s}$ is estimated as the slope calculated from a geometric mean regression. Because the slope of the Miller–Tans model identifies the isotopic source of CO_2 based on the samples that have been enriched in $^{13}CO_2$ we must correct for enrichment by subtracting 4.4‰ from the mixing model estimate.

2.4. Mini-tower: aboveground sampling of CO_2

The mini-tower is a 0.10 m diameter by 1 m tall PVC cylinder with 10 swagelock ports fitted with septa for collection of gas samples above the soil surface. We attached a 1 m^2 rubber sheet around the bottom of the mini-tower where it contacts the litter surface to prevent atmospheric incursion into the soil and to avoid disturbing the vertical CO_2 flux that may occur due to placement of the mini-tower (i.e. a lateral flux that may develop within the soil that would in effect go around the mini-tower footprint). The mini-tower was located in the center of the sheet which provided a maximum 0.95 m buffer zone.

We installed the mini-tower in the center of the plot without removing the litter layer. We placed weights on the rubber sheet to create a temporary seal between the tower and litter surface. We first sampled the mini-tower without inducing advective gas transport. We let the soil gas diffuse into the mini-tower for 45 min, after which we sampled the mini-tower from the bottom to the top. We used the same method to sample gas as used for the soil probe. Isotopic and concentration values were then used to estimate $\delta^{13}C_{R\text{-}s}$ using the Keeling plot method with an ordinary least squares regression (Keeling, 1958; Zobitz et al., 2006). For estimates of the Keeling intercept standard error, we bootstrapped the Keeling plot regression (10,000 iterations) using S-Plus (Insightful Corporation, Seattle, WA, USA).

We induced advection after this initial sampling by inserting a semi-rigid rubber disk with a slightly larger diameter than the inside of the mini-tower to the bottom of the tower. We then pulled the disk up with an attached handle to generate a vacuum in the tower (-4 kPa), thereby pulling soil air into the mini-tower. We measured the vacuum with a gauge attached to a vacuum hose inline with a swagelock port at the bottom of the mini-tower. Leaving the rubber disk at the top of the mini-tower, we proceeded to sample for CO_2 concentration and ^{13}C as described previously.

2.5. Isotopic steady-state diffusion models

Diffusion of CO_2 at steady-state is described by Fick's first law:

$$D_s\frac{\partial^2 C}{\partial z^2} = -\phi \tag{1}$$

where D_s is the bulk diffusion coefficient of soil ($cm^2\ s^{-1}$), $C =$ the concentration of CO_2 at a given depth in the soil profile ($mol\ cm^{-3}$), $z =$ depth in the soil profile (cm) and $\phi =$ production of CO_2 ($mol\ cm^{-3}\ s^{-1}$). Cerling (1984) developed a production–diffusion model of $^{13}CO_2$ based on the observation that the ^{12}C and ^{13}C diffuse along their own concentration gradients. In the review of isotopes of soil C and CO_2, Amundson et al. (1998) tested a similar model through simulations:

$$R^{13}_{(Z)} = \frac{\dfrac{\phi R^{13}_S}{D^{13}_S}\left(Lz - \dfrac{z^2}{2}\right) + C_{\text{atm}}R^{13}_{\text{atm}}}{\dfrac{\phi}{D_s}\left(Lz - \dfrac{z^2}{2}\right) + C_{\text{atm}}} \tag{2}$$

The model describes the isotopic ratio of ^{13}C to ^{12}C of a gas sample in the profile withdrawn from depth z. The model assumes that bulk CO_2 production and concentration represent ^{12}C given that it is the most relative abundant isotope of terrestrial carbon (98.9%). The isotopic ratio of $^{13}CO_2$ (R^{13}) is a function of the production rate, the isotopic ratio of the source (R^{13}_S), and the diffusion coefficient of $^{13}CO_2$ ($D^{13}_S =$ bulk soil $D_s/1.0044$, which accounts for the greater mass of ^{13}C and its subsequent slower diffusivity). In this model, the isotopic signal of respired CO_2 is mixed with the atmospheric (atm) background CO_2 concentration and isotopic ratio. The upper boundary for the model ($z = 0$) is the atmospheric isotopic signature and the lower boundary is the lower limit of respiration ($z = L$) where the concentration gradient is constant. We express the isotopic source and other isotopic data in delta (δ) notation where $\delta^{13}C = (R_{\text{sample}}/R_{\text{VPDB}} - 1) \times 1000‰$ and R is the molar ratio of $^{13}C/^{12}C$ and VPDB is the Vienna Pee Dee Belemnite isotopic standard.

We measured soil respiration using a portable infrared gas analyzer (Li-6250, LI-COR Inc, Lincoln, NE) incorporated into a photosynthesis system (Li-6200) and attached to a closed, dynamic soil respiration chamber (Li-6200-09). The chamber was placed on a 10 cm diameter by 5 cm tall PVC collar that was installed 2 cm into the mineral soil. We used the production value estimated from the gas analyzer and fit the isotopic and concentration profile samples to the above diffusion model. We used a non-linear regression to determine D_s, L and R^{13}_S (i.e. $\delta^{13}C_{R\text{-}s}$).

2.6. Advection–diffusion isotopic steady-state model

We used and advection–diffusion model to determine if advective gas transport was present during sampling of $\delta^{13}CO_2$ from the soil probe. Gas transport that includes both advection and

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diffusion at steady-state is described by Darcy's law and Fick's first law of diffusion:

$$v\frac{\partial C}{\partial z} - D_s\frac{\partial^2 C}{\partial z^2} = 0 \tag{3}$$

where the symbols are similar to the diffusion model described above and v = the Darcy velocity. Camarda et al. (2007) developed an isotopic steady-state model for CO_2 flux described by both advection and diffusion for a single dimension:

$$R(z) = \frac{\dfrac{[^{13}CO_2]_{atm}+[^{13}CO_2]_{Z_2}-[^{13}CO_2]_{atm}}{e^{\frac{v}{D^{13}CO_2}z_2}-1}\left(e^{\frac{v}{D^{13}CO_2}z}-1\right)}{\dfrac{[^{12}CO_2]_{atm}+[^{12}CO_2]_{Z_2}-[^{12}CO_2]_{atm}}{e^{\frac{v}{D^{12}CO_2}z_2}-1}\left(e^{\frac{v}{D^{12}CO_2}z}-1\right)} \tag{4}$$

The model describes the steady isotopic profile from a generic depth z_2 (m) to the soil surface, where $[^{x}CO_2]$ = the concentration of either ^{13}C or ^{12}C for the gas sample (vol%), $D^{13}CO_2$ = the diffusion coefficient of $^{13}CO_2$ as described above for the diffusion model ($m^2\ s^{-1}$), and v = Darcy velocity ($m\ s^{-1}$). For this steady-state model the pressure gradient and gas velocity that describe v are assumed to be constant with depth.

The approach is similar to the diffusion model in that both isotopes are modeled independently. The concentration of each isotope is calculated from samples withdrawn from the soil profile by the formulas:

$$[^{13}CO_2]_z = \frac{A[CO_2]_z}{1+A}, \quad [^{12}CO_2]_z = \frac{[CO_2]_z}{1+A} \tag{5}$$

Where $A = R_{PDB} \times ((\delta^{13}CO_2)_z/1000 + 1)$. The model assumes that the source of $\delta^{13}C_{R\text{-}s}$ is equivalent to the isotopic value at $z = -\infty$.

We used the D_s calculated from the diffusion model to fit the above model to the isotopic and concentration profiles from the soil profile at our site and estimated v and $\delta^{13}C_{R\text{-}s}$.

2.7. Carbon pool sampling

Our intent of sampling the forest carbon pools was to capture a wide range in isotopic values, from tree to soil, for the partitioning analysis. We considered foliage as the most depleted potential source signal and represents a lower bound of the isotopic range. Current year-foliage from the three nearest trees was collected using a shot gun and we analyzed the isotopic signature of carbon extracted from the foliage using hot water (Gessler et al., 2004; Brandes et al., 2006).

The isotopic composition of leaf sugars contains recent photo-assimilates, or "new" carbon, but it may not represent the isotopic signal of carbon that is respired or exuded by roots. To obtain a better representation of root respired/exuded carbon we collected samples of tree phloem and analyzed the water extractable carbon, similar to the foliage. Samples of phloem were from the same trees the foliage was collected from. By sampling phloem instead of roots we avoided any ambiguity related to which tree or species the roots belong to, allowing us to quantify the isotopic difference between the foliage and phloem carbon pools. Phloem was sampled by using a standard tree borer to remove a small core from the bole of the tree at 1.4 m height. The inner phloem was separated from xylem and bark in the field. Both foliage and phloem samples were first submerged in liquid N, then placed in a cooler filled with ice until they were transported back to the laboratory where they were stored in a 0 °C freezer until they were prepared for isotopic analysis.

Soil organic matter samples were taken from the site at 5, 15 and 30 cm depth, the same depths from which soil gas samples were taken. The samples were air dried, then ground to a fine powder for isotopic analysis. We considered the isotopic signal of soil organic matter as the most enriched organic source and represents an upper bound of the isotopic range we sampled.

We used the IsoSource stable isotope mixing model described by Phillips and Gregg (2003) to evaluate potential contributions to $\delta^{13}C_{R\text{-}s}$ estimated by 1) soil probe and 2) mini-tower. For the partitioning of the soil probe estimate of $\delta^{13}C_{R\text{-}s}$ we used the SOM at three depths, the foliage and phloem hot water extracts. We assumed that soluble carbon from phloem extracts represented the source respired by roots in this analysis. We consider this carbon source a reasonable proxy given the potential fractionations, mixing, and time delay that may occur between fixation by leaves and respiration by roots (Badeck et al., 2005; Gottlicher et al., 2006). The isotopic signature from foliage represents the lower bound, i.e. most depleted, of potential isotopic sources to $\delta^{13}C_{R\text{-}s}$. For the IsoSource analysis, we aggregated the SOM samples *a posteriori* (Phillips et al., 2005) into a group that represented old carbon sources while foliage and phloem extracts represented independent new carbon sources.

For partitioning of the mini-tower estimate, we grouped the foliage and phloem into an aboveground source, the SOM into a belowground source and we included an atmospheric component which represented a third source by itself. We used software settings of 0.1‰ tolerance, 1% intervals and used a component precision of 0.1‰. The output of the model is expressed as the percent frequency of all possible solutions and we report the range of proportions for each source.

2.8. Isotopic analysis

For $\delta^{13}C$ analysis of CO_2 samples, we used a Finnigan/MAT DeltaPlus XL isotope ratio mass spectrometer interfaced to a GasBench-II automated headspace sampler at the College of Oceanic and Atmospheric Sciences isotope facility, Oregon State University. The GasBench-II is a continuous flow interface that allows injections of several aliquots of a single gas sample into a mass spectrometer for automated isotope determinations of small gas samples. Exetainers of sampled gas were loaded onto a Combi-PAL auto-sampler attached to the GasBench. Helium pushed the sample air out of the exetainer and into the mass spectrometer. A typical analysis consisted of three gas standards (tank CO_2–He mixtures), five sample replicates and an additional 2 gas standards for every sample. The CO_2 concentration of each sample was calculated from the peak volt area produced by the mass spectrometer analysis of each sample.

The carbon isotope composition of organic matter was run at the Idaho Stable Isotope Laboratory, where samples were run on a continuous-flow stable isotopic analyses utilizing the Finnigan-MAT, Delta plus isotope ratio mass spectrometer (IRMS). The tree and soil organic matter samples were flash-combusted using CE Instrument's NC 2500 elemental analyzer, interfaced through the Conflo II and sent to the IRMS. Analysis of internal laboratory standards ensured that the estimates of the organic isotopic were accurate to within 0.1‰.

3. Results

3.1. Soil probe and profile models

The soil gas CO_2 concentration had a range of approximately 5000 μmol mol^{-1} with a corresponding isotopic range of 7‰ as depicted by the isotopic and concentration profiles (Fig. 1).

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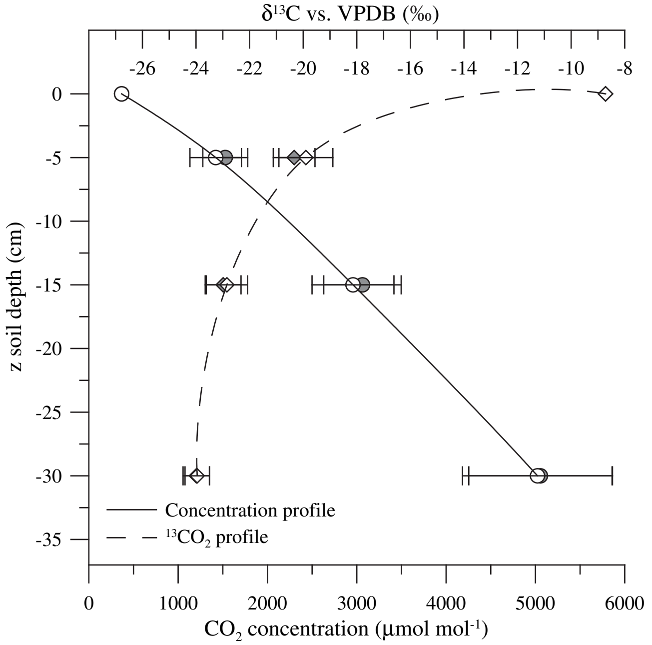

Fig. 1. Soil CO_2 concentration (circles) and isotopic (diamonds) profiles of the first 45 m (shaded) and second 45 min (open) sampling period. Samples were drawn from three soil probes located within 1 m of each other during which soil gas transport was by diffusion. Error bars represent one standard deviation of the mean.

A difference in concentration of 1500 µmol mol^{-1} was apparent between soil probe 3 at 30 cm and the two other probes. The effect of this difference was small as the average of the mixing model estimate was −25.3‰ (Table 1). The Miller–Tans mixing model estimate of $\delta^{13}C_{R\text{-}s}$ from the soil probes was on average 3.8‰ enriched relative to the diffusive model source estimate and 4.0‰ with respect to the advection–diffusion estimate.

The soil respiration rate was 4.1 µmol m^2 s^{-1}. The diffusivity of the soil averaged 8.1×10^{-6} m s^{-1} (4.5×10^{-6} m s^{-1} se) while the Darcy velocity averaged -2.2×10^{-5} m s^{-1} (9.6×10^{-6} m s^{-1} se) indicating a flux of atmospheric carbon into the soil profile. Given that there was no evidence of advection out of the soil and the similarity in the measured profile with the diffusion model predictions, we adjusted the source estimate of the advection–diffusion model by −4.4‰. The average source of $\delta^{13}C_{R\text{-}s}$ estimated from the steady-state diffusive model of the soil probe data was 29.1‰ and 29.3‰ from the advection–diffusion model.

Table 1
Averages and standard error (se) of the isotopic signal of soil respiration ($\delta^{13}C_{R\text{-}s}$) estimated by both belowground (soil probe) and aboveground techniques (mini-tower). Apparent fractionation is the difference between the profile model and soil probe estimate. The type of gas transport (diffusive vs. advection) and sampling period (1 = 1st 45 min, 2 = 2nd 45 min) are listed for each mini-tower estimate. Estimates are in units of $\delta^{13}C$ vs. VPDB (‰).

Method	$\delta^{13}C_{R\text{-}s}$ (‰)	se
Soil probe	−25.3	0.2
Diffusion model	−29.1	0.1
Apparent fractionation	−3.8	0.2
Adv–diffusion model	−29.3	0.6
Apparent fractionation	−4.0	0.6
Mini-tower$_{(\text{diffusion 1})}$	−23.3	1.2
Mini-tower$_{(\text{advection 1})}$	−23.6	1.4
Mini-tower$_{(\text{diffusion 2})}$	−24.0	0.7
Mini-tower$_{(\text{advection 2})}$	−25.0	0.8

The variation of the advection–diffusion model was greater than the diffusion model and depleted by 0.2‰ on average with a range of −1.88‰ to 1.24‰. The advection–diffusion model more closely predicted the 5 cm depth of the isotopic soil profile (Fig. 2).

3.2. Mini-tower

We sampled the soil respired CO_2 twice within 90 min using the mini-tower technique. The concentration gradient between the mini-tower and the background atmosphere was on the order of 375 µmol mol^{-1} with a corresponding isotopic range of 8.5‰ (Figs. 3 and 4). There was a high degree of variability within the mini-tower profiles and distinct geometric gradients did not develop from the soil surface to the top of the mini-tower. We omitted the 4.5 cm sample during the first 45 min sampling from further analysis because it was uncharacteristically depleted with respect to the other samples and most likely experienced fractionation. Despite the variation of the mini-tower profiles between the first and second 45 min sampling the Keeling plot estimates (Figs. 5 and 6) of the $\delta^{13}C_{R\text{-}s}$ were fairly similar, (Table 1) yielding a difference of 0.72‰ between the two.

3.3. Induced advection

Advection induced within the mini-tower resulted in changes in the concentration and isotopic profiles. The profiles of the first 45 min sampling were variable, but in comparison to the diffusive mini-tower sampling, the sample concentration values were greater near the soil surface and decreased with height from 30 cm (Fig. 3). The isotopic values were all depleted relative to the diffusive mini-tower samples, with samples below 21 cm having the lowest concentration of ^{13}C. The second advective mini-tower sampling resulted in more consistent profiles where all samples had greater CO_2 concentration values and depleted isotopic values relative to the diffusive sampling of the mini-tower (Fig. 4). The variation in the mini-tower concentration and isotopic profiles resulted in a 0.3‰ between the first and second

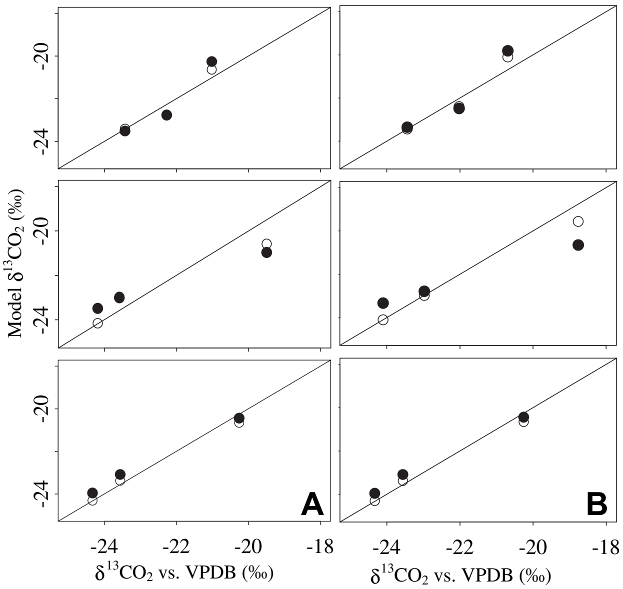

Fig. 2. Diffusion and advection–diffusion profile model results. The results from the first 45 min sampling period are shown in the left column (A) and the second 45 min period in the right column (B). The graphs show the 1:1 fit of the model predictions along the *y*-axis (solid symbols = diffusion steady-state model, open symbols = advection–diffusion model) and field sample values along the *x*-axis.

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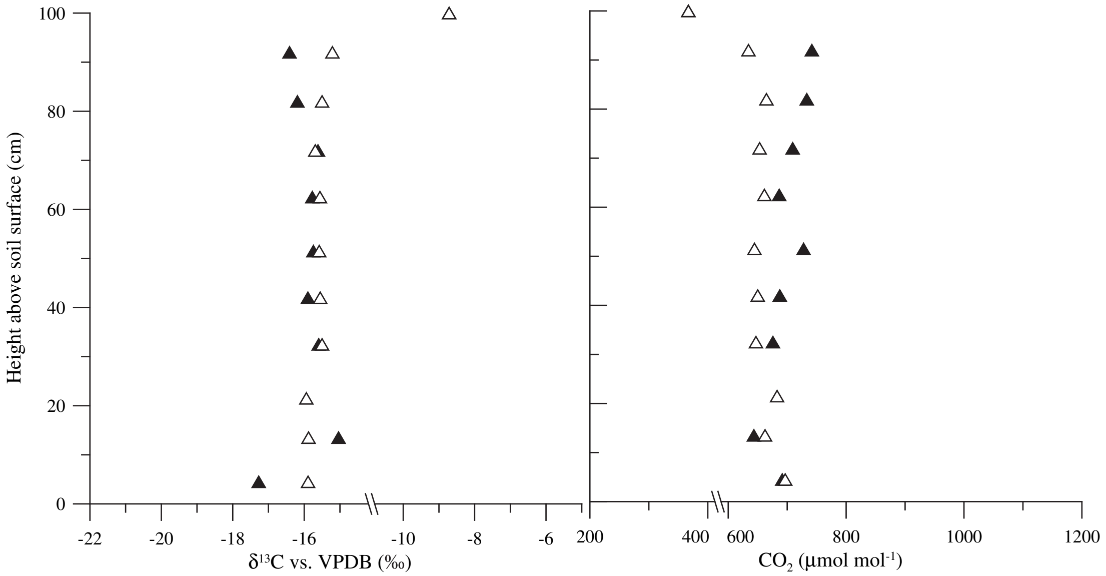

Fig. 3. Mini-tower concentration (left panel) and isotopic (right panel) profiles for the first 45 min sampling period. Solid symbols are samples made during diffusive gas transport and open symbols refer to samples made during the induced advection experiment.

diffusive mini-tower sampling and a −1‰ difference between the first and second advective sampling (Table 1).

3.4. Organic matter

The isotopic signal of tree foliage was −29.6‰ (0.8‰ sd) and SOM from 5 cm was −26.5‰ (1.2‰ standard deviation (sd)), 15 cm was −25.5‰, and 30 cm was −25.0‰ (0.8‰ sd). Organic samples became increasingly enriched along the plant–soil continuum resulting in a 4.5‰ gradient from tree foliage to soil at 30 cm. The isotopic signature of the phloem water-soluble extract was −28.0‰ (1.2‰ sd) and was on average 1.6‰ enriched relative to the water-soluble extracts of foliage. The isotopic signal of SOM became increasingly enriched with soil depth representing an isotopic gradient of 1.3‰.

3.5. Component contribution

We used the isotopic values from the organic carbon sources to partition the isotopic signature of soil respiration (−29.2‰). The predominate contribution (69–98%) to $\delta^{13}C_{R\text{-}s}$ was from a depleted source that was similar to the isotopic signature of the foliar soluble extracts. For this analysis we used the average source estimate from the diffusion and advection–diffusion models (−29.2‰) (Fig. 7). The phloem contribution ranged from 0 to 31% and the contribution from belowground sources ranged from 0 to 16%. The partitioning results were much more uncertain when we implemented the mini-tower source estimate measured under diffusive conditions. In this case the aboveground component (phloem and foliar extracts) contribution ranged from 0 to 78% and the belowground component contribution ranged from 0 to 90%. In partitioning the

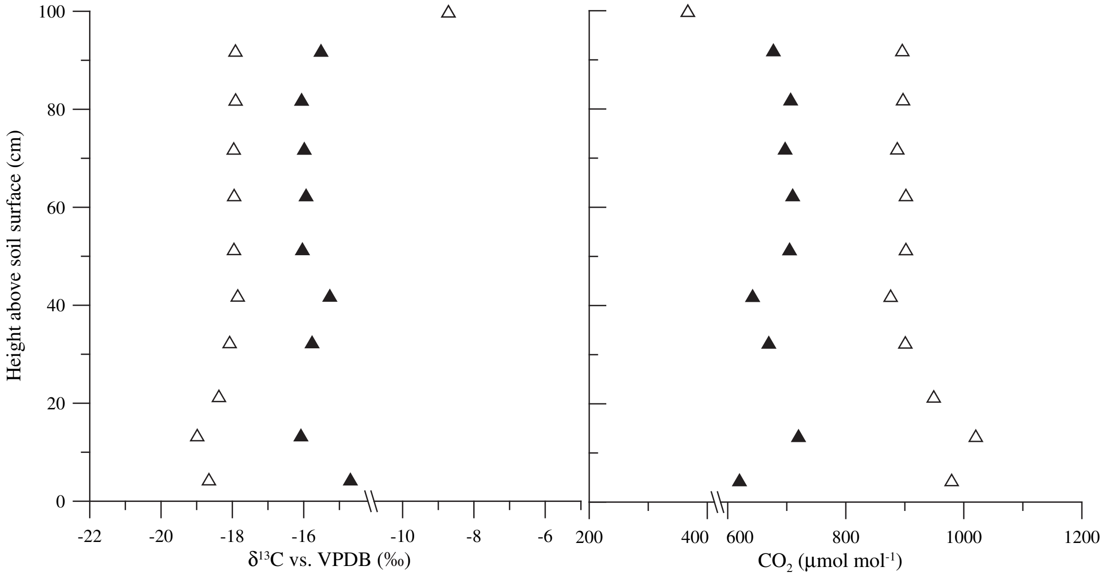

Fig. 4. Mini-tower concentration (left panel) and isotopic (right panel) profiles for the second 45 min sampling period. Solid symbols are samples made during diffusive gas transport and open symbols refer to samples made during the induced advection experiment.

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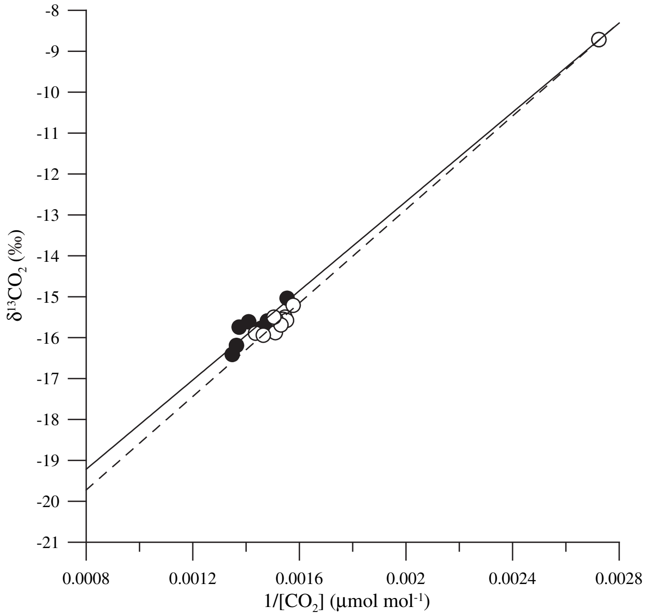

Fig. 5. Mini-tower Keeling plots for the first 45 min sampling period. Solid symbols refer to samples made during diffusive gas transport and open symbols refer to samples made during the induced advection experiment.

mini-tower source estimate, we also considered the contribution from the ambient atmosphere (−8.7‰) which was estimated to range from 8 to 26%.

4. Discussion

4.1. Soil $^{13}CO_2$ transport and measurement

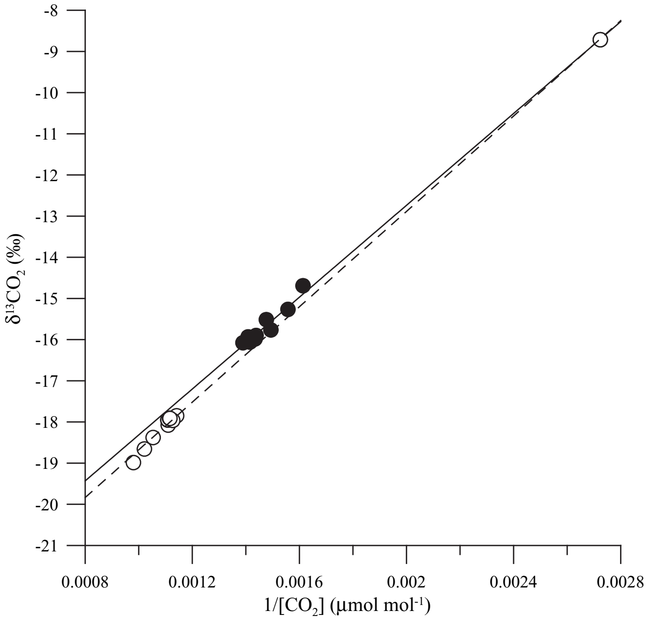

Fig. 6. Mini-tower Keeling plots for the second 45 min sampling period. Solid symbols refer to samples made during diffusive gas transport and open symbols refer to samples made during the induced advection experiment.

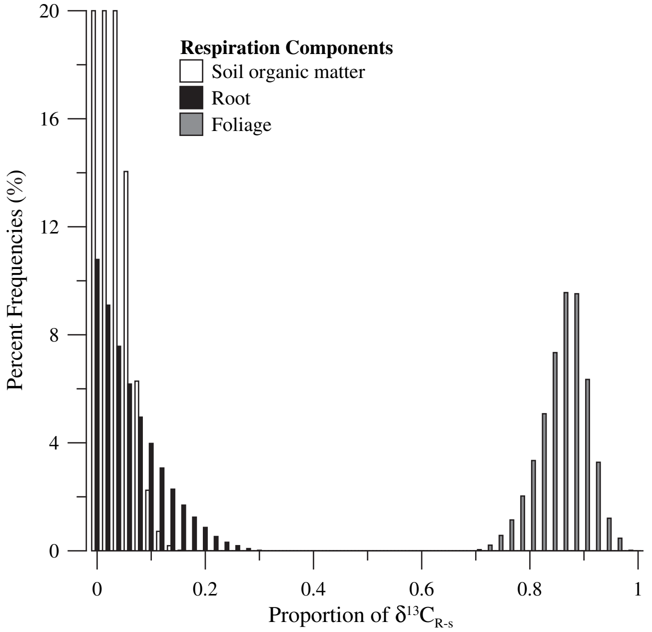

Fig. 7. Proportions of three carbon components of $\delta^{13}C_{R\text{-}s}$ estimated by isotopic steady-state gas transport models. The SOM component is an aggregation of the bulk soil $\delta^{13}C$ from 5, 15 and 30 cm depths. The component contributions are expressed as percent frequency of all possible solutions. For clarity, the entire percent frequency range of SOM was not included in this graph; these values extend to 73% at a proportion of 0.

We hypothesized that there would not be a difference between the corrected soil probe and mini-tower estimates. These two methods were compared with a model that predicts the isotopic signature of soil respiration when steady-state assumptions are met. We found good agreement between the soil probe estimate and the soil profile model. We found the average difference between the theoretical 4.4‰ correction and the correction calculated from the mixing model to be 0.6‰. This value is approximate to our analytic and measurement uncertainty and therefore, the soil was essentially at isotopic steady-state. However, we found the maximum difference between the soil profile estimate of $\delta^{13}C_{R\text{-}s}$ and aboveground techniques to be +5.7‰; in this case the *aboveground* estimate was *enriched* relative to the belowground estimate. In a similar field comparison, Mortazavi et al. (2004) found good agreement between estimates made from the soil profile and a mini-tower they employed, and one explanation for our contrasting results may be due to differences in site conditions or mini-tower construction and implementation.

An enriched isotopic source that was present in the mini-tower samples but absent from the belowground gas samples is also a potential explanation for the differences between aboveground and belowground estimates. However, most isotopic sources such as organic matter in the litter layer are lighter than SOM (Bowling et al., 2008; Ehleringer et al., 2000; Gleixner, 2005). Isotope fractionation from microbial respiration represents an enriched $\delta^{13}CO_2$ source, but the magnitude of this potential fractionation is small (Schweizer et al., 1999; Fernandez and Cadisch, 2003; Högberg et al., 2005) and cannot explain the measurement differences. Additionally, enriched respiratory sources, such as sporocarps (Bostrom et al., 2008), were not present during the measurement. Thus, it is unlikely that an enriched organic source will be found that explains the difference between the two estimates of $\delta^{13}C_{R\text{-}s}$ given that carbon isotopic signatures above the soil mineral layer will be depleted with respect to the mini-tower estimate. The background atmosphere is a highly enriched source relative to the carbon in the soil–plant continuum and it is possible that atmospheric incursion (Livingston et al., 2006) due to natural

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phenomena or to disturbance during the placement of the mini-tower diluted the source signal estimated by the mini-tower. Incursion violates the steady-state assumption of mixing-models, specifically that all sources are flux weighted (Pataki et al., 2003). Such atmospheric incursion has been documented in previous soil isotopic studies (Millard et al., 2008; Susfalk et al., 2002; Dudziak and Halas, 1996) and may represent up to 26% of the mini-tower estimate as determined from the IsoSource mixing model results.

The tools available to verify the CO_2 profile in the mini-tower are limited and, therefore, a laboratory experiment may be necessary to understand the diffusion of soil gas from the surface into the mini-tower. Furthermore, these results demonstrate that care must be taken when using aboveground approaches, such as closed chambers (Kayler et al., 2008; Nickerson and Risk, 2009), or open systems, such as the mini-tower to avoid perturbations to the soil CO_2 concentration and isotopic profile. The soil probe has been shown to accurately reflect CO_2 concentrations in the soil profile (Kayler et al., 2008) which can be used to check measurement assumptions; this is a distinct advantage over traditional surface methods and suggests that the soil probe is a robust method to measure $\delta^{13}C_{R\text{-}s}$ at steady-state.

4.2. Advection effect

Transport due to advection, the mass flow due to pressure gradients, may drive heavy atmospheric $^{13}CO_2$ into the soil or withdraw unfractionated soil $^{13}CO_2$ out. We tested for potential effects of natural occurring advection (*sensu* Takle et al., 2004) on estimates of $\delta^{13}C_{R\text{-}s}$ made from the soil profile. The estimates from the two soil profile models largely agreed with each other over the measurement period, indicating fractionation due to advection within the soil profile was minimal to non-existent. Interestingly, the fit of the advection–diffusion model to the 5 cm data was slightly better than the diffusive model. The near surface depths of the soil are important in defining the model curves, thus estimates made with the soil probe method will improve with more samples from this soil region. The advection–diffusion model also estimated a negative Darcy velocity, which we interpret as a flux of atmospheric CO_2 into the soil profile. This observation is consistent with the incursion discussion mentioned previously, but further investigation is needed to determine the potential magnitude of this effect on $\delta^{13}C_{R\text{-}s}$.

Information concerning the role of advection in $\delta^{13}C_{R\text{-}s}$ is sparse, despite the potential of advective gas transport to reduce the correction factor (−4.4‰) to mixing model estimates (Bowling et al., 2009). Our experiment in which we induced advection at the soil surface was an effort to quantify any isotopic effect on $\delta^{13}C_{R\text{-}s}$ from advection. The difference between the advective and diffusive sampling of the mini-tower was at most depleted by 1‰. These results were surprising; we expected an enriched estimate of $\delta^{13}C_{R\text{-}s}$ which would result from soil gas enriched in ^{13}C pulled up into the mini-tower for sampling. We clearly pulled soil gas into the mini-tower: CO_2 samples were both greater in concentration and depleted in ^{13}C compared with samples collected prior to inducing advection. Samples collected after inducing advection yielded an estimate of $\delta^{13}C_{R\text{-}s}$ that was nearly identical to the uncorrected estimate produced by the soil probe. This indicates that the induced advection experiment introduced soil gas that was uncontaminated by the isotopic signature of the background atmosphere.

In the advection experiment of this study, we generated a negative pressure of approximately −4 kPa. This value is far greater than the −5 Pa on $\delta^{18}O$ used to model effects on soil respiration (Stern et al., 1999) and −15 Pa induced for a field experiment that investigated the effects of pressure pumping on soil respiration (Takle et al., 2004). Thus, the −1‰ difference between the mini-tower estimate under diffusive and advective conditions may represent an upper bounds of the effects of advection on the apparent fractionation of ^{13}C. With natural fluctuations of pressure that are five orders of magnitude less than the negative pressure we generated, we can infer that gradients due to atmospheric pressure alone will not pull representative soil gas toward the soil surface. We were not able to sample the soil profile during the advection experiment; however, there was little change in the soil concentration and isotopic profiles after the first 45 min advective experiment indicating that, either the soil probes were located at such a distance that they were not influenced by the advection experiment or the time between sampling allowed for the effects of advection on the soil $^{13}CO_2$ profile to dissipate.

4.3. Partitioning the contribution of new and old carbon sources to $\delta^{13}C_{R\text{-}s}$

We calculated that 69–98% of the carbon respired from soil over the 90 min period we measured was from a depleted isotopic source that was similar to the signature of foliar extracts. The root isotopic contribution, for which we used phloem extractable carbon as a proxy for the carbon substrate respired, was estimated to account for 0–31% and falls well within the range of estimates from previous studies (Bond-Lamberty et al., 2004; Subke et al., 2006). However, even if root respiration accounted for 31% of $\delta^{13}C_{R\text{-}s}$ then 69% of soil respiration came from a source with a much more depleted isotopic signature.

Our inability to resolve the belowground carbon source to $\delta^{13}C_{R\text{-}s}$ is a symptom of the complexity of carbon sources and biochemistry belowground. Moss and lichen from an old-growth forest near this site (Crow et al., 2006) have isotopic signatures that are depleted in ^{13}C and, assuming a similar isotopic signature at our site, could be an important component we overlooked. Similarly, dissolved organic carbon has a depleted isotopic signature (Cleveland et al., 2004; Kaiser et al., 2001) and is hypothesized to have a high turnover rate in forest soils (Bengtson and Bengtsson, 2007) potentially explaining our partitioning results. It is also possible that only certain fractions of the soil organic matter were respired. Soil organic matter is a complex mixture of old and new carbon of varying degrees of decomposability (Sollins et al., 2006) and corresponding isotopic signatures further complicating identification of soil carbon contributions (Crow et al., 2006; Formanek and Ambus, 2004). A kinetic fractionation that occurs during root respiration that results in a depleted CO_2 isotopic signature could also account for the depleted source we identify in this study. Klumpp et al. (2005) found in a laboratory experiment that root respiration was depleted by up to 2.7‰ from bulk root biomass. Bulk biomass; however, represents carbon sequestered over a long time period and is bound to be more enriched than not only root respiration but also soluble carbon extracts from phloem.

4.4. Conclusions

The use of natural abundance as a means to partition soil respiration has many advantages since no alterations are made to the carbon substrate biochemistry or delivery in contrast to labeling techniques, and contributions can be monitored over the long-term in contrast to fumigation approaches. However, two goals need to be achieved to constrain partitioning estimates from natural abundance methods. The first is to ensure accurate and precise values of $\delta^{13}C_{R\text{-}s}$ estimates. The soil probe is able to achieve this goal with both the mixing model and soil profile model when the respiration is at isotopic steady-state. We did not find a large impact of natural fluctuations in advection on soil profile estimates of $\delta^{13}C_{R\text{-}s}$ suggesting that advection plays a minor role in forest

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estimates $\delta^{13}C_{R\text{-}s}$. Furthermore, we provide experimental evidence of a maximum 1‰ effect from advection on estimates of $\delta^{13}C_{R\text{-}s}$. The second goal is to sample potential carbon sources at a fine resolution. For example, SOM can be separated into several physical and chemical fractions (Ellerbrock and Kaiser, 2005). These fractions may contain carbon with distinct isotopic signatures (Haile-Mariam et al., 2008) that contribute to $\delta^{13}C_{R\text{-}s}$. Our study has shown carbon source partitioning using natural abundance measures of $\delta^{13}C_{R\text{-}s}$ is possible, but our samples were either too integrative or we missed a potential source when sampling.

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