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Dual-chamber measurements of $\delta^{13}C$ of soil-respired CO_2 partitioned using a field-based three end-member model

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ABSTRACT

The contribution of old soil C (SOM) to total soil respiration (R_S) in forest has been a crucial topic in global change research, but remains uncertain. Isotopic methods, such as natural variations in carbon isotope composition ($\delta^{13}C$) of soil respiration, are more frequently being applied, and show promise in separating heterotrophic and autotrophic contributions to R_S. However, natural and artificial modification of $\delta^{13}C_{Rs}$ can cause isotopic disequilibria in the soil-atmosphere system generating a mismatch between what is usually measured and what process-based models will predict. Here we report the partitioning of the soil surface CO_2 flux in a warm Mediterranean forest into components derived from root, litter/humus, and SOM sources using a new, three end-member mixing model, and compare this with the conventional partitioning into autotrophic and heterotrophic components. The three end-member mixing model takes into account both the discrimination during CO_2 respiration/decomposition of the three components, as well as the fractions of their CO_2 fluxes integrated over the total soil profile mass. In addition, we used a novel dual-chamber technique to ensure that $\delta^{13}C_{Rs}$ was subjected to minimal artefacts during measurement.

We observed that by using measured soil surface CO_2 concentrations as a baseline level for the dual-chamber operation, it was possible to achieve and monitor the necessary conservation of the soil CO_2 steady-state diffusion conditions during the measurements, without using permanent collars inserted deeply into the soil. When R_S (8.64 g CO_2 m^2 d^{-1}) was partitioned into two components, the mean autotrophic and heterotrophic respiration was 56 and 44%, respectively. When R_S was partitioned using the three-way model, however, roots, litter/humus, and SOM contributed 30, 33, and 37% of the total flux. Our results confirm that to improve the estimates of the partitioning method, it is important to distinguish the fractional contribution of the long-term SOM-derived flux from younger and more labile sources.

1. Introduction

Natural variations in carbon isotope composition ($\delta^{13}C$) provide a powerful tool for studying C dynamics in the soil–plant–atmosphere system (Hanson et al., 2000; Ehleringer et al., 2000; Bowling et al., 2008), including partitioning of the autotrophic and heterotrophic components of the surface flux, R_S. Reliably estimating the heterotrophic component of R_S is crucial for the characterisation of an ecosystem's net C balance. The difference between the C fluxes arising from soil heterotrophic respiration and net primary production defines an ecosystem's net exchange of C, which determines if the system is a net source of, or sink for, atmospheric CO_2. It is therefore crucial to estimate the contributions of individual components of R_S, e.g., CO_2 derived from autotrophic root respiration or from heterotrophic respiration of microbes decomposing recent litter, or old soil organic matter (SOM). Normally, three-way partitioning can be achieved only using two isotopes. Here we report a new approach that combines $\delta^{13}C$ measurements of CO_2 derived from SOM, roots and litter with the corresponding mass-dependent CO_2 fluxes to estimate their relative and absolute contributions to R_S.

Generally, chemical and biochemical processes accumulate the lighter isotope in the product, leaving the substrate enriched in the heavier isotope (Högberg and Ekblad, 1996). This fractionation can differ among biological processes. Recently, Werth and Kuzyakov (2010) reviewed $^{13}C/^{12}C$ fractionations by biotic processes occurring at the root–microbe–soil interface. They reported that organic C in the root tissues of C_3 vegetation are generally ^{13}C-enriched by $1.2 \pm 0.6‰$ compared with the aboveground tissues, whereas the CO_2 respired by roots is depleted by $2.1 \pm 2.6‰$, compared with root organic C. Under C_3 vegetation, microbial CO_2 is enriched by $0.7 \pm 2.8‰$ in comparison to the C in SOM. The causes of $\delta^{13}C$

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variation in respired CO_2 can be summarised as: (i) isotopic effects during the metabolic synthesis of secondary compounds (e.g., lipids, lignin, cellulose); (ii) variation in photosynthetic discrimination against ^{13}C combined with temporal lags in the movement of photosynthates through plant and soil pools; (iii) shifts in microbial $\delta^{13}C$ due to anaplerotic CO_2 fixation; (iv) preferential use of certain respiratory substrates by microbes; and (v) kinetic fractionation of $^{13}C/^{12}C$ during microbial respiration (Tu and Dawson, 2005; Bowling et al., 2008). Most of the C in SOM derives from the microbial degradation of plant litter which consists of polysaccharides and more depleted lignin ^{13}C. Since easily decomposable substances like glucose or sucrose are preferentially decomposed by microbes, the balance between litter-derived compounds retained in the soil has a direct impact, via isotopic mass balance, on the net $\delta^{13}C$ of SOM (Cotrufo et al., 2005). However, the $\delta^{13}C$ of SOM in forest ecosystems generally increases (i.e., becomes more negative) with soil depth by approximately 1–3‰ relative to that of the litter layer (Nadelhoffer and Fry, 1988). The reasons for this are still unclear (Boström et al., 2007). Possible explanations include the accumulated influence of isotopic discrimination during selective microbial decomposition of specific substrates within the SOM (Nadelhoffer and Fry, 1988; Buchmann et al., 1997; Lin et al., 1999), and more importantly by the increased contribution of microbially derived C to SOM with depth (Ehleringer et al., 2000; Högberg et al., 2005).

The use of $\delta^{13}C$ for partitioning the contribution of different sources of R_S has the advantage of permitting *in situ* measurements with minimal disturbance to soil and roots (Hanson et al., 2000; Paterson et al., 2009). Its success relies on distinguishing small differences in $\delta^{13}C$ of CO_2 produced by autotrophic and heterotrophic respiration, and it is frequently applied in combination with incubation of excised roots, litter, and SOM samples. Studies of variations in $\delta^{13}C$ of R_S commonly use mass balance equations to estimate the proportions of two (and rarely more than two) C sources that contribute to the soil surface CO_2 flux (Lin et al., 1999; Ngao et al., 2005; Sakata et al., 2007; Millard et al., 2010). Isotopic linear mixing models partition the contributions of $n + 1$ sources, when n isotopically distinct tracers (e.g., $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$) are used as end-members, and assume that source signatures that fall closest to that of the mixture provide the greatest contribution. Factors such as the seasonal variability of the photosynthetic discrimination, as well as environmental conditions such as soil temperature and soil moisture, can vary the differences in $\delta^{13}C$ between CO_2 derived from different C pools, and are therefore important constraints on the partitioning method (Phillips and Gregg, 2001; Trumbore, 2006). The isotopic approach also needs to overcome uncertainties associated with the binary diffusion of $^{12}CO_2$ and $^{13}CO_2$ through the soil's gas-filled pore space and across the soil–air interface. Generally, the slower molecular diffusivity of $^{13}CO_2$ can induce CO_2 in bulk soil to be up to 4.4‰ more enriched in ^{13}C than that released at the soil surface $\delta^{13}C_{Rs}$ (Amundson et al., 1998; Cerling et al., 1991).

Some complications in the interpretation of diffusive flux profiles were discussed by Koehler et al. (2010). Transient conditions in diffusive flux profiles can be caused by time-varying respiratory CO_2 production (Moyes et al., 2010). In addition, changes in atmospheric pressure and wind effects (advection) can alter the diffusion of soil CO_2 (Dudziak and Halas, 1996), From a diffusion experiment involving artificial soil and CO_2 source, Kayler et al. (2008) concluded that non-steady-state effects must be considered in field investigations of $\delta^{13}C_{Rs}$ in soils. However, the correct interpretation of $\delta^{13}C_{Rs}$ data is likely determined by the possibility of using measuring techniques which avoid perturbation of the steady-state diffusion conditions of respiration. Soil surface chambers are the most commonly used technique to measure $\delta^{13}C_{Rs}$. Such approaches include: (i) purging of atmospheric $\delta^{13}C$–CO_2 in the chamber headspace (Flanagan et al., 1996; Buchmann and Ehleringer, 1998), (ii) long-term chamber deployment for $\delta^{13}C_{Rs}$ equilibration (Mora and Raich, 2007), and (iii) the maintenance of chamber headspace at atmospheric CO_2 concentration, [CO_2] (Subke et al., 2004a; Bertolini et al., 2006; Midwood et al., 2008). However, several decades of experience with chamber-based measurements have revealed numerous potential sources of errors in measuring soil respiration (Davidson et al., 2002). Some recent studies on the use of closed- and open-chamber systems reported evidence of perturbation of the $\delta^{13}C$ steady-state diffusion profile and $^{12}CO_2$ and $^{13}CO_2$ gradients (Ohlsson et al., 2005; Nickerson and Risk, 2009; Gamnitzer et al., 2011). In addition, the installation of soil collars several centimetres into the soil, to minimize CO_2 leakage in and out of the chamber (Hutchinson and Livingston, 2001), has raised concerns regarding the possibility of measuring $\delta^{13}C_{Rs}$ beyond the collar insertion depth (Kayler et al., 2008), and their utility in soil respiration partitioning studies (Heinemeyer et al., 2011).

To address these concerns, we developed and used a dual-chamber technique to minimize the artefacts and biases in chamber-based measurements of $\delta^{13}C_{Rs}$. In addition, we aimed to partition the soil surface CO_2 flux into its components derived from root, litter/humus, and SOM sources and, alternatively, into autotrophic and heterotrophic components.

2. Materials and methods

2.1. Background and principles of the dual-chamber development

In general, to correctly measure $\delta^{13}C_{Rs}$ it is essential to maintain unbiased the diffusion conditions of R_S at soil surface. The reason for this assumption is as follows. The mass of ^{13}C is larger than that of ^{12}C and diffuses through the soil at a slower rate leading to a theoretical kinetic fractionation of ^{13}C and ^{12}C. This means that for estimates of $\delta^{13}C_{Rs}$ obtained using gas samples withdrawn from the soil profile, a correction of up to 4.4‰ is necessary to account for this fractionation (Amundson et al., 1998). Although transient changes in soil diffusivity and CO_2 concentration gradient might constantly alter the relative diffusion rates of $^{12}CO_2$ and $^{13}CO_2$ isotopologues, Cerling et al. (1991) demonstrated that if soil respiration is at a diffusive steady-state, the $\delta^{13}C$ of soil surface flux should theoretically match the $\delta^{13}C$ produced within the soil. Thus the measurements made at the soil surface do not need to be corrected for fractionation due to diffusion. This assumption means that a chamber technique used for $\delta^{13}C_{Rs}$ measurements has to achieve and maintain over few hours after its deployment the equilibrium between the soil CO_2 efflux and the R_S captured inside the chamber headspace.

Depending on the type of soil surface chamber used, R_S measurements are generally subject to disturbances which can have a large impact on the certainty of $\delta^{13}C_{Rs}$ estimates and on subsequent source partitioning. Some artefacts and biases in steady-state and non-steady-state respiration chambers measurements were discussed by Davidson et al. (2002). For steady-state chambers, these can be summarized as: (i) lateral and vertical diffusion of CO_2 produced below the chamber base due to the alteration of the diffusion gradient (Hutchinson and Livingston, 2001; Davidson et al., 2002; Nickerson and Risk, 2009); (ii) the risk of pressure differentials between the headspace volume and the surrounding atmosphere due to the improper control of gas flow rates (Hutchinson et al., 2000); (iii) over-pressurization of the headspace volume when pressurized CO_2-free air is forced through to control the CO_2 concentration inside the chambers (Lund et al., 1999); (iv) the exchange of gas through the vent in over-pressurized conditions which can dilute chamber air with ambient air (Longdoz et al., 2000); and (v) the alteration of concentration gradients at the soil surface due to excessive turbulence caused by fans inside the chambers used