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Partitioning soil surface CO_2 efflux into autotrophic and heterotrophic components, using natural gradients in soil $\delta^{13}C$ in an undisturbed savannah soil

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ABSTRACT

We used natural gradients in soil and vegetation $\delta^{13}C$ signatures in a savannah ecosystem in Texas to partition soil respiration into the autotrophic (Ra) and heterotrophic (Rh) components. We measured soil respiration along short transects from under clusters of C_3 trees into the C_4 dominated grassland. The site chosen for the study was experiencing a prolonged drought, so an irrigation treatment was applied at two positions of each transect. Soil surface CO_2 efflux was measured along transects and CO_2 collected for analysis of the $\delta^{13}C$ signature in order to: (i) determine how soil respiration rates varied along transects and were affected by localised change in soil moisture and (ii) partition the soil surface CO_2 efflux into Ra and Rh, which required measurement of the $\delta^{13}C$ signature of root- and soil-derived CO_2 for use in a mass balance model.

The soil at the site was unusually dry, with mean volumetric soil water content of 8.2%. Soil respiration rates were fastest in the centre of the tree cluster (1.5 ± 0.18 μmol m^{-2} s^{-1}; mean ± SE) and slowest at the cluster–grassland transition (0.6 ± 0.12 μmol m^{-2} s^{-1}). Irrigation produced a 7–11 fold increase in the soil respiration rate. There were no significant differences ($p > 0.5$) between the $\delta^{13}C$ signature of root biomass and respired CO_2, but differences ($p < 0.01$) were observed between the respired CO_2 and soil when sampled at the edge of the clusters and in the grassland. Therefore, end member values were measured by root and soil incubations, with times kept constant at 30 min for roots and 2 h for soils. The $\delta^{13}C$ signature of the soil surface CO_2 efflux and the two end member values were used to calculate that, in the irrigated soils, Rh comprised $51 \pm 13.5\%$ of the soil surface CO_2 efflux at the mid canopy position and $57 \pm 7.4\%$ at the drip line. In non-irrigated soil it was not possible to partition soil respiration, because the $\delta^{13}C$ signature of the soil surface CO_2 efflux was enriched compared to both the end member values. This was probably due to a combination of the very dry porous soils at our study site (which may have been particularly susceptible to ingress of atmospheric CO_2) and the very slow respiration rates of the non-irrigated soils.

1. Introduction

Soils are the largest pool of carbon (C) in terrestrial ecosystems, globally containing more than two-thirds of their total C (Amundson, 2001) and thus storing the equivalent of about 300 times the amount of C now released each year through the burning of fossil fuels (Schimel, 1995). Understanding soil C dynamics is, therefore, important as they can have a major influence on the global C cycle, the regulation of atmospheric CO_2 concentration and climate change (Grace and Rayment, 2000; Cox et al., 2000). The rate at which C accumulates in soil is a balance between the inputs of C from vegetation (directly through rhizodeposition or via mycorrhizal fungi, or indirectly via leaf litter decomposition and root turnover) and losses due to soil respiration. Soil respiration comprises two main components: (i) autotrophic respiration, Ra, from roots and their associated mycorrhizal fungi, including respiration from other microbes in the rhizosphere directly dependent on labile C leaked from roots; (ii) heterotrophic respiration, Rh, due to the breakdown of soil organic matter (SOM). Being able to partition soil respiration into Ra and Rh is important, therefore, for understanding the processes regulating SOM turnover and, ultimately, whether soil in a particular ecosystem will become a net source or sink for C with changes in climate or land use (Millard et al., 2007).

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A range of methods has been used to partition the soil surface CO_2 efflux into Ra and Rh (Hanson et al., 2000; Trumbore, 2005; Kuzyakov, 2006; Subke et al., 2006). These have included experiments to exclude root respiration and so eliminate Ra, by girdling trees (Bhupinderpal-Singh et al., 2003; Subke et al., 2004; Scott-Denton et al., 2006), or trenching to remove roots (Jiang et al., 2005; Sulzman et al., 2005; Tang et al., 2005). Alternatively, elevated CO_2 supplied with its own unique $\delta^{13}C$ signature has been used as a tracer in labelling experiments (Andrews et al., 1999; Lin et al., 1999; Pendall et al., 2001; Søe et al., 2004; Trueman and Gonzalez-Meler, 2005) or at ambient concentrations in short term experiments with small trees (Lin et al., 1999; Phillips and Fahey, 2005). All of these approaches introduce a significant disturbance into the system (Hanson et al., 2000; Kuzyakov, 2006), which can affect soil respiration directly. This is particularly the case for elevated CO_2 studies, which accelerate soil respiration due to priming of the ecosystem with additional C (King et al., 2004). An alternative isotopic approach has been the use of ^{13}C natural abundance discrimination, utilising differences in the $\delta^{13}C$ signature of SOM and plant roots to partition the soil surface CO_2 efflux into Ra and Rh. Using this approach C_3 plants have been grown in pots with soil originating from under C_4 vegetation (Fu and Cheng, 2002) or by transplanting C_4 soil into a forest (Susfalk et al., 2002). In agricultural systems a C_4 crop (maize) has been grown in soil from under C_3 vegetation (C_3 soil) in the field to produce the necessary isotopic differences (Rochette and Flanagan, 1997).

Use of ^{13}C natural abundance discrimination to partition soil respiration in undisturbed ecosystems relies on the presence of a natural difference in the $\delta^{13}C$ signature of SOM and plant roots, which Kuzyakov (2006) and Hanson et al. (2000) have suggested is unusual in natural ecosystems and, therefore, a limitation to the use of the technique. However, savannah ecosystems comprise a dynamic mixture of C_3 trees and C_4 grassland and at the global scale cover an area of some 33 million km^2 (Beerling and Osborne, 2006). In these biomes woody cover is a main determinant of ecosystem properties (Sankaran et al., 2005) and, depending on the precipitation, can either decrease (at wetter sites) or increase (at dryer sites) the amount of SOM when woody vegetation invades grassland (Jackson et al., 2002). For example, in the subtropical Rio Grande Plains of Southern Texas, during the last 150 years C_3 trees and shrubs have invaded areas previously dominated by C_4 grasses, to produce a savannah ecosystem (Archer, 1995). This has resulted in significant changes in soil biogeochemistry, with an increase in SOM (McCully et al., 2004) and depletion in the soil $\delta^{13}C$ signature under the trees compared with the grassland (Boutton et al., 1998, 1999). This makes the ecosystem ideal for using the differences in natural gradients in soil $\delta^{13}C$ signature of Ra and Rh to partition their contribution to total soil surface CO_2 efflux.

The approach taken was to measure soil respiration along a short transect from under C_3 trees into the C_4 dominated grassland in a savannah ecosystem in Texas. Because soil respiration rate can vary considerably in relation to soil moisture (Tedeschi et al., 2006) and the site chosen for the study was experiencing a prolonged drought, an irrigation treatment was applied at two positions in the transect. Soil surface CO_2 efflux was measured along the transect and respired CO_2 collected for analysis of the $\delta^{13}C$ signature in order to: (i) determine how soil respiration rates varied along the transect and were affected by localised change in soil moisture and (ii) partition the soil surface CO_2 efflux into Ra and Rh using a mass balance model (Lin et al., 1999), which required measurement of the $\delta^{13}C$ signature of root- and soil-derived CO_2. The $\delta^{13}CO_2$ value of soil (Ehleringer et al., 2000) and root (Klump et al., 2005) respiration can differ from that of the solid material and can also vary with the length of incubation (Klump et al., 2005). Therefore, as a preliminary to partitioning the soil surface CO_2 efflux, it was also necessary to determine if there was a difference between the $\delta^{13}C$ signature of respired CO_2 and solid samples of soil and roots and establish the conditions for measuring their $\delta^{13}C$ signature.

2. Materials and methods

2.1. Site description and transect layout

The soil respiration measurements were made at the Texas Agricultural Experimental Station La Copita Research Area (lat. 27°40′N, long. 98°12′W, elevation 75 m) in southern Texas. The site is a subtropical savannah ecosystem that has been described extensively before (Boutton et al., 1998, 1999; Hibbard et al., 2001; Liao et al., 2006). The mean annual temperature is 22.4 °C and the mean annual precipitation is 716 mm, with rainfall peaks in May–June and September. The soil is a sandy-loam (Typic and Pachic Argiustolls) and the current vegetation is C_4 grassland (dominated by *Paspalum*, *Bouteloua*, *Chloris* and *Eragrotis* species, but also containing a number of C_3 forbs (Boutton et al., 1999)), interspersed with small, discrete clusters of woody, C_3 plants. The N_2-fixing tree legume, *Prosopis glandulosa* (Torr.) var. *glandulosa* (honey mesquite), is the first woody plant to colonise and subsequently facilitates recruitment of other woody plants beneath its canopy (such as *Condalia hookeri* (M.C. Johnst.), *Celtis pallida* (Torr.), *Zanthoxylum fagara* (L.), *Diospyros texana* (Scheele.), and *Mahonia trifoliolata* (Moric.) (Fedde)) (Boutton et al., 1999). These thorny clusters expand laterally to form larger groves of woody vegetation, producing a mosaic of woody vegetation within the grassland. As a result, there is a variation in the $\delta^{13}C$ signature of the soil, which under the woody clusters reflects the C_3 vegetation, but in the grassland is intermediate between a typical C_3 and C_4 signature (Boutton et al., 1999).

In June 2006 six replicate tree clusters were selected on the basis of the similarity of their age (as assessed by the basal diameter of the *P. glandulosa* trunk). For each cluster a series of six soil respiration collars were positioned as shown in Fig. 1. Each transect had four positions, which were: (i) adjacent to the trunk of the *P. glandulosa* tree (bole); (ii) half way between the tree trunk and the edge of the tree canopy (mid canopy); (iii) at the edge of the tree canopy, adjacent to the grassland (drip line); and (iv) in the grassland. The length of each transect varied from 4.5 to 9.0 m, depending upon the size and shape of the tree canopy and the nearby presence of grassland with C_4 species. At both the mid canopy and drip line positions an additional ring was positioned for irrigation, by applying 792 cm^3 of water to a collar, the equivalent of 50 mm of rain, to simulate a subtropical rainstorm. Irrigation was applied 6 h before the respiration measurements commenced, to allow for the physical displacement of CO_2 from soil pores as water moved down the soil profile. Preliminary measurements showed that this occurred as a peak in CO_2 efflux from the soil surface for up to some 2–3 h after irrigation (Boutton, personal communication).

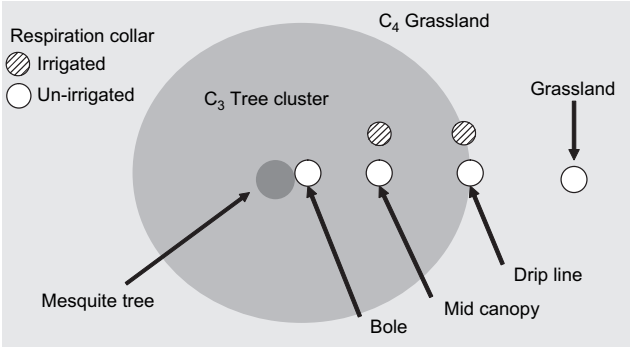

Fig. 1. The positioning of the soil respiration collars (○) in a transect from the central tree bole of a cluster into the surrounding grassland, including the two collars in each transect that were irrigated (▨).

2.2. Measurement of soil respiration and soil analyses

An open path, continuous flow chamber, based on the design by Fang and Moncrieff (1996) was used to measure and sample the soil surface CO_2 efflux. Air was supplied to the chamber from a tank of compressed medical grade air with a known CO_2 partial pressure and isotope ratio. A needle valve and flow meter were used to control the inlet airflow at about 13 $cm^3 s^{-1}$. Air was drawn out at the same rate using a small pump (TD-4x2NA(10), Brailsford & Co. Inc, Rye, NY, USA) and a mass flow controller (max. 33 $cm^3 s^{-1}$, FMA5516, Omega Engineering Inc., USA). Low CO_2 absorption tubing was used throughout the sampling system. Immediately before the chamber, a sample of inlet air (0.3 $cm^3 s^{-1}$) was directed through an absolute infrared gas analyser (Gashound Li-840, Li-Cor, Lincoln, NB, USA) and into the reference side of a differential CO_2/H_2O analyser (Li-6262, Li-Cor, Lincoln, NB, USA) for measurements of CO_2 and water vapour partial pressure, then exhausted to waste. The air exiting the chamber flowed through the sample cell of the same differential analyser and then exhausted to waste through a separate tee-valve. The development of laminar flow inside the chamber was prevented by a four-paddle fan (Igarashi IG33, Omega Engineering Inc., USA) suspended from the top of the chamber and rotating at 5 rpm.

The chamber fitted onto a circular steel soil respiration collar (diameter = 0.142 m), placed permanently in the soil to a depth of 25 mm. Each collar had an external water trough to form an airtight seal with the chamber. Before the chamber was positioned the inflow and outflow rates were changed to reduce the differential pressure between inside and atmospheric to below 0.1 Pa, to avoid significant advection of CO_2 from the soil (Fang and Moncrieff, 1996). The CO_2 partial pressure increased within the chamber and was monitored until it reached a steady-state (typically 60 min to reach between 7 and 20 Pa above the inlet partial pressure). After a steady-state CO_2 partial pressure was reached, the CO_2 and water vapour partial pressures, flow rate, soil and chamber temperatures and atmospheric air pressure were measured, then the inlet and soil respired air were sampled downstream from the differential CO_2 analyser. Rates of soil surface CO_2 efflux were calculated from the change in constituent concentrations before (absolute analyser) and after the chamber (differential analyser) following Pearcy et al. (1994).

The reference and sample airflows were directed through a drying agent (magnesium perchloride) and into separate 4 dm^3 enema bags, made of clear polyethylene with zip-lock closures (Allegiance Healthcare Corp., IL, USA). The gas was then sampled from the bags for isotopic analysis. Before closing the bags to sample the gas, five glass, 10 cm^3 septum capped vials (Labco, Ltd., High Wycombe, Bucks, UK) were placed inside. All vials were pretreated by heating the caps and septa to ensure stability of the $\delta^{13}CO_2$ signature (Midwood et al., 2006) and pre-purged of CO_2. The bags were flushed three times with air from the chamber and then the vial caps were removed inside the sealed bag and a 50 mm extension tube inside the enema bag used to flush fill each vial. Because the vials were opened and resealed inside the bag there was no possibility of contamination or loss of seal integrity. The $\delta^{13}CO_2$ signature of the soil efflux was calculated using a mass balance approach by knowing the signature of the air before (reference) and after passage through the chamber (sample).

After soil respiration rates had been measured and the soil surface CO_2 efflux sampled, two soil cores (10 cm diameter, 30 cm depth) were taken from each of the respiration rings and a third just adjacent to the ring. The first was used for sub-sampling roots and soil for determining the $\delta^{13}C$ signature of their respired CO_2. A sub sample of soil was also collected for measuring the $\delta^{13}C$ signature and total C content of the whole soil. The second soil core was cut into 25 mm lengths and volumetric water content of the soil was assessed by weighing before and after drying in an oven at 105 °C for 12 h. The third core was stored in a plastic bag at 4 °C until the root biomass was measured by washing the soil through a 0.5 mm screen, then drying and weighing the roots.

2.3. End member incubations and isotope analysis

Soil and root samples were incubated, in order to sample and measure the $\delta^{13}C$ signature of their respired CO_2 (the two 'end member' values used in the mass balance model to calculate Rh and Ra). A sub sample of roots (~0.7 g) and root-free soil (~3.0 g) was separated from the soil core and transferred to separate vials which were then flushed with CO_2-free air at a flow rate of 2.5 $cm^{-3} s^{-1}$ for several minutes. The vials were then incubated at room temperature and the CO_2 produced transferred using a peristaltic pump to a second vial to await analysis, as described by Midwood et al. (2006). As respiration rates were slow in the non-irrigated soils, several vials containing soil (up to five) were linked together with this system and the combined CO_2 production collected in a single receiving vial. As the incubations were kept as short as possible (consistent with having sufficient CO_2 for analysis) this approach also avoided the potential for very high partial pressures of CO_2 developing in the vial prior to analysis and ensured that the gas concentration did not exceed the bounds of linearity of the mass spectrometer system (Midwood et al., 2006).

Samples were shipped from Texas to Aberdeen (UK) for isotope analyses. Carbon isotope ratios were measured using an isotope ratio mass spectrometer (Thermo Finnigan Delta Plus^{XP}) interfaced to a Gas Bench II and PAL autosampler (Thermo Finnigan, Bremen, Germany); all isotope values are expressed as $\delta^{13}C_{V\text{-}PDB}$, defined as:

$$\delta^{13}C = \left[\left(R_{sample}/R_{standard}\right) - 1\right]1000 \quad (1)$$

where R was measured as the $^{13}C/^{12}C$ of the sample or standard. Typical precision for isotope analysis of compressed air was $-9.61 \pm 0.16‰$ (mean ± sd, $n = 28$). Solid sample $\delta^{13}C$ signatures and total C were measured using an isotope ratio mass spectrometer (Thermo Finnigan Delta Plus^{advantage}) interfaced to an elemental analyser (Thermo FlashEA1112, Thermo Finnigan, Bremen, Germany). Long term precision for quality control standards (milled flour) were: total C 39.9 ± 1.16% (mean ± sd, $n > 600$), $\delta^{13}C$ $-25.8 \pm 0.32‰$ (mean ± sd, $n > 600$).

2.4. Isotope mass balance calculations

The proportion of C_4 roots in a sample (F_{C_4}) from any point in the transect was calculated using the mass balance equation:

$$F_{C_4} = (\delta^{13}C_{sample} - \delta^{13}C_{C_3})/(\delta^{13}C_{C_4} - \delta^{13}C_{C_3}) \quad (2)$$

where the subscript samples, C_3 and C_4 were the $\delta^{13}C$ values of the whole root sample, C_3 roots and C_4 roots, respectively. These latter two values were assumed to be −29.4 and −14.0‰, respectively (Boutton et al., 1999).

The proportion of the soil surface CO_2 efflux due to the autotrophic respiration, A, was calculated using the approach taken by Lin et al. (1999):

$$A = \frac{\delta^{13}C_{R\text{-}soil} - \delta^{13}C_{R\text{-}SOM}}{\delta^{13}C_{R\text{-}root} - \delta^{13}C_{R\text{-}SOM}} \quad (3)$$

where the subscripts R-soil, R-SOM and R-root refer to the CO_2 respired from whole soil (and sampled from the chamber) and CO_2 sampled after incubation of the soil without roots and the roots, respectively.

3. Results

3.1. Soil characteristics and respiration along the transect

The soil at the site was unusually dry due to the prolonged drought (Fig. 2). Despite June normally being one of the wettest months of the year, there had been no measurable precipitation on site for the previous 5 months. The mean volumetric soil water content between 0 and 0.3 m depth was 8.2%. Across the transect there was a gradient in soil $\delta^{13}C$ signature (Fig. 3A), which increased from $-22.7 \pm 0.64‰$ (mean ± SE) at the tree bole to $-16.4 \pm 0.44‰$ in the grassland. This was a consequence of the switch from predominantly C_3 photosynthates supplying carbon to roots under the tree to a mixture of C_3/C_4 photosynthesis in the grassland, as shown by the ^{13}C signature of the roots (Fig. 3C). The value of $-19.9‰$ for the grassland roots was used to calculate F_{C_4} (Eq. (1)) with approximately 60% of the roots in the grassland being C_4 grass roots and the remainder C_3 forbs. The establishment of the trees also increased the organic C content of the soil, doubling from the grassland to under the tree (Fig. 3B). Root biomass was also much greater under the tree than in the grassland and declined quickly when moving away from the tree bole (Fig. 3D).

Soil respiration rates were fastest at the tree bole and slowest at the drip line (Table 1). There was a positive but weak linear relationship between soil respiration rate and soil organic C, with an r^2 of 0.454. There was no significant relationship between root biomass and soil respiration rate, due to the variability in the root biomass data under the trees (Fig. 3D), where thick roots were occasionally sampled.

3.2. Effect of irrigation on soil respiration

Irrigating the respiration collars produced soils that, 6 h later, were significantly ($p < 0.01$) wetter than the non-irrigated controls, down to a depth of 0.15 m (Fig. 2). As a consequence there was a 7–11 fold increase in the soil respiration rate of the irrigated compared to the non-irrigated collars at both the mid canopy and the drip line (Table 1).

3.3. Soil and root incubations

In order to determine if there was a difference between the $\delta^{13}C$ signatures of CO_2 respired from soil and roots and the total C content of the solid material, samples were taken along each transect and incubated as described above (Fig. 3A and C). For roots, there were no statistically significant differences ($p > 0.5$) between the $\delta^{13}C$ signature of solid material and respired CO_2. There was little difference between solid and respired CO_2 signatures in the soil under the trees, but significant ($p < 0.01$) differences at the drip line and in the grassland soil. The length of time of incubation was also studied as a possible factor affecting the $\delta^{13}CO_2$ signature of soil and root respiration (Table 2). The $\delta^{13}C$ signature of respired CO_2 did not vary significantly over time from the roots sampled under the trees, or in the grassland (except after 1 h when the grass root CO_2 was enriched with ^{13}C). However, the $\delta^{13}C$ signatures became more variable through time, as shown by the increased standard errors of the means. For the soil there was no significant difference ($p > 0.5$) in the $\delta^{13}C$ signature of respired CO_2 from grassland soil through time, but the CO_2 from the mid canopy soil became significantly ($p < 0.1$) enriched with ^{13}C. As a consequence, incubation times for root and soil end members were kept constant across the transect (so any errors were constant) and minimised to 30 min for roots and 2 h for soils, thereby providing enough CO_2 for the isotopic analyses.

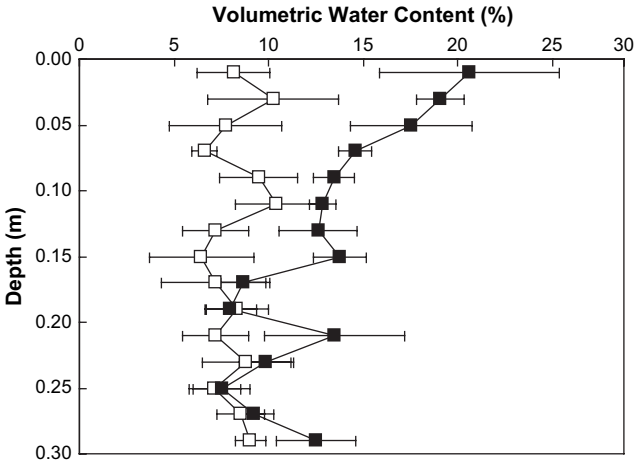

Fig. 2. The effect of irrigation on the volumetric water content of soil at the mid canopy position 6 h after irrigation. Values are for non-irrigated (□) and irrigated soil (■) and are the mean (±1 SE) of three replicates.

3.4. Partitioning soil respiration

Table 3 shows the $\delta^{13}C$ value of soil surface CO_2 efflux at each position in the transect. With the exception of one transect, the non-irrigated soils were all enriched with ^{13}C relative to both the whole soil and whole root $\delta^{13}C$ signatures (Fig. 3). Their $\delta^{13}C$ values were also more variable, as shown by the larger standard errors of the means compared with those from the irrigated rings (Table 3). As a result of the $\delta^{13}C$ value of soil surface CO_2 efflux not lying between the two end member values, it was not possible to partition the soil surface CO_2 efflux from the non-irrigated rings. In contrast, the irrigated rings had $\delta^{13}CO_2$ values for the soil surface efflux which allowed partitioning (Table 4). The heterotrophic respiration comprised 51 ± 13.5% of the soil surface CO_2 efflux at the mid canopy position and 57 ± 7.4% at the drip line. This compared to the value of 40% for the single, non-irrigated ring at the drip line for which it was possible to calculate the partitioning.

4. Discussion

4.1. The response of soil respiration to irrigation

Soil at the La Copita site was very dry during the sampling campaign (ranging from 5 to 12% volumetric water content). In such dry soil, moisture limits the overall rate of respiration, which is stimulated more by irrigation under the clusters than in the grassland (McCully et al., 2007). In our study irrigation under the clusters increased soil respiration rate 7–11 fold, but was unlikely to have affected Ra and Rh to the same extent. Rh responds to very small rain events, but the relationship between pulse size and duration of activity likely saturates at moderate rain events (Huxman et al., 2004). In contrast, Ra generally increases following relatively larger rain events or series of small events (Huxman et al., 2004). However, although our experiment simulated a large rain event, it did so at a very localised scale (within an individual respiration collar). It is likely, therefore, that the main response to irrigation would have been an increase in Rh, while Ra would respond to larger rain events; this would be due to increases in canopy photosynthesis and C allocation to roots, unlikely to be found by irrigation on such a localised scale. This is borne out by our data, which showed that in the irrigated collars, Rh comprised over half the total soil surface CO_2 efflux. This is at the upper end of the values found in the few other studies of forest systems that have used non-invasive, isotopic methods to partition soil respiration.

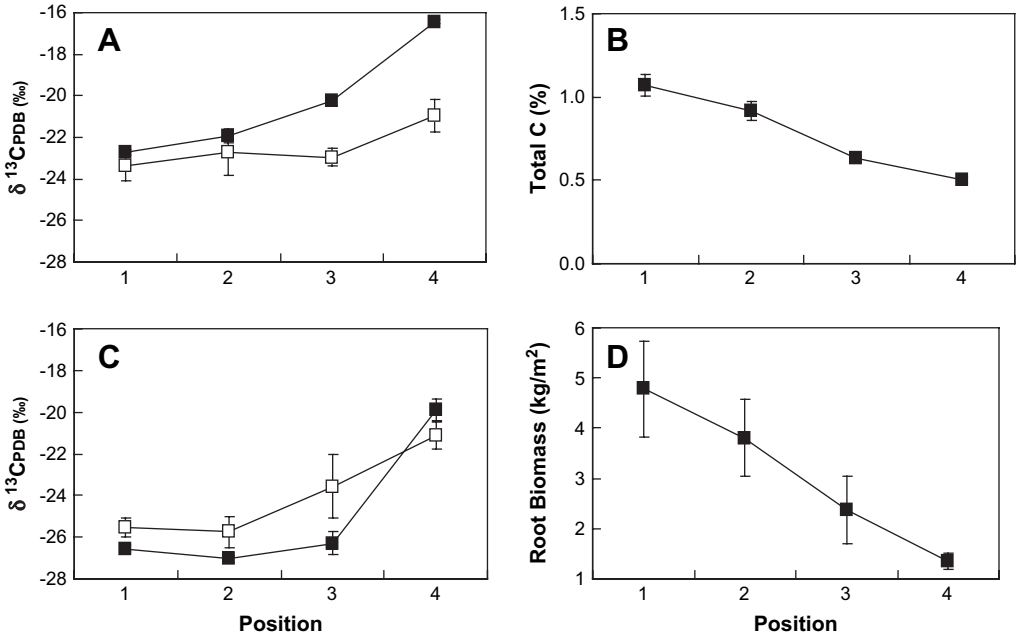

Fig. 3. The effect of position within the transect on: (A) the $\delta^{13}C$ of whole soil (□) and soil respired CO_2 (■); (B) whole soil organic C (%); (C) the $\delta^{13}C$ of whole roots (□) and root respired CO_2 (■); and (D) root distribution ($kg\,m^{-2}$). The positions were: (1) the tree bole; (2) the mid canopy; (3) the drip line; and (4) in the grassland, as shown in Fig. 1. Values are mean (±1 SE) from six replicate clusters.

4.2. Partition of soil efflux using natural isotope discrimination

Recently there has been an increase in the use of isotopic methods in natural systems to partition soil respiration. Natural abundance ^{14}C studies have used changes in the atmospheric radiocarbon values over the last few decades to partition Ra and Rh and to calculate the contribution of SOM turnover to soil respiration (Schuur and Trumbore, 2006). A transect study in the Sierra Nevada used radiocarbon signatures to calculate Rh between 20 and 50% of soil respiration in the summer (Wang et al., 2000). Subsequently, radiocarbon signatures have been used to estimate Rh in boreal black spruce forest in Alaska as 47–63% total soil respiration (Schuur and Trumbore, 2006) and as 20–50% in Manitoba, Canada (Czimczik et al., 2006) and 29–57% in a temperate, deciduous mixed forest in Michigan (Borken et al., 2006). These values are similar at the upper range to those we calculated for Rh in the irrigated respiration collars (51–57%).

We have demonstrated, at a site undergoing a transition from C_4 grassland to mixed C_3/C_4 vegetation, that natural differences in isotope signature between current autotrophic C inputs and SOM were sufficient to allow partitioning of the soil surface CO_2 efflux in Ra and Rh; albeit only for collars which had been irrigated. At our site the differences between the $\delta^{13}C$ of the respired CO_2 from the autotrophic and heterotrophic end members for the non-irrigated collars ranged between 0.2 and 8.8‰, with an averaged difference of 2.6‰. For the irrigated collars the differences ranged between 0.7 and 5.2‰ with an average difference of 2.6‰ and were not significantly different from the non-irrigated rings.

4.3. Soil and root incubations

Partitioning soil efflux using isotope techniques relies on defining the isotopic signature of the end members. A number of isotope studies aimed at partitioning soil efflux have used the $\delta^{13}C$ signature of the total C content of root biomass and soil to define these end members (Rochette and Flanagan, 1997; Lin et al., 1999; Søe et al., 2004). However, the use of the total C isotope values may not always be good proxies for the $\delta^{13}C$ of respired CO_2. Klump et al. (2005) measured $\delta^{13}C$ differences in excess of 5‰ between root biomass and respired CO_2 in perennial ryegrass. Smaller differences were noted in our study; the overall average difference (in both non-irrigated and irrigated respiration collars) was only 1‰. From the bole to the drip line the respired CO_2 was enriched relative to the root biomass. However, in the grassland the biomass was more enriched than the respired CO_2. Isotopic fractionation during biosynthetic processes within the plant leads to differences between the whole plant biomass and respired CO_2. For example, plant storage compounds such as starches and sugars are typically enriched relative to whole plant biomass (Gleixner et al., 1993), whereas structural compounds such as lignin are depleted (Benner et al., 1987). Changing weather patterns, for example, have been shown to affect the $\delta^{13}C$ of phloem sap at the base of mature trees; a process coupled with variations in canopy photosynthesis

Table 1
The effect of position in the transect and irrigation upon soil surface respiration rates

Position	Respiration rate ($\mu mol\,m^{-2}\,s^{-1}$)	
	Non-irrigated	Irrigated
Bole	1.5 ± 0.18	–
Mid canopy	1.0 ± 0.14	7.1 ± 0.79
Drip line	0.6 ± 0.12	7.0 ± 0.54
Grassland	0.8 ± 0.12	–

Values are the mean (±1 SE) from six replicate transects.

Table 2
The effect of incubation time on the $\delta^{13}C$ signature of respired CO_2 from roots and soil sampled either at the mid canopy position or in the grassland

Time (h)	Mid canopy roots	Grass roots	Time (h)	Mid canopy soil	Grassland soil
0.5	−28.5 ± 0.19	−15.2 ± 0.09	0.5	−23.3 ± 0.30	−17.0 ± 0.75
1	−28.9 ± 0.46	−13.9 ± 0.26	7	−20.6 ± 0.32	−18.1 ± 0.08
2	−29.1 ± 0.32	−14.7 ± 0.60	14	−21.2 ± 0.17	−17.8 ± 0.65
3	−28.1 ± 0.44	−15.2 ± 0.32			

Values are means (±1 SE) of three replicates taken from a single cluster.

Table 3
The effect of position in the transect and irrigation on the $\delta^{13}C$ signature of soil surface CO_2 efflux

Position	Non-irrigated	Irrigated
Bole	-17.7 ± 0.55	–
Mid canopy	-14.6 ± 1.01	-24.8 ± 0.19
Drip line	-15.9 ± 1.39	-24.9 ± 0.35
Grassland	-13.1 ± 0.63	–

Values are the mean (±1 SE) from six replicate transects.

(Barbour et al., 2005). Isotopic fractionation effects within plants are also known to have the potential to result in isotopic disequilibria between current photosynthate supply and respiratory substrates (Pataki et al., 2003). At our site the stable very dry weather patterns probably reduced the difference between the root biomass and respired CO_2, since these conditions would have limited growth and biosynthetic processes. Nevertheless root biomass may well have structural components with different carbon isotope signatures to that of the substrates fuelling respiration, and the magnitude of this difference may vary temporally in response to external factors such as climate.

Soil organic matter is made up of a variety of fractions which decompose at different rates and have different isotopic signatures (Boutton et al., 1999; Crow et al., 2006). In our soils this was exacerbated by the change in vegetation structure. Differences in the $\delta^{13}C$ between the respired CO_2 and total C content of the Texas soils were negligible in the centre of the woody plant cluster, at 0.6‰. However, in the grassland the difference was 4.5‰. We know the fine and coarse clay components of the soil at this study site are associated with highly recalcitrant aliphatic hydrocarbons. These soil fractions have slow turnover rates and relatively enriched $\delta^{13}C$ values, with a range from −18 to −14‰, reflecting the original C_4 landscape (Boutton et al., 1998). More recent carbon inputs are associated with the soil sand fraction and are much more labile. The soil from the grassland respiration collar had a relatively low organic C content (0.5% w/w), a significant proportion of which was attributable to the recalcitrant clay fraction. Thus variances in the soil and respired ^{13}C signatures were probably due to historical, recalcitrant C_4 derived C predominating in the solid samples, while the more recent and labile C originating from C_3 forbs and tree roots provided a significant proportion of the C substrates for respiration, even in the grassland. In the centre of the woody plant cluster the soil total organic C content had increased to 1.5% due to the presence of the woody plants. Much of this C being a relatively recent addition to the soil is associated with the sand fraction and is labile.

One assumption with our approach was that the isotopic values of component respiration sources were not sensitive to the disturbance caused by isolating and separating roots and soil for end member determinations. We tested this by incubating roots and soil over different time periods and monitoring the $\delta^{13}C$ of the respired CO_2. For the roots obtained from the grassland and mid canopy positions little change was detected after incubation for up to 3 h. Högberg et al. (2001) have shown in girdled trees that respiration fuelled by starch reserves can continue for several days at appreciable rates in roots starved of photosynthetic products but with an intact xylem.

Over 14 h, CO_2 respired from soil taken from the mid canopy position became more enriched, whilst for the grassland soil the opposite trend was observed. A range of factors such as microbial community and substrate supply will influence the isotopic signature of soil respired CO_2. Crow et al. (2006) noted marked changes in $\delta^{13}C$ of respired CO_2 with time from incubated soil fractions derived from both a coniferous and deciduous forest soil. Initial isotopic depletion of the respired CO_2 was followed by gradual enrichment but the rate of change was different for different size classes and soil origin. Crow et al. (2006) attributed these observations to a combination of criteria, including organic matter quality, isotopic discrimination by microbes and differences in the microbial community composition. During our experiments the soils were all incubated for just 2 h in an attempt to minimise the impact of any time-dependent changes in isotopic signatures. Our system of linking together tubes of soil (Midwood et al., 2006) meant that by manipulating the amount of soil, sufficient CO_2 for analysis was obtained within the required timeframe.

A number of alternative approaches have been used to determine the end member values. Schuur and Trumbore (2006) in a radiocarbon study incubated intact soil cores for up to 6 days before sampling the CO_2; the authors reasoned that after this time active root respiration has significantly declined so that respired CO_2 is of primarily heterotrophic origin. For our stable isotope study this approach would not have worked well. Even assuming no contribution from the roots, our evidence indicates that the $\delta^{13}C$ of the SOM respired CO_2 changed significantly in less than 24 h.

4.4. Failure to partition soil efflux in dry soil

Without irrigation it was impossible to partition between Ra and Rh, due to the $\delta^{13}C$ value of soil surface CO_2 efflux being more enriched than that of the roots or the SOM. At steady-state conditions the $\delta^{13}C$ of the soil efflux will equal that of the source, a condition imposed by isotopic mass balance and readily demonstrated in two component mixing models (Amundson et al., 1998). Here it appears steady-state conditions were not established before the soil surface CO_2 efflux was sampled. The most likely cause of this was probably twofold: (1) the very dry porous soils at our study site which may have been particularly susceptible to ingress of atmospheric CO_2 with a $\delta^{13}C$ value of approximately −9‰ and (2) very low respiration rates in the non-irrigated soils. Mixing of atmospheric and soil-derived CO_2 has been reported previously as being a problem for partitioning respiration from dry, coarse-textured sandy clay loam soil by Susfalk et al. (2002), who estimated that in summer atmospheric CO_2 contributed up to 35% of the near-surface soil gas at their forest site. Dudziak and Halas (1996) calculated changes of a several permil to a depth of 0.3 m due to the mixing of atmospheric CO_2 in forest soils with low respiration rates. In our study, the soil was porous, being a sandy-loam with 75% sand in the A-horizons down to 0.3 m depth (Boutton et al., 1998). It was also very dry (Fig. 2) due to the prolonged drought. Using a two compartment mixing model (see Eq. (1)) we estimated that in the dry soil, atmospheric CO_2 contributed 41% of the soil surface efflux at the bole, 60% at the mid canopy, 48% at the drip line and 61% in the grassland (assuming source $\delta^{13}C$ value of 50:50, autotrophic and heterotrophic respiration and air value of −9‰). Irrigation produced an initial pulse in CO_2 efflux which was some 10–15 times faster than from non-irrigated soils and then stabilised to the rates

Table 4
Partitioning of the total soil surface CO_2 efflux into autotrophic and heterotrophic components

Position	Treatment	Source of CO_2	Respiration rate ($\mu mol\ m^{-2}\ s^{-1}$)
Mid canopy	Irrigated	Autotrophic	3.5 ± 0.83
		Heterotrophic	3.6 ± 1.28
	Non-irrigated	Autotrophic	–
		Heterotrophic	–
Drip line	Irrigated	Autotrophic	3.0 ± 0.34
		Heterotrophic	4.0 ± 0.73
	Non-irrigated	Autotrophic	0.3 (n = 1)
		Heterotrophic	0.2 (n = 1)

Values are the mean (± SE) of six replicates unless indicated in the table.

reported in Table 1 after some 2–3 h (Boutton, personal communication). Much of this initial CO_2 efflux would have been due to displacement of gas from soil pores by the water, which would have had the effect of removing the atmospheric-derived CO_2 from the upper layers of the soil. This, coupled with their faster respiration rate, probably explains why we were successful in partitioning Ra and Rh in the irrigated soil, but not the non-irrigated rings. Further work will be required to establish how this can be avoided in the future; this may simply involve taking more time for the soil efflux collection. Insertion of the respiration collars deeper into the soil surface may also help to reduce lateral CO_2 movement. However, this technique may only be applicable to soils with lower air-filled porosity or higher respiration rates.

5. Conclusion

We have shown that it is possible to use natural abundance isotopic discrimination in an undisturbed ecosystem to partition surface soil CO_2 efflux into Ra and Rh. This technique will provide a powerful new tool to enable the C dynamics of soil respiration to be better understood. This will ultimately provide new insights into how soils will respond to anticipated changes in climate, whether or not they become source or sinks of C and how this ultimately feedbacks to moderating or exacerbating the rate of climate change. This study serves to illustrate a number of key factors to be considered when applying this technique to the partitioning of soil surface CO_2 efflux. The isotope mass balance model should use values based on the $\delta^{13}C$ of respired CO_2, not solid soil and root material and care should be taken to keep the incubation times to a minimum. In dry soils further work is required to overcome the problem of atmospheric air ingress into the upper layers.

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References

Amundson, R., 2001. The carbon budget in soils. Annual Review of Earth and Planetary Sciences 29, 535–562.

Amundson, R., Stern, L., Baisden, T., Wang, Y., 1998. The isotopic composition of soil and soil-respired CO_2. Geoderma 82, 83–114.

Andrews, J.A., Harrison, K., Matamala, R., Schlesinger, W.H., 1999. Separation of root respiration from total soil respiration using carbon-13 labeling during free-air carbon dioxide enrichment (FACE). Soil Science Society of America Journal 63, 1429–1435.

Archer, S., 1995. Tree–grass dynamics in a *Prosopis*-thornscrub savanna parkland – reconstructing the past and predicting the future. Ecoscience 2, 83–99.

Barbour, M.M., Hunt, J.E., Dungan, R.J., Turnbull, M.H., Brailsford, G.W., Farquhar, G. D., Whitehead, D., 2005. Variation in the degree of coupling between $\delta^{13}C$ of phloem sap and ecosystem respiration in two mature *Nothofagus* forests. New Phytologist 166, 497–512.

Beerling, D.J., Osborne, C.P., 2006. The origin of the savanna biome. Global Change Biology 12, 2023–2031.

Benner, R., Fogel, M.L., Sprague, E.K., Hodson, R.E., 1987. Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. Nature 329, 708–710.

Bhupinderpal-Singh, Nordgren, A., Lofvenius, M.O., Höbgerg, M.N., Mellander, P.E., Högberg, P., 2003. Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year. Plant Cell and Environment 26, 1287–1296.

Borken, W., Savage, K., Davidson, E.A., Trumbore, S.E., 2006. Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. Global Change Biology 12, 177–193.

Boutton, T.W., Archer, S.R., Midwood, A.J., Zitter, S.F., Bohl, R., 1998. $\delta^{13}C$ values of soil organic carbon and their use in documenting vegetation change in a subtropical savanna ecosystem. Geoderma 82, 5–41.

Boutton, T.W., Archer, S.R., Midwood, A.J., 1999. Stable isotopes in ecosystem science; structure, function and dynamics of a subtropical savanna. Rapid Communications in Mass Spectrometry 13, 1263–1277.

Cox, P.M., Betts, R.A., Jones, C.D., Spall, S.A., Totterdell, I.J., 2000. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. Nature 408, 184–187.

Crow, S.E., Sulzman, E.W., Rugh, W.D., Bowden, R.D., Lajtha, K., 2006. Isotope analysis of respired CO_2 during decomposition of separated soil organic matter pools. Soil Biology and Biochemistry 38, 3279–3281.

Czimczik, C.L., Trumbore, S.E., Carbone, M.S., Winston, G.C., 2006. Changing sources of soil respiration with time since fire in a boreal forest. Global Change Biology 12, 1–15.

Dudziak, A., Halas, S., 1996. Diurnal cycle of carbon isotope ratio in soil CO_2 in various ecosystems. Plant and Soil 183, 291–299.

Ehleringer, J.R., Buchmann, N., Flanagan, L.E., 2000. Carbon isotope ratios in belowground carbon cycling processes. Ecological Applications 10, 412–422.

Fang, C., Moncrieff, J.B., 1996. An improved dynamic chamber technique for measuring CO_2 efflux from the soil surface. Functional Ecology 10, 297–305.

Fu, S.L., Cheng, W.X., 2002. Rhizosphere priming effects on the decomposition of soil organic matter in C_4 and C_3 grassland soils. Plant and Soil 238, 289–294.

Gleixner, G., Danier, H.-J., Werner, R.A., Schmidt, H.-L., 1993. Correlations between the ^{13}C content of primary and secondary plant products in different cell compartments and that in decomposing basidiomycetes. Plant Physiology 102, 1287–1290.

Grace, J., Rayment, M., 2000. Respiration in the balance. Nature 404, 819–820.

Hanson, P.J., Edwards, N.T., Garten, C.T., Andrews, J.A., 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogeochemistry 48, 115–146.

Hibbard, K.A., Archer, S., Schimel, D.S., Valentine, D.W., 2001. Biogeochemical changes accompanying woody plant encroachment in a subtropical savanna. Ecology 82, 1999–2011.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., Read, D.J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789–792.

Huxman, T.E., Snyder, K.A., Tissue, D., Leffler, A.J., Ogle, K., Pockman, W.T., Sandquist, D.R., Potts, D.L., Schwinning, S., 2004. Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. Oecologia 141, 254–268.

Jackson, R.B., Banner, J.L., Jobbágy, E.G., Pockman, W.T., Wall, D.H., 2002. Ecosystem carbon loss with woody plant invasion of grasslands. Nature 418, 623–626.

Jiang, L., Shi, F., Li, B., Luo, Y., Chen, J., Chen, J., 2005. Separating rhizosphere respiration from total soil respiration in two larch plantations in northeastern China. Tree Physiology 25, 1187–1195.

King, J.S., Hanson, P.J., Bernhardt, E., DeAngelis, P., Norby, R.J., Prequitzer, K.S., 2004. A multiyear synthesis of soil respiration responses to elevated atmospheric CO_2 from four forest FACE experiments. Global Change Biology 10, 1027–1042.

Klump, K., Schäufele, R., Lötscher, M., Lattanzi, F.A., Schnyder, H., 2005. C-isotope composition of CO_2 respired by shoots and roots: fractionation during dark respiration? Plant Cell and Environment 28, 241–250.

Kuzyakov, Y., 2006. Sources of CO_2 efflux from soil and review of partitioning methods. Soil Biology and Biochemistry 38, 425–448.

Liao, J.D., Boutton, T.W., Jastrow, J.D., 2006. Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. Soil Biology and Biochemistry 38, 3184–3196.

Lin, G.H., Ehleringer, J.R., Rygiewicz, P.T., Johnson, M.G., Tingey, D.T., 1999. Elevated CO_2 and temperature impacts on different components of soil CO_2 efflux in Douglas-fir terracosms. Global Change Biology 5, 157–168.

McCully, R.L., Archer, S.R., Boutton, T.W., Hons, F.M., Zuberer, D.A., 2004. Soil respiration and nutrient cycling in wooded communities developing in grassland. Ecology 85, 2804–2817.

McCully, R.L., Boutton, T.W., Archer, S.R., 2007. Soil respiration in a subtropical savanna parkland: response to water additions. Soil Science Society of America Journal 71, 820–828.

Midwood, A.J., Gebbing, T., Wendler, R., Sommerkorn, M., Hunt, J.E., Millard, P., 2006. Collection and storage of CO_2 for ^{13}C analysis: an application to separate soil CO_2 efflux into root- and soil-derived components. Rapid Communications in Mass Spectrometry 20, 3379–3384.

Millard, P., Sommerkorn, M., Grelet, G.-A., 2007. Environmental change and carbon limitation in trees: a biochemical, ecophysiological and ecosystem appraisal. New Phytologist 175, 11–28.

Pataki, D.E., Ehleringer, J.R., Flanagan, L.B., Yakir, D., Bowling, D.R., Still, C.J., Buchmann, N., Kaplan, J.O., Berry, J.A., 2003. The application and interpretation of Keeling plots in terrestrial carbon cycle research. Global Biogeochemical Cycles 17, 1022.

Pearcy, R.W., Ehleringer, J., Mooney, H.A., Rundel, P.W., 1994. Plant Physiological Ecology. Field Methods and Instrumentation. Chapman & Hall, London, 457 pp.

Pendall, E., Leavitt, S.W., Brooks, T., Kimball, B.A., Pinter, P.J., Wall, G.W., LaMorte, R.L., Wechsung, G., Wechsung, F., Adamsen, F., Matthias, A.D., Thompson, T.L., 2001. Elevated CO_2 stimulates soil respiration in a FACE wheat field. Basic and Applied Ecology 2, 193–201.

Phillips, R.P., Fahey, T.J., 2005. Patterns of rhizosphere carbon flux in sugar maple (*Acer saccharum*) and yellow birch (*Betula allegheniensis*) saplings. Global Change Biology 11, 983–995.

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

Sankaran, M., Hanan, N.P., Scholes, R.J., Ratnam, J., Augustine, D.J., Cade, B.S., Gignoux, J., Higgins, S.I., Le Roux, X., Ludwig, F., Ardo, J., Banyikwa, F., Bronn, A.,

Bucini, G., Caylor, K.K., Coughenour, M.B., Diouf, A., Ekaya, W., Feral, C.J., February, E.C., Frost, P.G.H., Hiernaux, P., Hrabar, H., Metzger, K.L., Prins, H.H.T., Ringrose, S., Sea, W., Tews, J., Worden, J., Zambatis, N., 2005. Determinants of woody cover in African savannas. Nature 438, 846–849.

Schimel, D.S., 1995. Terrestrial ecosystems and the carbon cycle. Global Change Biology 1, 77–91.

Schuur, E.A.G., Trumbore, S.E., 2006. Partitioning sources of soil respiration in boreal black spruce forest using radiocarbon. Global Change Biology 12, 165–176.

Scott-Denton, L.E., Rosenstiel, T.D., Monson, R.K., 2006. Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. Global Change Biology 12, 205–216.

Søe, A.R.B., Giesemann, A., Anderson, T.H., Weigel, H.J., Buchmann, N., 2004. Soil respiration under elevated CO_2 and its partitioning into recently assimilated and older carbon sources. Plant and Soil 262, 85–94.

Subke, J.-A., Hahn, V., Battipaglia, G., Linder, S., Buchmann, N., Cotrufo, M.F., 2004. Feedback interactions between needle litter decomposition and rhizosphere activity. Oecologia 139, 551–559.

Subke, J.-A., Inglima, I., Cotrufo, F., 2006. Trends and methodological impacts in soil CO_2 efflux partitioning: a metaanalytical review. Global Change Biology 12, 1–23.

Sulzman, E.W., Brant, J.B., Bowden, R.D., Lajtha, K., 2005. Contribution of above ground litter, belowground liter and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest. Biogeochemistry 73, 231–256.

Susfalk, R.B., Cheng, W.X., Johnson, D.W., Walker, R.F., Verburg, P., Fu, S., 2002. Lateral diffusion and atmospheric CO_2 mixing compromise estimates of rhizosphere respiration in a forest soil. Canadian Journal of Forest Research 32, 1005–1015.

Tang, J., Misson, L., Gerhenson, A., Cheng, W., Goldstein, A.H., 2005. Continuous measurements of soil respiration with and without roots in a ponderosa pine plantation in the Sierra Nevada Mountains. Agricultural and Forest Meteorology 132, 212–227.

Tedeschi, V., Rey, A., Manca, G., Valentini, R., Jarvis, P.G., Borghetti, M., 2006. Soil respiration in a Mediterranean oak forest at different developmental stages after coppicing. Global Change Biology 12, 110–121.

Trumbore, S., 2005. Carbon respired by terrestrial ecosystems – recent progress and challenges. Global Change Biology 11, 1–13.

Trueman, R.J., Gonzalez-Meler, M.A., 2005. Accelerated belowground C cycling in a managed agriforest ecosystem exposed to elevated carbon dioxide concentrations. Global Change Biology 11, 1258–1271.

Wang, Y., Amundson, R., Niu, X.-F., 2000. Seasonal and altitudinal variation in decomposition of soil organic matter inferred from radiocarbon measurements of soil CO_2 flux. Global Biogeochemical Cycles 14, 199–212.