# Lateral diffusion and atmospheric $CO_2$ mixing compromise estimates of rhizosphere respiration in a forest soil

**R.B. Susfalk, W.X. Cheng, D.W. Johnson, R.F. Walker, P. Verburg, and S. Fu**

**Abstract**: Measurements of rhizosphere carbon efflux are critical to the determination of soil carbon balance by $CO_2$ flux measurements. We attempted to measure rhizosphere respiration in a forest ecosystem by transplanting $^{13}C$-enriched soils from a tallgrass prairie into a mixed-conifer forest soil but found that atmospheric air mixing and lateral diffusion confounded $\delta^{13}C$-$CO_2$ measurements. Surface $CO_2$ efflux ($\delta^{13}C \approx -20‰$) was enriched 6‰ relative to soil $CO_2$ measured at depth because of the presence of atmospheric-derived $CO_2$ (–8‰) near the soil surface. The $\delta^{13}C$-$CO_2$ value of transplanted soil $CO_2$ did not reflect its $^{13}C$-enriched carbon source but was within 1‰ of native soil $CO_2$ because of lateral diffusion from the surrounding native soil. A two-component steady-state model of lateral diffusion supported our assertion that this soil was susceptible to atmospheric air mixing and lateral diffusion because of its high effective porosity and relatively low concentration of soil $CO_2$. Percent rhizosphere respiration was estimated at 35 and 45% after applying corrections for atmospheric air mixing and (or) lateral diffusion. These confounding effects may be reduced or eliminated by utilizing a larger transplanted soil pit and by reducing soil $CO_2$ diffusivity, for example, by increasing water content.

**Résumé** : La mesure des émanations de carbone provenant de la rhizosphère est essentielle pour estimer le bilan du carbone du sol via des mesures de flux de $CO_2$. Nous avons essayé de mesurer la respiration de la rhizosphère dans un écosystème forestier en transplantant des sols enrichis en $^{13}C$ et provenant d'une prairie d'herbes hautes dans un sol de forêt mélangée de conifères mais nous avons trouvé que les apports de l'air atmosphérique et de la diffusion latérale se confondaient avec les mesures de $\delta^{13}C$-$CO_2$. Les émanations de $CO_2$ ($\delta^{13}C \approx -20‰$) à la surface étaient enrichies de 6‰ en comparaison du $CO_2$ du sol mesuré en profondeur à cause de la présence à proximité de la surface du sol de $CO_2$ provenant de l'atmosphère (–8‰). La valeur de $\delta^{13}C$-$CO_2$ du $CO_2$ provenant du sol transplanté ne reflétait pas sa source en carbone enrichi de $^{13}C$, mais se situait à moins de 1‰ du $CO_2$ du sol natif du fait de la diffusion latérale à partir du sol natif environnant. Un modèle à deux composantes de la diffusion latérale à l'état d'équilibre supporte notre affirmation concernant la susceptibilité de ce sol au mélange avec l'air atmosphérique et à la diffusion latérale du fait de sa forte porosité effective et de la concentration relativement faible en $CO_2$ du sol. La respiration relative de la rhizosphère a été estimée entre 35 et 45% après l'application des corrections pour le mélange avec l'air atmosphérique ou la diffusion latérale. Ces effets confondants peuvent être réduits ou éliminés en utilisant un trou plus large pour le sol transplanté et en réduisant la capacité de diffusion du $CO_2$ du sol en augmentant, par exemple, la teneur en eau.

[Traduit par la Rédaction]

## Introduction

The role of soils in the global carbon (C) cycle is substantial, as soils contain more C than live biomass (Eswaran et al. 1993), and the emission of $CO_2$ from soil is a major flux of C to the atmosphere (Schlesinger and Andrews 2000). Soil $CO_2$ efflux has been widely measured in forest systems, but our understanding of the relative contributions by individual sources and how these sources will be affected by global change is poor. Carbon dioxide in soils results from root respiration and respiration by soil organisms that utilizes C derived from plant or soil organic matter. Rhizosphere respiration, the combined respiration from roots and microbial respiration utilizing C from root exudates, may account for about half of total soil respiration in forest soils (Hanson et al. 2000), but contributions up to 90% have

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been reported (Thierron and Laudelout 1996). It is likely that the various processes responsible for soil $CO_2$ will respond differently to climate change (e.g., Boone et al. 1998), necessitating a better understanding of individual processes to adequately assess the response of soils to global change.

The measurement of rhizosphere respiration has been a challenging task. Current methodologies include component integration (Hendrickson and Robinson 1984; Edwards and Harris 1977; Anderson 1973), root exclusion (Ewel et al. 1987; Edwards 1975; Anderson 1973; Wiant 1967), and isotopic tracer (Horwath et al. 1994; Dörr and Münnich 1987, 1986; Edwards and Harris 1977) techniques. Component integration and root exclusion suffer from excessive disturbance, with changes in temperature and soil moisture possibly altering respiration rates. Carbon isotopic methods utilizing either the radioactive $^{14}C$ isotope or the stable $^{13}C$ isotope are preferred, as they measure respiration rates in situ and do not require the alteration of the soil environment (Hanson et al. 2000). The natural stable isotope technique is the superior isotopic method, as soils and vegetation possess a natural $^{13}C$ label, whereas the artificial addition of radioactive $^{14}C$ may pose health and waste-disposal hazards.

Techniques based on natural stable carbon isotopes take advantage of the photosynthetic discrimination against the heavier $^{13}C$ isotope (Farquhar et al. 1989). Carbon-13 abundance is commonly reported as $\delta^{13}C$, or the $^{13}C/^{12}C$ ratio relative to the Pee Dee Belemnite (PDB) standard. Vegetation with $C_3$ ($\delta^{13}C \approx -27‰$) and $C_4$ ($\delta^{13}C \approx -12‰$) photosynthetic pathways exhibit different levels of discrimination against the heavier $^{13}C$ isotope. Soil respired $CO_2$ has a $\delta^{13}C$ value close to its organic substrate, as there is no net isotopic discrimination during respiration (Lin and Ehleringer 1997). A potential obstacle to the stable isotope approach is the small separation of $\delta^{13}C$ values between plant and soil organic matter derived C relative to the isotopic fractionation background in soils (Hanson et al. 2000). As a result, stable isotope experiments generally rely on the introduction of C from a non-native photosynthetic pathway. Examples include growing $C_4$ corn in a $^{13}C$-depleted soil previously supporting $C_3$ vegetation (Rochette et al. 1999; Rochette and Flanagan 1997) and the utilization of $\delta^{13}C$-depleted $CO_2$ generated from natural gas ($\delta^{13}C \approx -43‰$) in elevated $CO_2$ studies (Andrews et al. 1999; Lin et al. 1999).

In an attempt to quantify the contribution of rhizosphere respiration within a forest ecosystem, we transplanted $^{13}C$-enriched soils ($\delta^{13}C \approx -14‰$) from a $C_4$-dominated tallgrass prairie into a mixed $C_3$ conifer stand ($\delta^{13}C \approx -27‰$) in the western Sierra Nevada mountain range. To test this natural $^{13}C$ approach, we investigated the potential influences of lateral diffusion and the mixing of atmospheric-derived $CO_2$ on the $\delta^{13}C$ values of soil $CO_2$. Lateral soil $CO_2$ diffusion has not been traditionally considered in efflux and soil $pCO_2$ experiments in forested sites, as most studies consider the lateral extent of soil horizons to either be homogeneous or dwarfed by the large vertical gradient between soil $CO_2$ (> 10 000 $\mu L \cdot L^{-1}$; $\delta^{13}C = -23‰$) and the bulk atmosphere (ca. 365 $\mu L \cdot L^{-1}$; $\delta^{13}C \approx -8‰$). However, lateral diffusion from atmospheric air has been shown to significantly reduce soil $pCO_2$ over 150 cm away from an exposed soil pit wall (Davidson and Trumbore 1995). Rochette and Flanagan (1997) found that total soil respiration under corn, within the inter-rows and in nearby controls were not significantly different, indicating a homogenization of soil $CO_2$ by lateral diffusion. In an undisturbed system, lateral diffusion may be an important process in homogenizing $\delta^{13}C$-$CO_2$ produced from spatially diverse sources.

The magnitude of the vertical mixing of atmospheric $CO_2$ with soil $CO_2$ will be small because of large vertical diffusion gradient between soil and atmospheric $CO_2$. The relative abundance of $^{13}C$ can be used to assess this vertical mixing, as atmospheric $CO_2$ ($\delta^{13}C \approx -8‰$) is more enriched in $^{13}C$ than soil $CO_2$ ($\delta^{13}C \approx -23‰$) in $C_3$-dominated ecosystems. The intensity of atmospheric air mixing has been reported to be a function of soil respiration rate and the rate of gaseous diffusion within the soil (Amundson et al. 1998; Dudziak and Halas 1996*b*; Cerling 1984). Diurnal variations in soil $\delta^{13}C$-$CO_2$ may also result from atmospheric air mixing, as there will be a less intense mixing during daytime periods of higher rhizosphere respiration (Dudziak and Halas 1996*a*). The objectives of this paper were to (*i*) test the transplanted soil pit method; (*ii*) examine the mixing of atmospheric-derived $CO_2$ with soil $CO_2$; (*iii*) investigate the potential influence of lateral diffusion on the $\delta^{13}C$ value of soil $CO_2$ when soils of two dissimilar isotopic carbon signatures were juxtaposed; and (*iv*) discuss the implications that these processes may have on our ability to resolve the various C sources (e.g., air, roots, soil organic matter) contributing to soil $CO_2$.

## Materials and methods

### Site and soil description

Four paired native and transplanted soil plots were established in November 1999 in a mixed Sierra Nevada conifer stand at the Blodgett Experimental Forest, Georgetown, Calif., U.S.A. Transplanted soil plots consisted of 75 × 75 cm wide by 40 cm deep pits that were carefully excavated in such a manner that visible roots remained intact. To prevent vertical gaseous diffusion from underlying native soil, the pit bottom was lined with a vinyl barrier. Each pit was filled with a $^{13}C$-enriched ($^{13}C_E$) soil obtained from the surface layer of a Mollisol soil (approximately 0–30 cm) at the Konza Prairie Long-Term Ecological Research Site in Kansas. Vegetation at this site had been dominated by $C_4$ grasses for possibly thousands of years. The soil was sieved (<2 mm), picked free of roots, homogenized, and air-dried prior to use. This clay loam soil had a pH of 7.6 and contained 23 $mg \cdot g^{-1}$ of organic C and 2.0 $mg \cdot g^{-1}$ of N. The $\delta^{13}C$ value of the total soil organic C was –14.7‰. To apply the results obtained from the prairie soil to the natural forest setting, undisturbed forest soil plots near the transplanted soil pits were marked as native $^{13}C$-depleted ($^{13}C_D$) plots. The undisturbed native forest plots were not intended as a control plot for the transplanted soil but, rather, as a way to relate discrete rhizosphere respiration measurements within the transplanted prairie soil to continuous soil efflux measurements taken in the native forest soil. Table 1 lists selected properties of the native forest soil.

### Surface and subsurface $CO_2$ trapping

Surface $CO_2$ efflux was trapped in August 2000 utilizing a closed-circulation, inverted plastic box technique following Cheng et al. (2000). The inverted box was 26.1 cm in length,

**Table 1.** Selected properties of the native forest soil (Holland series).

| Depth (cm) | Horizon | Percentage of <2 mm fraction | | | Bulk density ($g \cdot cm^{-3}$) | pH ($H_2O$) | Carbon ($mg \cdot g^{-1}$) | Nitrogen ($mg \cdot g^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | | Clay | Silt | Sand | | | | |
| 0–5 | A11 | 11.2 | 26.4 | 62.4 | — | 6.0 | 27.7 | 2.0 |
| 5–25 | A12 | 12.4 | 25.4 | 62.2 | 1.46 | 5.9 | 10.8 | 0.9 |
| 25–53 | B1 | 15.4 | 25.1 | 59.5 | 1.45 | 6.0 | 3.6 | 0.3 |

**Note:** Data are from the National Soil Survey Center, Natural Resources Conservation Service, U.S. Department of Agriculture, Lincoln, Nebr.

15.2 cm in width, 6.2 cm in height, and was inserted 1 cm into the soil. A vacuum pump pulled air from within the inverted box through a sand column filled with 4 M NaOH to trap $CO_2$. The inverted box apparatus was purged with $CO_2$-free air prior to the 48-h trapping period. The flow rate was maintained at 0.20 $L \cdot min^{-1}$ with an inline needle valve. An aliquot of NaOH solution from each column was analyzed for inorganic C utilizing a Shimadzu 5050A TOC analyzer. Raw values were corrected for laboratory and field exposure to atmospheric air by subtracting out the C present in field controls. Another aliquot of NaOH solution was mixed with $SrCl_2$ to generate the $SrCO_3$ precipitate that was analyzed for $\delta^{13}C$ by a PDZ Europa (Cheshire, U.K.) Hydra 20–20 continuous flow isotope ratio mass spectrometer (IRMS) (Harris et al. 1997) at the University of California, Davis. Surface respiration was measured using a separate automated, open-flow cuvette system (Cheng et al. 2000) that produced surface respiration rates comparable with LI-COR 6200 (LI-COR, Inc., Lincoln, Nebr.) equipped with a static soil respiration chamber.

Subsurface $CO_2$ was collected using gas wells (Johnson et al. 1994). In June 2000, gas wells at 5, 10, and 15 cm away from the $^{13}C_E$ (transplanted) and $^{13}C_D$ (native) soil boundary were inserted to 15 and 30 cm depths. Additional gas wells were placed at 15 and 30 cm depths in the native soil, at least 0.6 m from the adjacent $^{13}C_E$ soil plot. In August 2000, all gas wells within the $^{13}C_E$ plots were removed and were replaced by a single 30 cm deep gas well placed at the center of the plot. After insertion of each well, 15 mL of soil gas was immediately purged, followed by at least 8 h before the first gas collection. Seven millilitres of soil gas was purged from the gas well to displace the dead volume, followed by the collection of 11 mL that was immediately transferred into 10-mL vacutainers. The $\delta^{13}C$ of $CO_2$ in the vacutainers was analyzed by a PDZ Europa TGII trace gas analyzer coupled to a Europa Geo IRMS at the University of California, Davis. As the diffusion of $CO_2$ within the soil naturally discriminates against the heavier $^{13}C$-$CO_2$ isotope by 4.4 ‰ (Cerling 1984; Cerling et al. 1991), observed $\delta^{13}C$-$CO_2$ values from the gas wells were adjusted to account for this influence. The $\delta^{13}C$ values presented in the tables and figures include this correction, unless noted. Surface efflux measurements were considered to be at steady state and were not subject to this correction.

### Statistical analyses

Mean comparisons of $\delta^{13}C$-$CO_2$ values between soil types and depths were accomplished by a pooled *t* test ($\alpha = 0.05$) using the Data Desk software (Data Description, Inc., Ithaca, N.Y.). A Student's *t* test ($\alpha = 0.05$) was used to compare the means of soil $\delta^{13}C$ values, carbon content, and root density with depth. Mean comparisons of soil $\delta^{13}C$-$CO_2$ between depths and distances from the native–transplanted boundary were accomplished by a post-hoc Bonferroni ($\alpha = 0.05$) following a three-way analysis of variance (native and transplanted soils by three distances from the boundary by two depths) using the Data Desk software. Treatments were considered significant only when $p \leq 0.05$ (according to the *F* test).

### Partitioning rhizosphere and soil–microbial respiration

Based on Cerri et al. (1985), the following equation can be used to partition $^{13}C$ enriched soil-derived ($C_4$) carbon from $^{13}C$ depleted plant-derived ($C_3$) carbon:

$$[1] \qquad \% \text{ RR} = \frac{(\delta_t - \delta_4)}{(\delta_3 - \delta_4)} \times 100$$

where % RR is the percentage of C derived from $C_3$ plant carbon; $\delta_3$ and $\delta_4$ are the $\delta^{13}C$ values of $C_3$ plant C and $C_4$-derived soil C, respectively; and $\delta_t$ was the $\delta^{13}C$ value of the $CO_2$ sample. As there is no isotope discrimination during respiration (Lin and Ehleringer 1997), $\delta_3$ and $\delta_4$ were considered to be the $\delta^{13}C$ of the soil organic matter ($\delta^{13}C \approx -14$‰) and the $C_3$ roots ($\delta^{13}C \approx -27$‰) present in the transplanted soil pits.

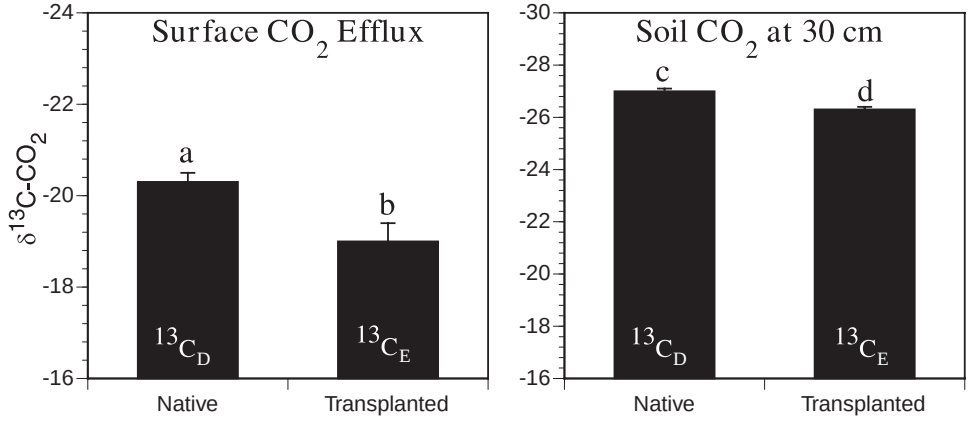
### Calculation of vertical and lateral $^{13}C$-$CO_2$ diffusion

Cerling (1984) developed a steady-state $CO_2$ isotope diffusion model to investigate the vertical $\delta^{13}C$ trends in soils containing pedogenic carbonates. This model concurred with field observations that soil $\delta^{13}C$-$CO_2$ is susceptible to isotopic discrimination by diffusion (Cerling et al. 1991; Cerling 1984; Dörr and Münnich 1980) and to diurnal and seasonal trends resulting from changes in atmospheric air mixing (Dudziak and Halas 1996*a*; Mook and Koopmans 1983). This model incorporated the different diffusivities of $^{12}C$- and $^{13}C$-$CO_2$ and assumed a constant production rate of $CO_2$ from soil respiration (Amundson et al. 1998):

$$[2] \qquad R_s^{13} = \frac{\dfrac{\phi R_p^{13}}{D_s^{13}}\left(Lx - \dfrac{x^2}{2}\right) + C_b R_b^{13}}{\dfrac{\phi}{D_s}\left(Lx - \dfrac{x^2}{2}\right) + C_b}$$

where $R_s^{13}$ is the ratio of $^{13}C$ to $^{12}C$ in soil $CO_2$ at a given depth, $\phi$ is the $CO_2$ production in the soil ($mol \cdot cm^{-3} \cdot s^{-1}$); $D_s^{13}$ and $D_s$ were the diffusion coefficients of $^{13}CO_2$ and bulk

**Fig. 1.** Surface efflux and soil $\delta^{13}C\text{-}CO_2$ in native $\delta^{13}C_D$ and transplanted $\delta^{13}C_E$ soils. Measurements within the transplanted soils were taken at least 20 cm from the native–transplanted soil interface. Soil $\delta^{13}C\text{-}CO_2$ values were adjusted by 4.4‰ to account for isotopic discrimination that occurred during diffusion. Means with different letters are statistically different by a pooled *t* test ($\alpha = 0.05$). Error bars are SEs ($n = 4$).




$CO_2$ ($cm^2 \cdot s^{-1}$), respectively; $R_p^{13}$ and $R_b^{13}$ were the $^{13}C/^{12}C$ ratios of soil respired $CO_2$ and atmospheric $CO_2$; $x$ was the distance increment away from the soil surface (cm), and $C_b$ is the atmospheric air $CO_2$ concentration ($mol \cdot cm^{-3}$). The boundary conditions were the $\delta^{13}C\text{-}CO_2$ at the soil surface ($x = 0$) and the maximum distance ($L$) at which soil respiration was equally distributed ($x = L$). Soil $CO_2$ production ($\phi$) was adjusted to correspond with measured $pCO_2$ levels at a 15 cm depth. This model varied from Cerling's derivation in that $[^{12}CO_2]$ was approximated by bulk $[CO_2]$ (Amundson et al. 1998). The diffusion coefficient of gaseous $CO_2$ was calculated from a number of variables, including porosity, temperature, and moisture content (Moldrup et al. 1996). Modeled scenarios differed by soil temperature and volumetric water content and included snowmelt, June, July, August, and "rainy September week" scenarios having diffusion coefficients of 0.0254, 0.0472, 0.0517, 0.0614, and 0.040 $cm^2 \cdot s^{-1}$, respectively. Scenarios were based on actual monthly or weekly data collected at the site except for the arbitrarily defined snowmelt scenario. $R_s^{13}$ was subsequently converted to standard notation:

$$[3] \qquad \delta^{13}C = \left( \frac{R_s^{13} - R_{std}}{R_{std}} \right) \times 1000$$

where $R_{std}$ is the $^{13}CO_2/^{12}CO_2$ ratios of PDB carbonate.

Equation 2 was adapted to calculate lateral diffusion in transplanted soils by replacing the atmospheric air boundary conditions by those found at the native–transplanted soil interface: $R_b^{13}$ and $C_b$ became the $^{13}C/^{12}C$ ratio and concentration of soil respired $CO_2$ at the native–transplanted soil interface, and $x$ became the distance increment away from the native–transplanted soil interface (cm). The $CO_2$ diffusion coefficient within transplanted soils was calculated to be 0.02 $cm^2 \cdot s^{-1}$.

## Results

### $\delta^{13}C$ of surface efflux and subsurface $CO_2$ from native soils

Surface $CO_2$ efflux from the native soil ($\delta^{13}C = -20.3‰$) was considerably more enriched in $^{13}C$ than soil $CO_2$ derived from soil organic matter (SOM; $\delta^{13}C = -26.1‰$; $P = 0.0002$) or plant roots ($\delta^{13}C \approx -27‰$) (Fig. 1). As isotopic discrimination did not occur during respiration (Lin and Ehleringer 1997; Cheng 1996), the observed enrichment in surface efflux could be attributed to experimental bias or atmospheric air mixing. The maximum contamination by atmospheric air calculated by eq. 1 was 35%, assuming negligible root respiration and $CO_2$ endmembers of atmospheric air and soil respiration. Native soil $\delta^{13}C\text{-}CO_2$ at 30 cm (Fig. 1) was consistent with source SOM (Table 2) and rhizosphere C from this $C_3$-dominated ecosystem.

Soil $\delta^{13}C\text{-}CO_2$ in the native soil was more $^{13}C$ enriched with depth ($P = 0.0307$) because of the greater $^{13}C$ enrichment in SOM and the diminishing role of rhizosphere respiration with depth (Table 2). The $\delta^{13}C$ value of SOM under forests is known to increase by about 1‰ with depth (Buchmann et al. 1988; Vitorello et al. 1989), because SOM deeper in the profile originated when atmospheric air was more enriched in $^{13}C$ (Ehleringer et al. 2000), and there is less labile $^{13}C$ in older, deeper SOM relative to younger litter-derived SOM (Schleser and Pohling 1980). Lower rooting density (Table 2) implies that rhizosphere respiration will decrease as $\delta^{13}C\text{-}CO_2$ values increase with depth.

### $\delta^{13}C$ values of surface efflux and subsurface $CO_2$ in transplanted soils

The $\delta^{13}C$ value of $CO_2$ in the centre of the transplanted soil pits were within 1‰ of native soil $CO_2$ in both the surface efflux and in soil $CO_2$ at a 30 cm depth (Fig. 1). The entire pit appeared to be contaminated by native soil $CO_2$, as measurements taken at different depths and distances from the pit boundary were within about 1‰ of each other (Table 3). Within the transplanted soil, surface efflux had a $\delta^{13}C$ value of –19.0‰, and soil $CO_2$ at a 30 cm depth had a value of –25.9‰. Rhizosphere respiration (eq. 1) contributed 35 and 91% of total soil respiration (% RR) at surface and 30 cm depths, respectively. The discrepancy between % RR at the surface and at a 30 cm depth, and their apparent disagreement with the 49% mean % RR in forest ecosystems (Hanson et al. 2000) suggested the presence of other $CO_2$ sources. The lateral diffusion of native soil $CO_2$ and the ver-

tical mixing of atmospheric $CO_2$ were considered the most likely sources.

### Vertical and lateral $\delta^{13}C$-$CO_2$ diffusion

The effect of atmospheric air on soil $\delta^{13}C$-$CO_2$ under various soil moisture conditions was investigated using an existing steady-state model (Fig. 2; Amundson et al. 1998; Cerling 1984). Near the soil surface, atmospheric air mixing reduced the $\delta^{13}C$-$CO_2$ values by 4‰ under dry conditions (August) and by 2.5‰ under moist conditions (snowmelt) (Table 4). The effect of atmospheric air mixing at depth was negligible, as both scenarios changed less than 0.3‰. In this model, drier soil conditions increased effective porosity, resulting in a greater mixing of atmospheric air and soil $CO_2$.

The effect of lateral diffusion on $\delta^{13}C$-$CO_2$ values was investigated by recasting the vertical diffusion model presented in eq. 2 as a lateral diffusion model. In this model, soil $\delta^{13}C$-$CO_2$ at the transplanted–native soil interface was set to native soil $\delta^{13}C$-$CO_2$ at a 15 cm depth, and became more enriched in $\delta^{13}C$ further into the transplanted soil because of the respiration of $^{13}C_E$ soil organic matter (Fig. 3). Using the 0.02 $cm^2 \cdot s^{-1}$ diffusion coefficient calculated for this soil, $\delta^{13}C$-$CO_2$ increased from –23.2‰ at the transplanted–native soil interface to –21.2‰ at 20 cm away from the interface towards the centre of the pit. The extent of $\delta^{13}C_D$- and $\delta^{13}C_E$-$CO_2$ mixing within the transplanted soil was dependent on the diffusion coefficient, which was the function of a number of variables, including porosity, temperature, and moisture content. Although observed $\delta^{13}C$ values were within model predictions, the calculated values were probably underestimated, as the model did not account for lateral diffusion from other soil pit faces and assumed that soil $CO_2$ production was entirely from SOM sources.

## Discussion

### Methodological implications

The original objective of this research was to assess the contribution of rhizosphere respiration in a forest ecosystem by partitioning surface $CO_2$ efflux into its root- and soil-derived components using a natural $^{13}C$-tracer method. The natural $^{13}C$ tracer was introduced by a soil transplanting approach and was necessary to measure rhizosphere respiration per unit of root volume (length, root C, or root N). Rhizosphere respiration from the native, undisturbed forest soil was to be calculated by applying a series of discrete stable isotope measurements from the transplanted soil analog to continuous soil efflux measurements taken in both transplanted and native soils by combining isotope and root dynamics measurements. Respiration measurements of the prairie soil may have been biased by the alteration of root distribution and soil disturbance during the excavation and subsequent transplanting of soils. The unintentional severing and removal of fine roots during excavation will decrease rhizosphere respiration relative to the respiration of soil-derived C. This was partly mitigated by a settling period to allow for new root growth. Disturbance of the prairie soil was expected to temporally increase soil respiration because of the increased decomposition of labile organic matter (Blet-Charaudeau et al. 1990). We believe that the 8-month period between transplanting and measurement was adequate for soil disturbance effects to subside. Two previous mesocosom-scale greenhouse experiments (e.g., Cheng et al. 2000) using the same prairie soil exhibited strong soil respiration and nitrate leaching immediately after soil transplanting, which subsequently declined into stable equilibrium levels within 2–4 months (unpublished data).

There was a 6‰ discrepancy between surface efflux and subsurface $CO_2$ measured for native soil ($P < 0.0001$; Fig. 1), which may have been partly due to the experimental techniques used. Surface efflux and subsurface measurements reflected $CO_2$ trapped during different time scales; this was because gas well measurements were an instantaneous sample of the subsurface $CO_2$, whereas the inverted box technique provided a mean $\delta^{13}C$ value of $CO_2$ trapped over two diurnal periods. Diurnal variation has been found to alter the $\delta^{13}C$ value of surface efflux by up to 2.5‰ in a deciduous forest, partly attributed to the greater mixing of atmospheric air in the surface soil during lower nighttime biological activity (Dudziak and Halas 1996*a*). If the observed discrepancy was entirely attributed to the effects of diurnal variation, nighttime soil respiration at this site would have a $\delta^{13}C$ value of –35‰. As there was no known mechanism to deplete $\delta^{13}C$-$CO_2$ by over 5‰ at night, diurnal varia-

**Table 2.** $\delta^{13}C$, carbon, and rooting density in native and transplanted soils.

| Depth (cm) | $\delta^{13}C$ (‰) | Carbon ($mg \cdot g^{-1}$) | Root density ($mm\ root \cdot cm^{-3}$) |
|---|---|---|---|
| **Native $\delta^{13}C_D$ soils** | | | |
| 0–15 | –26.02 (0.10)*a* | 46.1 (0.3)*a* | 11.92 (1.03)*a* |
| 15–30 | –25.08 (0.31)*b* | 29.8 (1.1)*b* | 7.41 (0.60)*b* |
| 30–45 | –24.36 (0.07)*b* | 15.8 (0.5)*c* | 4.02 (0.33)*c* |
| **Transplanted $\delta^{13}C_E$ soils** | | | |
| Pre-experiment | –14.7 (0.07)*a* | 20.0 (0.8)*a* | — |
| 0–15 | –15.2 (0.11)*b* | 21.3 (0.8)*a* | — |

**Note:** Values are means with SE given in parentheses. Values within each soil type with the same letter were not significantly different as determined by a Student's *t* test ($\alpha = 0.05$).

**Table 3.** $\delta^{13}C$-$CO_2$ values from native soils and in transplanted soil pits at 5, 10, and 15 cm from the native–transplanted soil boundary.

| | | $\delta^{13}C$-$CO_2$ (‰) | |
|---|---|---|---|
| Depth (cm) | Distance to boundary (cm) | Native | Transplanted |
| 15 | Native | –28.5 (0.2)*ab* | |
| 15 | 5 | | –27.6 (0.5)*ac* |
| 15 | 10 | | –27.4 (0.5)*ac* |
| 15 | 15 | | –28.7 (0.3)*a* |
| 30 | Native | –27.2 (0.3)*c* | |
| 30 | 5 | | –27.6 (0.2)*bc* |
| 30 | 15 | | –27.3 (0.6)*ac* |

**Note:** Values are means with SE given in parentheses. Soil $\delta^{13}C$-$CO_2$ values were adjusted by 4.4‰ to account for isotopic discrimination that occurred during diffusion. Values with the same letter were not significantly different as determined by a post-hoc Bonferroni ($\alpha = 0.05$) following a three-way analysis of variance.

**Table 4.** Change in $\delta^{13}C\text{-}CO_2$ at selected depths and depth increments for the model scenarios described in Fig. 2.

| Depth or depth increment | Snowmelt | June | July | August | Rainy September week |
|---|---|---|---|---|---|
| **Change in $\delta^{13}C\text{-}CO_2$ relative to August scenario (‰)** | | | | | |
| At 5 cm | –1.01 | –0.38 | –0.25 | — | –0.58 |
| At 15 cm | –0.43 | –0.17 | –0.11 | — | –0.25 |
| At 30 cm | –0.27 | –0.11 | –0.07 | — | –0.16 |
| **Change in $\delta^{13}C\text{-}CO_2$ by depth increment (‰)** | | | | | |
| $\Delta_{(1–5\ cm)}$ | –2.42 | –3.60 | –3.78 | –4.13 | –3.27 |
| $\Delta_{(5–15\ cm)}$ | –0.50 | –0.87 | –0.94 | –1.08 | –0.75 |
| $\Delta_{(15–30\ cm)}$ | –0.12 | –0.22 | –0.24 | –0.28 | –0.19 |

**Fig. 2.** Calculated steady-state $pCO_2$ (A) and soil $\delta^{13}C\text{-}CO_2$ (B) within native $\delta^{13}C_D$ soils under different temperature and moisture conditions. The snowmelt scenario assumed a 10°C temperature ($T$) and 25% volumetric water content (θ). Other scenarios were based on measured averages (June: $T$ = 16.3°C, θ = 0.14; July: $T$ = 15.7°C, θ = 0.11; August: $T$ = 16.9°C, θ = 0.08; rainy September week: $T$ = 13.1°C, θ = 0.17). The diffusion coefficient was calculated following Moldrup et al. (1996) based on temperature, moisture, and a total porosity of 63%. This model was based on Amundson et al. (1998) and assumed that $CO_2$ was evenly produced over 50 cm, that the $CO_2$ ($\delta^{13}C$ = –27‰) production rate was $2.90 \times 10^{-3}$ mol·cm$^{-3}$·s$^{-1}$, and that atmospheric $CO_2$ ($\delta^{13}C$ = –8‰) had a concentration of 29 mmol·L$^{-1}$.

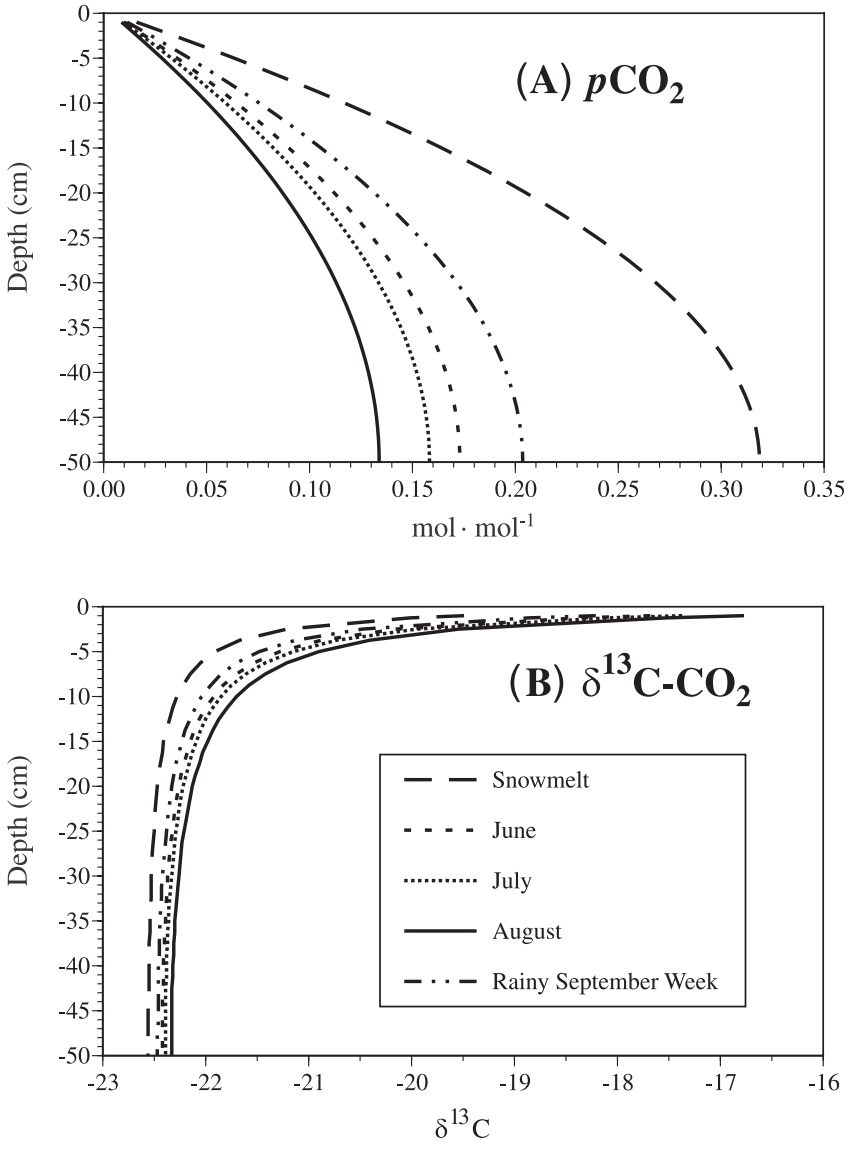
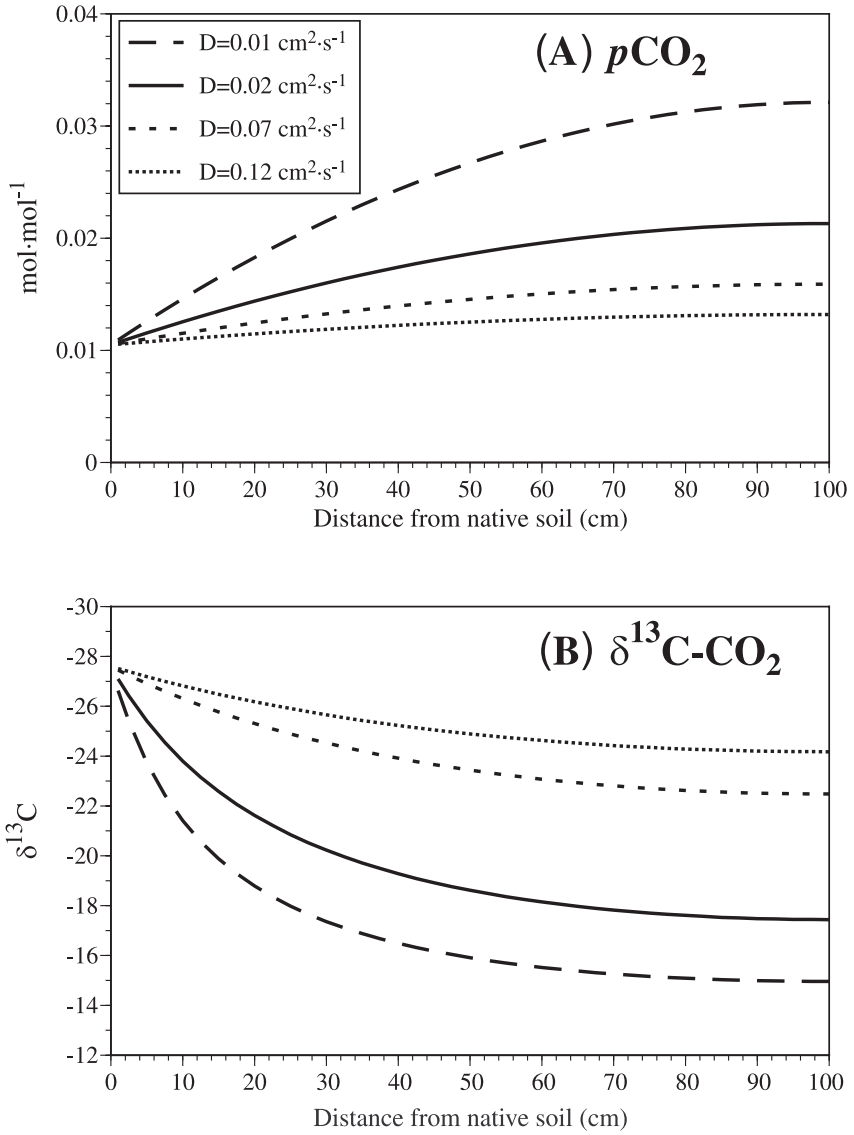


**Fig. 3.** Calculated steady-state $pCO_2$ (A) and soil $\delta^{13}C$-$CO_2$ (B) within transplanted $\delta^{13}C_E$ soils using the lateral diffusion model described in the text. It was assumed that $CO_2$ was evenly produced over 100 cm, that the $CO_2$ ($\delta^{13}C$ = –15.2‰) production rate was $2.94 \times 10^{-3}$ mol·cm$^{-3}$·s$^{-1}$, and the transplanted–native soil interface had a $pCO_2$ ($\delta^{13}C$ = –23.2‰) of 875 mmol·L$^{-1}$.




tion could not be solely responsible. Secondly, the more complex trapping methodology necessary to collect the surface efflux may have been susceptible to enrichment in $\delta^{13}C$ by isotopic discrimination, pre-experiment contamination (e.g., $CO_2$ in the NaOH pellets; Davidson 1995), or "leakage" of atmospheric air into the system during or after trapping. Previous studies utilizing this methodology have not been observed to fractionate $CO_2$ isotopes (Cheng 1996; Cheng and Johnson 1998), nor has there been any evidence that the inverted box has introduced a systematic contamination of atmospheric $CO_2$ during trapping (Cheng et al. 2000 and unpublished data). Field and laboratory contamination were accounted for by the use of blanks. Although methodological differences may account for a small discrepancy between surface efflux and soil $\delta^{13}C$-$CO_2$ values, the results from previous experiments indicated that methodological differences could not account for the apparent 6‰ enrichment in surface efflux relative to soil $CO_2$.

**Atmospheric air mixing in native and transplanted soils**

The most likely cause of this observed enrichment of surface efflux (Fig. 1) was the presence of atmospheric $CO_2$ ($\delta^{13}C$ = –8‰), estimated to contribute up to 35% of the near-surface soil gas. The soil atmosphere of dry, relatively unproductive soils can be composed of a significant concentration of atmospheric-derived $CO_2$ (Amundson et al. 1988) as predicted by a two-component steady-state diffusion model (Amundson et al. 1998; Cerling 1984). Soils at this forested site were of a sandy clay loam texture, having a soil moisture content of 0.08 m$^3$·m$^{-3}$ at the time of sampling, with a summer–fall mean soil respiration rate of 5.6 ± 0.5 μmol $CO_2$·cm$^{-2}$·s$^{-1}$, and a soil $pCO_2$ of 6.9 ± 1.1 mmol·mol$^{-1}$ at a 15 cm depth. The combination of coarse soil texture and low soil moisture content resulted in a high effective porosity (63% of the soil pore space was filled by air) and was the primary factor in this soil's susceptibility to atmospheric air mixing. The presence of atmospheric air within the inverted

box was ascribed to the lateral diffusion of $CO_2$ underneath the box edges in response to the artificially lowered $CO_2$ concentration within the inverted box (Healy et al. 1996) and (or) natural lateral diffusion processes. Lateral diffusion beneath the box edges most likely occurred during previous isotopic trapping experiments utilizing the inverted box (Cheng et al. 2000 and unpublished data). However, the finer soil texture and considerably greater soil moistures of those experiments reduced the effective soil porosity, most likely resulting in negligible atmospheric air mixing and a lower chamber-induced lateral diffusion rate. Atmospheric air contamination may be reduced or eliminated by either conducting the experiment under conditions less favorable to atmospheric air mixing (e.g., finer soil texture and greater soil moisture) or by reducing the importance of chamber-induced lateral diffusion by inserting the box edges deeper into the soil (Hutchinson and Livingston 2001). Deeper insertion of the box may sever roots and provide a barrier against future root growth and would not be effective against natural lateral diffusion processes.

Atmospheric air mixing should be spatially and temporally variable, as both net $CO_2$ production (Fang et al. 1998; Longdoz et al. 2000; Rayment and Jarvis 2000) and individual $CO_2$ sources are highly variable within soil. The extent of $C_3$ plant cover and moisture has been found to exert a strong influence on the $\delta^{13}C$ of the soil $CO_2$ in the Mojave Desert, California (Amundson et al. 1998). The response of atmospheric air mixing to the diurnal variation of soil $CO_2$ production has been attributed as one of the sources of diurnal variation in soil $\delta^{13}C$-$CO_2$ (Dudziak and Halas 1996*a*). Despite the soil $\delta^{13}C$-$CO_2$ being determined by local C sources, mixing with atmospheric-derived $CO_2$ can significantly alter the $\delta^{13}C$ value depending on several processes, including total respiration rate, soil moisture content, and soil texture. Soil moisture may be the most critical factor controlling the variability in $\delta^{13}C$-$CO_2$, as greater moisture contents independently stimulates biological activity and respiration rates, as well as reduces the rate of gaseous diffusion within the soil by physically occupying pore space, because gaseous diffusion in water is orders of magnitude smaller than through air.

Precipitation at this site was highly seasonal, resulting in a general pattern of the highest soil moisture content during spring snowmelt that steadily declined until winter precipitation. Therefore, the 6–8‰ enrichment attributed to atmospheric-derived $CO_2$ mixing during August was most likely representative only of the dry summer months. The two-component model (Fig. 2) affirmed that atmospheric air mixing increased $\delta^{13}C$-$CO_2$ by over 4‰ in the top 5 cm of dry soil (August scenario) compared with a 2.5‰ increase in moister soil (snowmelt scenario; Table 4). The snowmelt scenario was less susceptible to atmospheric air mixing, because its higher moisture levels reduced the ability of gas to diffuse through the soil, resulting in a greater soil $CO_2$ concentration. This response of $\delta^{13}C$-$CO_2$ to seasonal fluctuations in soil moisture content was greater than a seasonal shift of about 1‰ because of changes in rhizosphere respiration, modeled by adjusting % RR from 1 (winter) to 50% (summer). Seasonal changes due to atmospheric air mixing should actually be greater than predicted here, as the model did not account for seasonal changes in respiration rates or the source of $CO_2$ production in the soil.

**Rhizosphere respiration**

The difference in isotopic carbon signature of soil (–14‰) and plant-derived (–27‰) C sources present in the transplanted soil provided the basis from which to estimate the percentage of rhizosphere respiration (% RR). The % RR in surface efflux was 35% but was considered an underestimate because of the presence of atmospheric-derived $CO_2$. Assuming atmospheric air mixing had the same effect on both soil types, correcting this value for atmospheric-derived $CO_2$ mixing yielded a % RR of 83% (Table 5). Atmospheric air mixing in transplanted soils will likely be lower than in native soils because of a lower gaseous diffusion rate and finer texture of the transplanted soils. At a 30 cm depth, % RR was 95% after the soil $\delta^{13}C$-$CO_2$ value was adjusted for diffusive discrimination (Table 5). Both corrected estimates of % RR were greater than the 49% mean in forest soils (Hanson et al. 2000) and were not in agreement with a qualitative assessment of very low rooting density within the transplanted soil pits observed by minirhizotron (unpublished data). Rhizosphere respiration was expected to be lower than the forest mean, as the excavation and soil transplanting method invariably resulted in the severing and removal of some fraction of the highly active fine root biomass. Our inflated % RR estimates were most likely due to the presence of $^{13}C$-depleted $CO_2$ from the surrounding native soil. A vapor barrier beneath the transplanted soil minimized contamination by vertical diffusion from the underlying native soil $CO_2$. The diffusion of bulk $CO_2$ from the native soil into the pit was not a likely contamination mechanism, as the transplanted prairie soil had a greater $pCO_2$ concentration ($11.8 \pm 1.6$ mmol·mol$^{-1}$ (mean ± SE) at a 30 cm depth) than native soil ($9.0 \pm 1.1$ mmol·mol$^{-1}$). Instead, we believe that our inflated % RR measurements resulted from the lateral diffusion by the individual $CO_2$ isotopes between adjacent sources of $^{13}C$-enriched and $^{13}C$-depleted $CO_2$.

**Lateral diffusion**

The two-component mixing model of lateral diffusion (Fig. 3) supported our claim that lateral diffusion was the most likely mechanism causing the similar $\delta^{13}C$-$CO_2$ values in transplanted and native soils. For a soil with a 0.02 cm$^2$·s$^{-1}$ diffusion coefficient, the lateral diffusion with native $CO_2$ reduced the $\delta^{13}C$ value of soil $CO_2$ by two permil at the furthest extent away from the native soil (Fig. 3). The depletion of $\delta^{13}C$ was more precipitous closer to the native soil, as soil $CO_2$ decreased by 5‰ within the first 25 cm of the native–transplanted soil interface. Applying these trends to observed values, $\delta^{13}C$-$CO_2$ at a 30 cm depth was corrected for lateral diffusion, resulting in a corrected % RR of 45% (Table 5). At the surface, this 5‰ correction for lateral diffusion was nearly offset by the –5.9‰ correction for atmospheric air mixing. The correction for lateral diffusion was conservative, as the one-dimensional model did not account for diffusion from other pit faces. Although lateral diffusion confounded our results from this site, the model suggested that an experiment having a larger pit volume and a soil with a lower effective porosity may be less susceptible

**Table 5.** Corrections applied to the $\delta^{13}C$ values of soil $CO_2$.

| | Raw values | | Corrected for diffusive discrimination[c] | | Corrected for atmospheric air mixing[d] | | Corrected for lateral diffusion[e] | |
|---|---|---|---|---|---|---|---|---|
| | ‰ | % RR[b] | ‰ | % RR[b] | ‰ | % RR[b] | ‰ | % RR[b] |
| **Surface $CO_2$ efflux[a]** | | | | | | | | |
| Transplanted | –19.0 | 35 | — | — | –24.9 | 83 | –19.9 | 35 |
| Native | –20.3 | | — | | –27 | | — | |
| **Subsurface $CO_2$ (at a 30 cm depth)[a]** | | | | | | | | |
| Transplanted | –21.9 | 61 | –26.3 | 95 | — | — | –21.3 | 45 |
| Native | –22.6 | | –27.0 | | — | | — | |
| **Endmembers used in % RR calculation** | | | | | | | | |
| $C_3$ (plant derived) | –27 | | –27 | | –27 | | –27 | |
| $C_4$ (soil derived) | –14 | | –14 | | –14 | | –16[e] | |

[a]Corrections applied to $\delta^{13}C$-$CO_2$ are cumulative from left to right.
[b]Percent rhizosphere respiration (% RR) was calculated from eq. 1 with stated endmembers.
[c]The –4.4‰ correction for diffusive discrimination (Cerling 1991) was only applied to subsurface soil $CO_2$ values.
[d]The atmospheric air mixing correction was only applied to surface efflux measurements and was determined by difference from observed and diffusive discrimination corrected values at a 30 cm depth.
[e]The 5‰ correction for lateral diffusion was determined from the two-component steady-state model and included a correction for the $C_4$ endmember based on Fig. 3 (see text).

to lateral diffusion. Reduction of the diffusion coefficient from 0.02 to 0.01 $cm^2 \cdot s^{-1}$ (e.g., a soil with a 20% volumetric water content having a 10% greater bulk density than our soil) was predicted to be unaffected by lateral diffusion at the center of a 2-m pit.

This soil transplanting experiment demonstrated that soil $\delta^{13}C$-$CO_2$ reflected the integration of spatially different C sources and that any assumption of spatial homogeneity must be done so with caution. Of the few studies that have quantified spatial variation, the coefficients of variation from surface $CO_2$ efflux measurements have been typically large (Hanson et al. 1993; Rayment and Jarvis 2000). This spatial heterogeneity can be a function of root density, soil texture, soil organic matter, and the ratios of C/N and lignin/N in organic materials (Longdoz et al. 2000). In the present study, coefficients of variation ranged from 0.15 and 0.32 for soil $CO_2$ and from 0.26 to 0.43 for soil $CO_2$ efflux. The interpretation of spatial variation in soil $\delta^{13}C$-$CO_2$ is made more complex by numerous isotopic sources that may be present. These sources may include carbonates ($\delta^{13}C = 0$), root-derived $C_3$ and $C_4$ carbon ($\delta^{13}C \approx -27$ and –14‰, respectively), SOM ($\delta^{13}C \approx -10$ to –15 and –20 to –27‰), and atmospheric air ($\delta^{13}C \approx -8$‰). The spatial variation of C sources will be more highly variable in desert ecosystems having large interspaces, less variable in forests, and the least heterogeneous in grasslands. This spatial variation coupled with lateral diffusion suggests that soil $CO_2$ may not necessarily reflect the carbon isotopic signature of local C sources, especially in dry, porous soils.

**Summary**

We attempted to quantify the contribution of rhizosphere respiration within a forest ecosystem using stable carbon isotopes by transplanting $^{13}C$-enriched soils from a $C_4$-dominated tall-grass prairie into a $C_3$ mixed conifer stand. Rhizosphere respiration measurements were confounded by the high effective porosity that resulted from a relatively coarse soil texture coupled with low soil moisture. The high effective porosity was a critical factor in the susceptibility of this soil to atmospheric air mixing and a contributing factor to the lateral diffusion of atmospheric air beneath the inverted box edges. Although the juxtaposition of these soils was artificial, the homogenization of soil $\delta^{13}C$-$CO_2$ values by the lateral diffusion of $^{13}C$- and $^{12}C$-$CO_2$ isotopes suggested that soil $\delta^{13}C$-$CO_2$ may not necessarily reflect the carbon signature of local C sources. The dependence of atmospheric air mixing and lateral diffusion on biological and physical processes results in a temporal and spatial heterogeneity that will be difficult to account for. We predicted that the transplanted soil and inverted box techniques can be successful by utilizing a larger transplanted soil pit and by minimizing soil diffusion processes by selecting a site having a greater water content and a finer soil texture.

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