Soil Biology & Biochemistry 55 (2012) 40–47

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Soil organic carbon decomposition from recently added and older sources estimated by $\delta^{13}C$ values of CO_2 and organic matter

Johanna Pausch [a,b,*], Yakov Kuzyakov [a]

[a] Department of Soil Science of Temperate Ecosystems, University of Göttingen, Germany
[b] Department of Agroecosystem Research, BayCEER, University of Bayreuth, Universitätsstr. 30, 95440 Bayreuth, Germany

ARTICLE INFO

Article history:
Received 16 January 2012
Received in revised form
4 June 2012
Accepted 6 June 2012
Available online 27 June 2012

Keywords:
CO_2 partitioning
Miller/Tans model
C_3/C_4 vegetation change
Isotopic approaches

ABSTRACT

The production of CO_2 in soil strongly depends on the availability of organic carbon (C) for microorganisms. It is obvious, that C that entered the soil recently is more easily available for microorganisms in comparison to older C. However, only very few approaches allow for a quantitative estimation of the availability of C in relation to the time it is entering the soil. We hypothesized that $\delta^{13}C$ values of CO_2 and of soil organic matter (SOM) after a C_3 to C_4 vegetation change will enable to calculate the relative availability of younger (C_4-derived) and older C (C_3-derived) sources for microorganisms. Soil CO_2 was sampled over one vegetation period at depths of 10, 40–50 and 60–70 cm at three treatments: a C_3 reference (wheat), a C_4/fallow (fallow after one year of maize cropping), and a C_4/C_4 (two years of maize cropping). Based on the $\delta^{13}C$ of CO_2 purified from the admixture of atmospheric CO_2 by the Miller/Tans model and on the $\delta^{13}C$ values of SOM, the contributions of younger and older C sources to CO_2 and SOM were assessed. Depending on the soil depth and the presence of living roots, the contribution of younger C to soil CO_2 ranged from 16 to 50%, but that to SOM was less than 5%. By comparing the contributions of older and younger C to CO_2 and SOM, we found that the relative availability of organics recently introduced into the soil (C_4-derived) was about 7 times higher than the availability of C stabilized in soil for longer than one year (C_3-derived). We concluded that simultaneous analysis of the $\delta^{13}C$ values of both SOM and of CO_2 allows not only for the quantification of the CO_2 sources, but also for the estimation of the availability of soil C pools of different age for microorganisms.

1. Introduction

The availability of soil organic C for microbial decomposition is crucial for many processes within the C cycle since it controls the rate of CO_2 flux to the atmosphere, determines the sources contributing to soil CO_2, affects microbial activity and composition, and reflects C sequestration. Soil organic C consists of various heterogeneous pools which differ in their stability and availability and are characterized by particular turnover rates (Von Lützow et al., 2007; Blagodatskaya et al., 2011). Older, more recalcitrant C pools are less decomposable by microorganisms in comparison to younger C pools (Von Lützow et al., 2006; Jastrow et al., 2007). According to their turnover time various C pools contribute differently to soil CO_2 as the major product of microbial decomposition.

To distinguish between C pools and to determine their contribution to soil CO_2, isotopic tracer techniques have been applied. A vegetation change from C_3 to C_4 plants results in different isotopic composition of young and old C pools, which allows for their separation (Balesdent and Mariotti, 1987). Depending on the photosynthetic pathways, different isotopic ^{13}C fractionations occur during CO_2 assimilation, leading to a distinct isotopic composition of C_3 and C_4 plants (Farquhar et al., 1989). Therefore, when growing C_4 plants on soil originally formed in areas of C_3 vegetation (or vice versa), older (C_3-derived) and younger (C_4-derived) C can be differentiated based on their isotopic differences (Balesdent and Mariotti, 1987).

The first aim of this study was the partitioning of soil CO_2 and SOM into their C sources after the application of a C_3 to C_4 vegetation change. Over one vegetation period soil CO_2 was sampled at depths of 10, 40–50 and 60–70 cm at three treatments: 1) C_3 reference with long term C_3 plant cropping; 2) C_4/fallow with maize in the first year of the experiment, and a bare fallow in the second year; and 3) C_4/C_4 with two years' maize cropping.

A major problem for the evaluation of the isotopic composition of soil CO_2 and the subsequent determination of the contribution of

* Corresponding author. Department of Agroecosystem Research, BayCEER, University of Bayreuth, Universitätsstr. 30, 95440 Bayreuth, Germany. Tel.: +49 921 55 2177; fax: +49 921 55 2315.
E-mail addresses: johanna.pausch@uni-bayreuth.de, j.pausch@gmx.de (J. Pausch).

0038-0717/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2012.06.007

various C sources, is the admixture of atmospheric CO_2 to soil air resulting in the modification of its $\delta^{13}C$ value. This strongly limits the application of $\delta^{13}C$ values of CO_2 for the estimation of CO_2 sources and for the evaluation of their availability for microorganisms (Søe et al., 2004). Removal of atmospheric CO_2 is therefore a prerequisite for analyzing the soil CO_2. For this purpose, Keeling (1958, 1961) and Miller and Tans (2003) suggested different approaches based on two component isotopic mixing lines. When applying the commonly used Keeling plot approach, the isotopic composition of sampled air versus the inverse of the respective CO_2 concentration is used to estimate the *y*-axis intercept equivalent to the $\delta^{13}C$ value of pure soil CO_2. The Miller/Tans model is based on the linear regression between the product of CO_2 concentration and its $\delta^{13}C$ value plotted against the CO_2 concentration. The isotopic composition of pure CO_2 from the soil is then determined as the slope of the regression line. Although the Miller/Tans model has seldom been used before, its important advantage is that the calculated $\delta^{13}C$ values of soil CO_2 are less variable in comparison to the Keeling plot approach, especially if the measured CO_2 concentration varies across a broad range. As soil CO_2 concentrations usually do vary over a broad range (more than two orders of magnitude), we used the Miller/Tans model to remove the admixture of atmospheric CO_2 before calculating the contribution of older (C_3-derived) and younger (C_4-derived) sources to CO_2.

The C_3 to C_4 vegetation change approach has commonly been applied to determine either the sources of CO_2 or of SOM, but has very seldom been used to relate isotopic compositions of CO_2 to that of SOM in a way that allows for a quantitative estimation of the availability of C pools for microorganisms (Flessa et al., 2000; Kuzyakov, 2011). We therefore hypothesized that the purified $\delta^{13}C$ values of CO_2 and the $\delta^{13}C$ values of SOM after a C_3 to C_4 vegetation change can be used to estimate the relative availability of younger and older C sources for soil microorganisms.

2. Materials and methods

2.1. Experimental design

The experimental site was established on an arable field in the north-west of Göttingen, Germany (51°33′36.8″N, 9°53′46.9″E). The soil is a haplic Luvisol the organic carbon of which originates from permanent C_3 vegetation. The main soil properties are presented in Table 1.

A vegetation change in 2009 from C_3 to C_4 crops was used to introduce a distinct ^{13}C signal into the soil. The experimental site and design is described in detail by Kramer et al. (2012). In the first year of the study, plots (24 × 24 m) with maize (*Zea mays* L. cv. Ronaldinio) and reference plots (24 × 24 m) with winter wheat (*Triticum aestivum* L. cv. Julius) were established. Wheat seedlings, sown in October 2008, had been removed from the maize plots with a non-selective herbicide ("Roundup", Monsanto Agrar, Düsseldorf, Germany) before sowing maize in April 2009. Wheat was harvested in August and maize in early November 2009 and the straw was removed from the plots. In the second year of the study, in April 2010, maize (*Z. mays* L. cv. Fernandez) and summer wheat (*T. aestivum* L. cv. Melon) were replanted. A bare fallow area (2 × 5 m) was established within one of the maize plots. This area was manually kept free from vegetation during the growing season and roots from the neighboring plants were severed with a spade up to 20 cm depth every two weeks in order to eliminate lateral root ingrowth. Maize and wheat plants were harvested in November 2010. In this paper, the treatments will be referred to as 'C_3 reference' for the plots with continuous wheat cropping, 'C_4/C_4' for the plots with maize cropping in the first and in the second year of the experiment, and 'C_4/fallow' for the plots with maize cropping in the first year and bare fallow in the second year.

Five soil moisture sensors (EC-5, Decagon Devices, Pullman, USA) were installed at 48 cm depth at the C_3 reference. The water content of the soil was measured every 30 min and displayed as daily average.

2.2. Soil air samplers

To sample all three treatments during the same growing season, sampling was done in 2010, in the second year of maize cropping, after establishing the bare fallow (C_4/fallow). The soil air samplers were installed in April 2010 two weeks before the first sampling, in order to reduce disturbance. The air samplers were constructed according to the principle described by Kammann et al. (2001) with the difference that we did not roll them up to a spiral. The sampler, consisting of a silicone tube (length 14.5 cm, inner diameter 10 mm, wall thickness 3 mm), was sealed at one end with a silicone stopper (length 2 cm). Teflon rings (height 5 mm, inner diameter 8 mm) were placed inside the tube to prevent compression by the overlying soil. The other end of the sampler was connected to a non-permeable polyurethane- (PU) tube (consisting of a 5 cm tube with an inner diameter of 4 mm and a tube with ≤80 cm, depending on the sampling depth, with an inner diameter of 1.8 mm) fitted with a three-way stopcock with a cannula to allow for above soil sampling (Knorr et al., 2008).

The air samplers were installed with four replicates at randomly selected positions within all three treatments (C_3 reference, C_4/C_4, and C_4/fallow), at three depths of 10 cm, 40–50 cm and 60–70 cm. The samplers at 40–50 cm and 60–70 cm were vertically installed into a hole made by a Pürckhauer sampler (groove width 18 mm, ecoTech, Bonn, Germany). The hole was refilled with the soil core after installation and sealed with mud at the surface. Due to a steeper CO_2 concentration gradient in the upper soil depths, the 10 cm samplers were horizontally inserted after excavating a soil core (0.008 m^3). The soil core was placed back and the soil surface was slightly compressed.

2.3. Sampling and analysis

Soil air samples were taken twice per month throughout the maize growing season (from May to October 2010) and once after the maize harvest in November 2010. We sampled soil air by plugging evacuated 5 ml vials into the cannula. The stopcock was carefully opened and soil air was soaked into the vial.

Table 1
Selected properties (±SEM) of the haplic Luvisol determined before the start of the experiment (Kramer et al., 2012, modified). Significant differences between the horizons are indicated by different letters ($P < 0.05$).

Horizon	Depth [m]	pH ($CaCl_2$)	Bulk density [g cm^{-3}]	C_{org} [g kg^{-1}]	Total N [g kg^{-1}]	C/N	$\delta^{15}N$ [‰]	$\delta^{13}C$ [‰]
Ap1	0–0.25	6.0 ± 0.1 a	1.4 ± 0.0 a	12.4 ± 0.4 a	1.3 ± 0.0 a	9.8 a	8.0 ± 0.2 a	−27.4 ± 0.1 a
Ap2	0.25–0.37	6.2 ± 0.1 a	1.6 ± 0.0 b	6.9 ± 1.2 b	0.8 ± 0.1 b	9.2 a	7.9 ± 0.5 ab	−26.5 ± 0.2 b
Btw1	0.37–0.65	6.6 ± 0.1 b	1.7 ± 0.0 c	3.3 ± 0.5 c	0.4 ± 0.0 c	8.9 ab	6.4 ± 0.3 bc	−26.1 ± 0.1 bc
Btw2	>0.65	7.0 ± 0.1 c	1.6 ± 0.0 b	1.8 ± 0.4[a] c	0.3 ± 0.0 c	6.9 b	5.6 ± 0.6 c	−25.5 ± 0.3 c

[a] Small quantities of $CaCO_3$ may occur below 65 cm.

The relative ^{13}C abundances and total C concentrations of the CO_2 in the soil air samples were measured by a gas chromatograph 5890 Series II (Hewlett–Packard, Wilmington, USA) coupled to a Delta V Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, Bremen, Germany) via a Combustion Interface II (Thermo Fisher Scientific, Bremen, Germany).

Soil from each treatment was sampled four times during the growing season (May, June, July and August 2010) from depths of 0–10 cm, 40–50 cm and 60–70 cm using a Riverside auger (inner diameter 5 cm, Eijkelkamp, Giesbeek, The Netherlands). Roots from the 0–10 cm soil depth were separated from the soil and rinsed with deionized water. Soil and root material was dried at 60 °C for three days and homogenized in a ball mill. Relative C isotope abundances and total C contents of root and soil samples were measured using an elemental analyzer (Carlo Erba 1108, Milano, Italy) coupled to a Delta S Gas-isotope Ratio Mass Spectrometer (Finnigan MAT, Bremen, Germany) through a ConFlo III interface (Thermo Fisher Scientific, Bremen, Germany). The standard gases (Australian National University sucrose (ANU) and NBS 19) were calibrated with reference to the international standard (Vienna Pee Dee Belemnite (V-PDB)).

2.4. Calculations and statistics

2.4.1. Miller/Tans model

The $\delta^{13}C$ value of pure soil CO_2 was determined by correcting the measured $\delta^{13}C$ value for the admixture of atmospheric CO_2 based on the Miller/Tans model (Miller and Tans, 2003), using a geometric mean regression (GMR) as suggested for soil CO_2 (Kayler et al., 2010). A GMR through the individual data points of all samplings was calculated for each depth separately for the C_3 reference, C_4/fallow and C_4/C_4 treatments. The slope of the GMR is equivalent to a seasonally integrated $\delta^{13}C$ value of pure soil CO_2. Standard errors for the slope of the GMR were calculated from the respective ordinary least square regression (Sokal and Rohlf, 1995). These standard errors may not completely characterize the uncertainty (Zobitz et al., 2006).

2.4.2. Contribution of recently added C to total soil CO_2 and total SOM

The contributions of older (C_3-derived) and younger (C_4-derived) sources to total soil CO_2 or total SOM were calculated using linear two source isotopic mixing models.

$$\delta^{13}C_t \cdot C_t = \delta^{13}C_{C_3} \cdot C_{C_3} + \delta^{13}C_{C_4} \cdot C_{C_4} \quad (1)$$

$$C_t = C_{C_3} + C_{C_4} \quad (2)$$

$$f_{C_4} = \left(\delta^{13}C_t - \delta^{13}C_{C_3}\right) \Big/ \left(\delta^{13}C_{C_4} - \delta^{13}C_{C_3}\right) \quad (3)$$

$$f_{C_3} = 1 - f_{C_4} \quad (4)$$

where $\delta^{13}C_t$ is the isotopic composition of either total CO_2 or total SOM and C_t is the total CO_2 concentration or total C content of SOM. $\delta^{13}C_{C_3}$ and $\delta^{13}C_{C_4}$ are the isotopic compositions of the C_3 and C_4 sources, respectively. C_{C_3} and C_{C_4} are the CO_2 concentrations or C contents of SOM of the C_3 and C_4 sources. f_{C_3} and f_{C_4} are the proportional contributions of the C_3 and the C_4 source to total CO_2 or SOM.

The C_3 source ($\delta^{13}C_{C_3}$) was either defined by the calculated Miller/Tans $\delta^{13}C$ value of the C_3 reference soil when partitioning total CO_2, or by the $\delta^{13}C$ value of SOM of the C_3 reference soil when partitioning total SOM. For partitioning total CO_2 we used the isotopic composition of the maize roots as C_4 source ($\delta^{13}C_{C_4}$). Apparent ^{13}C fractionations between roots and SOM (F_{SOM}) from the pure C_3 system were assumed to be the same in a pure C_4 system and were therefore applied to maize roots to calculate the isotopic composition of the C_4 source ($\delta^{13}C_{C_4}$) when partitioning SOM:

$$F_{SOM} = \delta^{13}C_{C_3} - \delta^{13}C_{C_3\text{-Root}} \quad (‰) \quad (5)$$

$$\delta^{13}C_{C_4} = \delta^{13}C_{C_4\text{-Root}} + F_{SOM} \quad (‰) \quad (6)$$

where $\delta^{13}C_{C_3\text{-Root}}$ and $\delta^{13}C_{C_4\text{-Root}}$ are the $\delta^{13}C$ values of the wheat and the maize root, respectively.

Standard errors of f_{C_4} and f_{C_3} were calculated as described by Phillips and Gregg (2001).

2.4.3. Relative availability of older and younger C

The relative availability of SOM for soil microorganisms was estimated based on its $\delta^{13}C$ values and on the $\delta^{13}C$ of soil CO_2. In order to evaluate the availability of organics that entered the soil after the start of maize cropping (younger C) and to compare it with the availability of organics that had entered the soil before maize cropping (older C), the ratios of C_4- to C_3-derived C in SOM were related to that in CO_2. The respective C_4/C_3 ratios in SOM and in CO_2 were calculated using linear two source isotopic mixing models (Phillips and Gregg, 2001).

2.4.4. Statistics

The values presented in the figures and tables are given as means ± standard errors of means (±SEM). Significant differences of the soil properties (Table 1) between the horizons were obtained by a one-way analysis of variance (ANOVA) in combination with a *post hoc* unequal *N* HSD test. The slopes of the regression lines calculated by the Miller/Tans model showed *P*-values always lower than 0.001. A Fisher's z-transformation of the correlation coefficient (r) of the Miller/Tans models showed that r was always highly significant ($P \leq 0.001$). *T*-tests were used to evaluate differences between depths and treatments in the isotopic composition of CO_2 as well as of SOM and in the contribution of C_4-derived C to total soil CO_2 and SOM, respectively. Statistical analyses were performed with the statistical package STATISTICA for Windows (version 7.0; StatSoft Inc., OK, USA).

3. Results

3.1. Isotopic composition of soil CO_2 and removal of atmospheric CO_2 admixture

The raw data shows that the CO_2 concentrations of the three treatments C_3 reference, C_4/fallow and C_4/C_4 are in a similar range and display similar dynamics, i.e. the treatment itself had no impact (Fig. 1). Consistently lower CO_2 concentrations were detected at the 10 cm soil depth for all treatments, in comparison to the concentrations at the 40–50 cm and at the 60–70 cm depth, where the concentrations were twice as high. In August, the CO_2 concentration increased at all depths and treatments. This increase can be explained by an increasing soil water content as shown for the C_3 reference at a depth of 48 cm (Fig. 1).

The dynamics of the isotopic composition of CO_2 differed between the treatments (Fig. 1). Whereas the $\delta^{13}C$ values of the C_3 reference at the 40–50 cm and at the 60–70 cm soil depths, ranging from −30‰ to −24‰, did not show a trend over time, the $\delta^{13}C$ values of the C_4/C_4 treatment increased at mid-June and reached values of up to −16‰, and decreased again in August at all depths. The $\delta^{13}C$ of CO_2 in the C_4/fallow treatment decreased over the season due to the absence of new C_4 inputs in the second year,

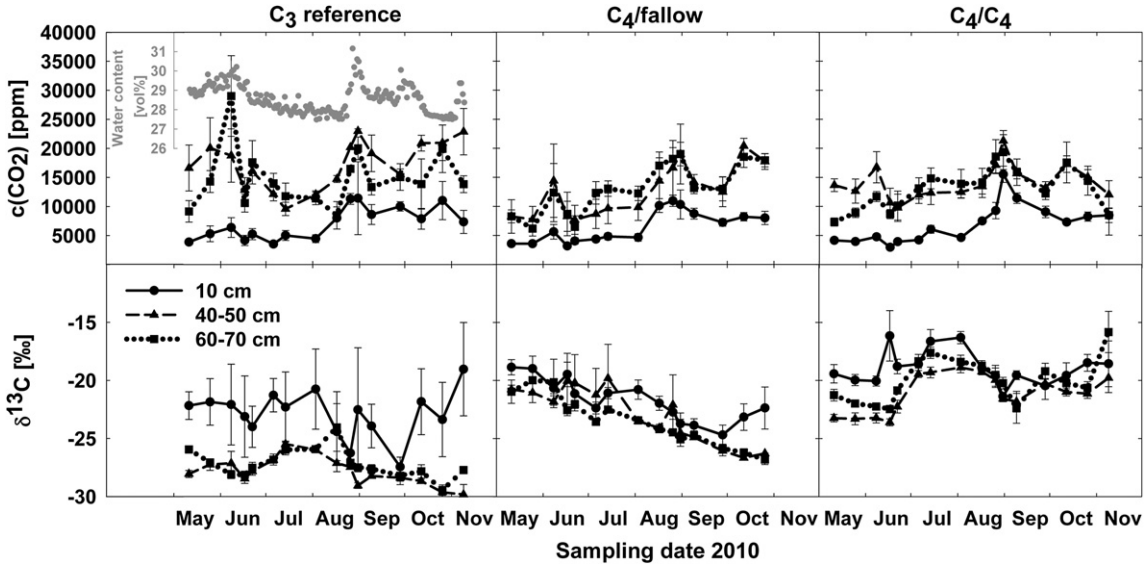

Fig. 1. Top: CO_2 concentration (mean ± SEM, $N = 4$) and bottom: $\delta^{13}C$ values (mean ± SEM, $N = 4$) of total soil CO_2 during the growing season 2010 at 10 cm, 40–50 cm and 60–70 cm depth at the C_3 reference (left), the C_4/fallow (middle) and the C_4/C_4 (right) treatment. Raw data without purification from the admixture of atmospheric CO_2 are presented. The soil water content at 48 cm depth under the C_3 reference is shown in gray color.

and due to the decomposition of the C_4 organics which remained after the first year of maize cropping. At the end of the vegetation period, $\delta^{13}C$ values of −27‰ were detected, which were within the $\delta^{13}C$ range of the C_3 reference. During the vegetation period the $\delta^{13}C$ values of CO_2 of the C_3 reference soil at the depth of 40–50 cm and 60–70 cm were lower compared to the values at the C_4/fallow and the C_4/C_4 treatments. The different dynamics of the $\delta^{13}C$ values indicate changes in the contribution of C_3 and C_4 sources to total CO_2.

For all three treatments, the highest $\delta^{13}C$ values were found at the 10 cm depth, but with the highest variation for the C_3 reference soil. High variation of CO_2 concentrations and especially of $\delta^{13}C$ values, due to the admixture of atmospheric CO_2, confirmed that a correction for the admixture was required to determine the $\delta^{13}C$ of pure soil CO_2. Using the Miller/Tans model the single CO_2 concentration data (shown as means in Fig. 1) was plotted against the $\delta^{13}C$ value multiplied by the CO_2 concentration (Miller and Tans, 2003) (Fig. 2). The best correlations were found for samples taken from the C_3 reference soil because of the highest difference of $\delta^{13}C$ values of organics utilized by microorganisms and atmospheric CO_2. The slope of the regression line equals the integrated $\delta^{13}C$ value of pure soil CO_2 without atmospheric CO_2 (Figs. 2 and 3A).

The purified $\delta^{13}C$ values of soil CO_2 consistently and strongly differed between the three treatments for all depths investigated (Fig. 3A). The lowest $\delta^{13}C$ values were calculated for CO_2 produced at the C_3 reference site. The C_4/fallow treatment represented an intermediate stage and the highest $\delta^{13}C$ values were detected for the C_4/C_4 treatment. Correspondingly to the $\delta^{13}C$ of SOM (Table 2) the $\delta^{13}C$ of CO_2 at the C_3 reference increased slightly with depth, from about −31‰ at the 10 cm depth to −30‰ at the 60–70 cm depth (Fig. 3A). However, there were no depth gradients for the C_4/fallow and the C_4/C_4 treatments with average $\delta^{13}C$ values of approximately −26‰ and −23‰, respectively.

3.2. Contribution of recently added C to total soil CO_2 and SOM and relative availability of younger and older C

Based on two component isotopic mixing lines, the contribution of younger C (C_4-derived) to soil CO_2, after the removal of the admixture of atmospheric air (Fig. 3B) and its contribution to SOM (Table 2) were calculated. The contribution of recently added C to CO_2 at the C_4/C_4 treatment site was approximately 50% at the 10 cm depth, which was twice as high compared to the C_4/fallow treatment. This can be explained by root-derived CO_2 (root and rhizomicrobial respiration) in the presence of living roots in the C_4/C_4 soil.

The contribution of younger C to soil CO_2 decreased with depth for both C_4 treatments. At the 40–50 cm and the 60–70 cm depth of the C_4/C_4 treatment, younger C contributed about 43% to soil CO_2, while at the C_4/fallow treatment only 16% C_4-derived C was detected at the 60–70 cm depth. Despite the high contribution of recently added C to soil CO_2, even at the 60–70 cm soil depth of the C_4/fallow treatment, the bulk SOM was only slightly (<5%) enriched by the C_4 signal after one year of maize cropping (Table 2).

By relating the contribution of recently added C to SOM to its contribution to CO_2, the availability of younger C relative to older C was estimated. This was done only for the 10 cm depth of the C_4/fallow treatment, because of the absence of significant differences in $\delta^{13}C$ of SOM below the plough horizon at 40–50 cm between the C_3 reference and the C_4/fallow treatment (Table 2) and because we cannot completely exclude the influence of carbonates at the 60–70 cm depth (see Table 1). The younger C, introduced into the soil at a depth of 10 cm in the first year of maize cropping, was about 7 times more available for microbial decomposition in comparison to more recalcitrant C which was older than two years.

4. Discussion

4.1. Dynamics of isotopic composition of soil CO_2

The $\delta^{13}C$ values of the measured soil CO_2 of the C_4/C_4 treatment showed a clear pattern over the growing season with increasing values at mid-June (Fig. 1). Similar patterns had been observed in earlier studies (Rochette and Flanagan, 1997; Rochette et al., 1999) and were explained by a higher contribution of root-derived respiration to total soil CO_2 with increasing root biomass during the growing season. At the end of the growing season, the $\delta^{13}C$ values of CO_2 declined again to the initial level due to reduced root-derived respiration.

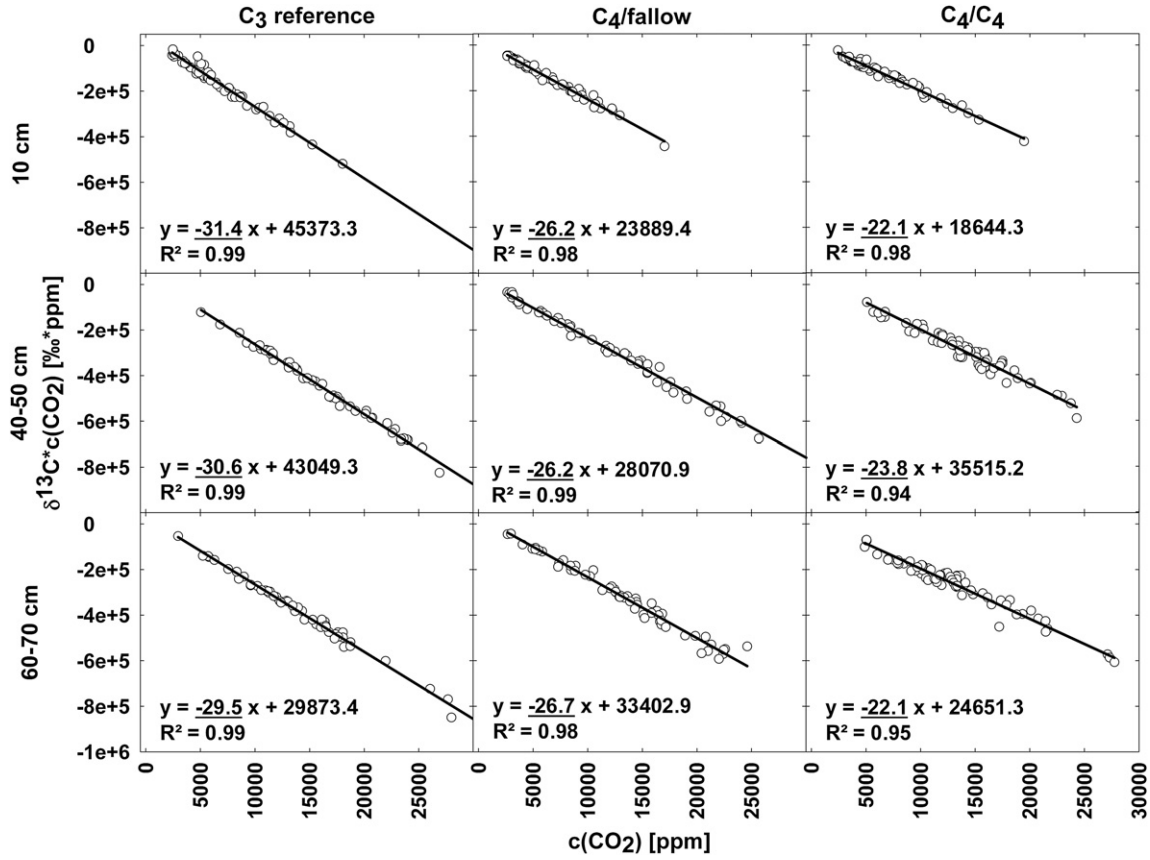

Fig. 2. Miller/Tans models for the C_3 reference, the C_4/fallow and the C_4/C_4 treatment at 10 cm, 40–50 cm and 60–70 cm soil depth. The slope of the regression line is equivalent to the $\delta^{13}C$ of pure soil CO_2 without admixture of atmospheric CO_2. Since the samples were taken over one growing period (2010), the slope represents a seasonally integrated $\delta^{13}C$ value. Note: for a better visualization (not for the calculations), the *x*-axis was truncated at 30,000 ppm, as only 5 points were higher.

The $\delta^{13}C$ of CO_2 in the C_4/fallow soil decreased consistently with time, and converged to the range of the $\delta^{13}C$ of CO_2 measured at the C_3 reference plot. The relative contribution of younger C to soil CO_2 at the C_4/fallow treatment decreased in the absence of new C_4 inputs, because younger (C_4-derived) organics remaining in the soil after the first year of maize cropping had higher availability and, consequently, faster decomposition rates compared to older (C_3-derived) organics. CO_2 produced at the beginning of the vegetation season mainly originated from the compounds with fast decomposition rates, while compounds with lower decomposition rates gained an increasing influence on the CO_2 with time (Werth and Kuzyakov, 2008). This led to a decrease of $\delta^{13}C$ values of soil CO_2 within the C_4/fallow treatment.

The CO_2 measured at the 10 cm depth of the C_3 reference soil was characterized by highly variable $\delta^{13}C$ values ranging from $-27‰$ to $-19‰$ (Fig. 1). This high spatial (indicated by large standard errors) and temporal variability can be explained by a higher variability in soil moisture and temperature at the wheat site compared to the C_4/C_4 and to the C_4/fallow treatment, which might have led to bigger differences in microbial activity and hence in C decomposition

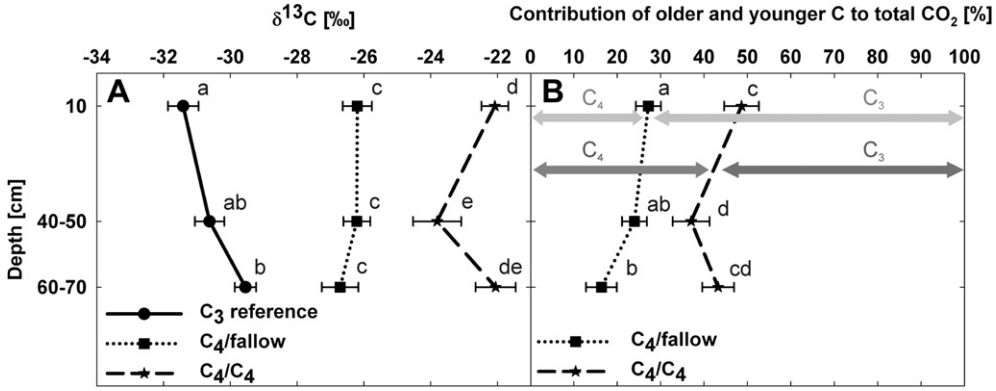

Fig. 3. A) $\delta^{13}C$ values (±SEM) of soil CO_2 calculated by Miller/Tans models for the C_3 reference, the C_4/fallow and the C_4/C_4 treatment at 10 cm, 40–50 cm and 60–70 cm depth, and B) contribution of younger and older C sources to total CO_2 at the C_4/fallow and C_4/C_4 treatment at 10 cm, 40–50 cm and 60–70 cm soil depth. Significant differences between the depths and treatments are marked by different letters ($P < 0.05$).

Table 2
$\delta^{13}C$ values of SOM (±SEM) of the C_3 reference, the C_4/fallow and the C_4/C_4 treatments and the contribution of C_4-derived C to total SOM (±SEM) on C_4/fallow and C_4/C_4 treatments at 10 cm, 40–50 cm and 60–70 cm depths. The $\delta^{13}C$ values of pure C_4 soil were: $-11.4 \pm 0.2‰$ for 10 cm; $-10.5 \pm 0.2‰$ for 40–50 cm; $-10.6 \pm 0.2‰$ for 60–70 cm. Significant differences between the depths within a treatment are marked by different lowercase letters ($P < 0.05$). Values followed by different uppercase letters indicate statistical differences between the treatments at a certain soil depth ($P < 0.05$).

Depth [cm]	$\delta^{13}C$ [‰]			Contribution of recently added C to total SOM [%]	
	C_3 reference	C_4/fallow	C_4/C_4	C_4/fallow	C_4/C_4
10	−27.0 ± 0.2 aA	−26.3 ± 0.1 aB	−26.4 ± 0.2 aAB	4.6 ± 1.3 aA	3.8 ± 1.1 aA
40–50	−26.2 ± 0.1 bA	−26.0 ± 0.2 abA	−26.2 ± 0.1 aA	1.4 ± 1.7 aA	0 ± 1.2 bA
60–70	−26.3 ± 0.1 bA	−25.7 ± 0.1 bB	−26.2 ± 0.1 aA	3.6 ± 1.1 aA	0.6 ± 1.0 bA

on the wheat site. Furthermore, soil moisture is a key factor controlling the intensity of atmospheric air mixing on all three sites, because it alters the rate of gaseous diffusion (Susfalk et al., 2002). The magnitude of the atmospheric air admixture mainly depends on the decomposition rates of SOM and on the rate of gaseous diffusion within the soil (Amundson et al., 1998; Cerling, 1984; Dudziak and Halas, 1996; Susfalk et al., 2002). The $\delta^{13}C$ values at the 10 cm depth of the C_3 reference increased at the end of the vegetation period due to a higher admixture of atmospheric CO_2 as a result of a decreasing water content (Fig. 1). The spatial and temporal variability of soil respiration, as well as of the effective soil porosity, led to a variation in the contribution of atmospheric air to total soil CO_2. A contribution of up to 35% of atmospheric air to near-surface soil gas has been reported (Susfalk et al., 2002). For grassland, Millard et al. (2008) calculated contributions of even 61%.

In this study, it was not possible to determine the percentage of atmospheric air mixed to soil CO_2 because the isotopic composition of atmospheric CO_2 in the vicinity of the soil surface was not measured. The use of the atmospheric $\delta^{13}C$ value of $-8‰$ and a CO_2 concentration of about 390 ppm, as an average for the bulk atmosphere, is inappropriate because the air close to the surface is strongly affected by CO_2 from the soil and by gaseous exchange with the vegetation.

4.2. Application of the Miller/Tans model to determine the isotopic composition of soil CO_2

The isotopic signature of pure soil CO_2, without admixing of atmospheric CO_2, can be determined using two component isotopic mixing lines (Fig. 2). The application of the various mixing models in combination with different regression approaches, such as ordinary least squares or GMR, was discussed in detail by Zobitz et al. (2006) and Kayler et al. (2010). For systems with broad CO_2 concentration ranges, such as soil CO_2, the application of the Miller/Tans model used with a GMR was recommended, since it provides the most accurate and precise estimate of the $\delta^{13}C$ value of purely soil-respired CO_2 (Kayler et al., 2010).

The basis of the mixing model is a mass balance equation which relies on the assumption of a simple mixing of only two gas components, soil CO_2 and atmospheric CO_2 (Pataki et al., 2003). During the sampling period, the contribution of the two components may change, but the isotope composition of the single components does not. In our experiment, besides the $\delta^{13}C$ value of the former C_3 vegetation and that of the atmospheric CO_2 mixed into the soil, a third source, with a C_4 isotopic signal, was added to soil by maize cropping. Since the contribution of the C_4 signal varied spatially and temporally, e.g. with changing root biomass and soil moisture (Fig. 1), the $\delta^{13}C$ of soil CO_2 did not remain constant. Nevertheless, both C_3- and C_4-derived CO_2 were accompanied by high CO_2 concentrations, whereas atmospheric CO_2, strongly enriched in ^{13}C compared to C_3–CO_2, had constantly lower concentrations. This enabled us to distinguish between soil CO_2 and atmospheric air mixed into the soil.

Additionally, the second component of the mixing model, atmospheric CO_2, may be a source of uncertainty since its $\delta^{13}C$ values can also differ spatially and temporally. The atmospheric CO_2 in the vicinity of the soil surface did not reflect the isotopic composition of the bulk atmospheric CO_2 of $-8‰$ but is, as a function of the distance from the soil surface, influenced by respiration and assimilation processes and the intensity of air mixing from higher atmospheric layers. Temporal variations in the isotopic composition of atmospheric CO_2 have also been reported, with increasing $\delta^{13}C$ values during periods of high photosynthetic activity (spring and summer), and declining $\delta^{13}C$ values during periods dominated by soil respiration (fall and winter) (Amundson et al., 1998). We concluded, that despite the mixing model requirement of constant $\delta^{13}C$ values for both components being violated, in particular for treatments with a C_4 source, reasonable results can nonetheless be obtained by applying the Miller/Tans model, mainly because of the broad CO_2 concentration range and the vicinity of our data to the $\delta^{13}C$ value and concentration of CO_2 from soil CO_2. Spatial and temporal fluctuations in the $\delta^{13}C$ values of both components of the mixing model may explain variations from the defined mixing line.

4.3. Contribution of recently added C to total soil CO_2

The application of a two source isotopic mixing model for partitioning total soil CO_2 into younger (C_4-derived) and older (C_3-derived) sources required the $\delta^{13}C$ values of both endmembers, i.e. (1) of the CO_2 from the decomposition of SOM from a C_3 bare fallow (C_3-derived), and (2) of pure root and/or rhizosphere respiration (C_4-derived). As a C_3 endmember for the mixing models (Eq. (1)) the isotopic composition of CO_2 from the C_3-reference site, obtained from the Miller/Tans models was used. This allowed accounting for ^{13}C fractionation between roots and soil CO_2. However, the ^{13}C fractionation between wheat roots and soil CO_2 under wheat not only comprised ^{13}C fractionation between SOM and SOM-derived CO_2, but also ^{13}C fractionation by root-derived respiration, i.e. between $\delta^{13}C$ of roots and that of root-derived CO_2. The latter may have lead to a slightly biased contribution of younger and older C to total CO_2 since ^{13}C fractionation by root-derived respiration should not have been accounted for in the C_3 endmember because of the absence of wheat roots at the C_4/fallow and at the C_4/C_4 treatment. To overcome this problem, the $\delta^{13}C$ of CO_2 from a bare fallow with long term C_3 history can be used as the C_3 endmember of the mixing model.

As done in most recent studies, we assumed that the net ^{13}C fractionation during respiration is negligible and, hence, the bulk isotopic composition of roots was used as the $\delta^{13}C$ value of the C_4 endmember when partitioning CO_2 (e.g. Buchmann and Ehleringer, 1998; Rochette et al., 1999; Rochette and Flanagan, 1997). Thus, this assumption may result in a slightly biased contribution of younger and older C to total CO_2 since ^{13}C fractionation in the C_4 endmember (maize roots) was not accounted for. To overcome this problem root-derived CO_2 measured in hydrocultures can be used

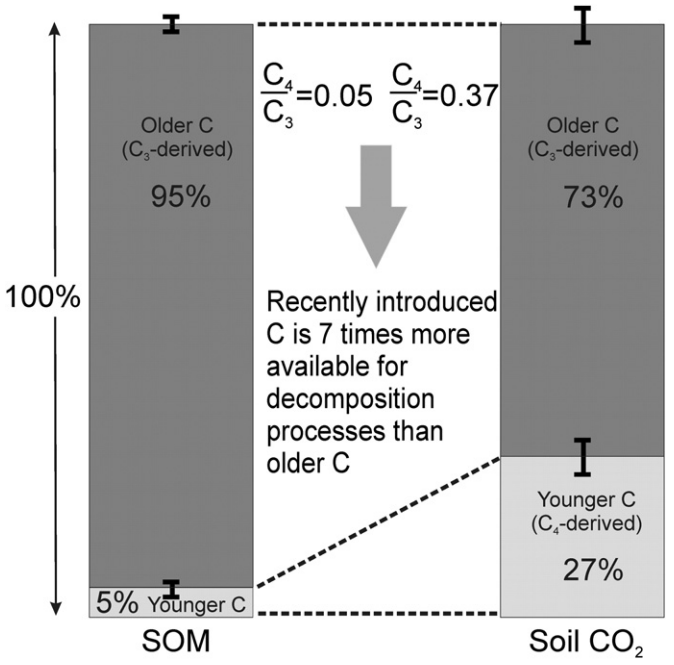

Fig. 4. Contribution of older and younger C to SOM and soil CO_2 at the 10 cm depth of the C_4/fallow treatment, and calculation of relative availability of recently added and older C. The seasonally integrated $\delta^{13}C$ value of CO_2 at this treatment and depth was $-26.2 \pm 0.4‰$. The $\delta^{13}C$ value of SOM was $-26.3 \pm 0.1‰$. The contribution of C_4-derived C to total CO_2 or SOM was calculated based on the following isotopic composition of the C_3 and C_4 sources: $-27.0 \pm 0.2‰$ and $-11.4 \pm 0.2‰$ for SOM in a pure C_3 system and a pure C_4 system, respectively; $-31.4 \pm 0.5‰$ and $-12.2 \pm 0.2‰$ for CO_2 in a pure C_3 system and maize roots, respectively.

as C_4 endmember. However, this was not applicable in the present experiment.

An estimated 50% of CO_2 released at the 10 cm depth of the C_4/C_4 soil was derived from recently added C (C_4-derived). This percentage decreased with depth, because there were fewer roots in deeper soil. Furthermore, the content of SOM decreased with depth (Table 1) and its turnover in deeper soil horizons is slower compared to upper horizons. Thus, the relative contribution of the roots to the CO_2 signature increased. Maize roots, and consequently rhizodeposition, are concentrated near the soil surface (Amos and Walters, 2006). The contribution of root-derived CO_2 under maize can account for up to 45% of total soil CO_2 (Rochette et al., 1999; Werth and Kuzyakov, 2009). Similar values have been reported for wheat (Kuzyakov and Cheng, 2001). Root-derived respiration explained the higher percentage of C_4-derived CO_2 for the C_4/C_4 treatment in comparison to the C_4/fallow treatment.

4.4. Relative availability of younger and older C

A high contribution of younger C to CO_2 was detected for the C_4/fallow as well as for the C_4/C_4 treatment at each sampling depth, whereas there was a much lower impact of younger C on SOM. This finding was confirmed by other studies. Flessa et al. (2000) reported that even after 37 years of maize cropping, the contribution of maize-derived C to SOM accounted for 15% of total C, but for about 58% of CO_2. A discussion by Kuzyakov (2011) showed that the C_4-to-C_3 ratio of SOM slowly increases and reaches saturation while it rises exponentially in CO_2 (outlined in Fig. 5 by Kuzyakov, 2011). Therefore, even a few decades after the C_3/C_4 vegetation change there will still be a high proportion of C_3-derived C in SOM, but a low contribution to the CO_2 efflux (Kuzyakov, 2011). This reflects the availability of recent and old SOM pools and allows to calculate the relative availability of C_4- and C_3-derived C by relating the C_4-to-C_3 ratio of CO_2 to that of SOM. Since root-derived CO_2 played a crucial role for the C_4/C_4 treatment, we only calculated the relative availability for the 10 cm depth of the C_4/fallow treatment (Fig. 4). While the ratio of C_4-to-C_3 at the 10 cm soil depth was 0.05 in SOM, it was about 0.5 in CO_2 (Fig. 4). Thus, the younger C, introduced into the soil in the previous year, was 10 times more available for microorganisms than the older C. The availability of soil C decreased with time as shown by the high proportion of younger C in CO_2 compared to older C and so indicated C stabilization.

5. Conclusions

In order to evaluate the availability of younger relative to older C for soil microorganisms a number of calculation steps were performed. In the first step – the removal of the admixture of atmospheric CO_2 and the estimation of pure soil CO_2 – the Miller/Tans model was successfully applied and provided a clear $\delta^{13}C$ signature of the soil CO_2. In the second step we estimated the contributions of younger (C_4-derived) and older (C_3-derived) carbon to CO_2 and to SOM. In the last step, we compared the contributions of older and younger sources to CO_2 and to SOM, and calculated the relative availability of recent and of old C. We showed that, despite the fact that the contribution of recent C to SOM was less than 5%, the contribution of recent C to produced CO_2 was about 27% at a soil depth of 10 cm within the C_4/fallow treatment. This indicates that one year after the C input into the soil, its availability for microorganisms was about 7 times higher than the availability of C sources older than one year.

Acknowledgments

We would like to thank Nils Peter and Ilse Thaufelder for their intensive help during the field and laboratory work, and Dr. Martina Gocke and Michael Riederer for suggestions on the earlier version of the manuscript. We thank Prof. Dr. Kai-Uwe Totsche and Andreas Schmalwasser for providing the soil water content data. We further thank two unknown reviewers for detailed comments and suggestions on this manuscript. The financial support for this project was provided by the DFG, within the Research Unit "Food-Webs".

References

Amos, B., Walters, D.T., 2006. Maize root biomass and net rhizodeposited carbon: an analysis of the literature. Soil Science Society of America Journal 70, 1489–1503.
Amundson, R., Stern, L., Baisden, T., Wang, Y., 1998. The isotopic composition of soil and soil-respired CO_2. Geoderma 82, 83–114.
Balesdent, J., Mariotti, A., 1987. Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics. Soil Biology & Biochemistry 19 (1), 25–30.
Blagodatskaya, E., Yuyukina, T., Blagodatsky, S., Kuzyakov, Y., 2011. Turnover of soil organic matter and microbial biomass under C_3–C_4 vegetation change: consideration of ^{13}C fractionation and preferential substrate utilization. Soil Biology & Biochemistry 43, 159–166.
Buchmann, N., Ehleringer, J.R., 1998. CO_2 concentration profiles, and carbon and oxygen isotopes in C_3 and C_4 crop canopies. Agricultural and Forest Meteorology 89, 45–58.
Cerling, T.E., 1984. The stable isotopic composition of modern soil carbonate and its relationship to climate. Earth and Planetary Science Letters 71, 229–240.
Dudziak, A., Halas, S., 1996. Influence of freezing and thawing on the carbon isotope composition in soil CO_2. Geoderma 69, 209–216.
Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40, 503–537.
Flessa, H., Ludwig, B., Heil, B., Merbach, W., 2000. The origin of soil organic C, dissolved organic C and respiration in a long-term maize experiment in Halle, Germany, determined by ^{13}C natural abundance. Journal of Plant Nutrition and Soil Science 163, 157–163.
Jastrow, J.D., Amonette, J.E., Bailey, V.L., 2007. Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. Climatic Change 80, 5–23.

Kammann, C., Grünhage, L., Jäger, H.-J., 2001. A new sampling technique to monitor concentrations of CH_4, N_2O and CO_2 in air at well-defined depths in soils with varied water potential. European Journal of Soil Science 52, 297–303.
Kayler, Z.E., Ganio, L., Hauck, M., Pypker, T.G., Sulzman, E.W., Mix, A.C., Bond, B.J., 2010. Bias and uncertainty of $\delta^{13}CO_2$ isotopic mixing models. Oecologia 163, 227–234.
Keeling, C.D., 1958. The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas. Geochimica et Cosmochimica Acta 13, 322–334.
Keeling, C.D., 1961. The concentration and isotopic abundances of carbon dioxide in rural and marine air. Geochimica et Cosmochimica Acta 24, 277–298.
Knorr, K.-H., Oosterwoud, M.R., Blodau, C., 2008. Experimental drought alters rates of soil respiration and methanogenesis but not carbon exchange in soil of a temperate fen. Soil Biology & Biochemistry 40, 1781–1791.
Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Schoene, J., Scheunemann, N., Schmalwasser, A., Totsche, K.U., Walker, F., Scheu, S., Kandeler, E., 2012. Carbon flow into microbial and fungal biomass as a basis to understand the belowground food web in an agroecosystem. Pedobiologia 55, 111–119.
Kuzyakov, Y., 2011. How to link soil C pools with CO_2 fluxes? Biogeosciences 8, 1523–1537.
Kuzyakov, Y., Cheng, W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology & Biochemistry 33, 1915–1925.
Millard, P., Midwood, A.J., Hunt, J.E., Whitehead, D., Boutton, T.W., 2008. Partitioning soil surface CO_2 efflux into autotrophic and heterotrophic components, using natural gradients in soil $\delta^{13}C$ in an undisturbed savannah soil. Soil Biology & Biochemistry 40, 1575–1582.
Miller, J.B., Tans, P.P., 2003. Calculating isotopic fractionation from atmospheric measurements at various scales. Tellus 55B, 207–214.
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. http://dx.doi.org/10.1029/2001GB001850.
Phillips, D.L., Gregg, J.W., 2001. Uncertainty in source partitioning using stable isotopes. Oecologia 127, 171–179.
Rochette, P., Flanagan, L.B., 1997. Quantifying rhizosphere respiration on a corn crop under field conditions. Soil Science Society of America Journal 61, 466–474.
Rochette, P., Flanagan, L.B., Gregorich, E.G., 1999. Separating soil respiration into plant and soil components using analyses of the natural abundance of carbon-13. Soil Science Society of America Journal 63, 1207–1213.
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.
Sokal, R.R., Rohlf, F.J., 1995. Biometry. W.H. Freeman, New York.
Susfalk, R.B., Cheng, W.X., Johnson, D.W., Walker, R.F., Verburg, P., Fu, S., 2002. Lateral diffusion and atmospheric CO_2 mixing comprise estimates of rhizosphere respiration in a forest soil. Canadian Journal of Forest Research 32, 1005–1015.
Von Lützow, M., Kogel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. European Journal of Soil Science 57, 426–445.
Von Lützow, M., Kogel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. Soil Biology & Biochemistry 39, 2183–2207.
Werth, M., Kuzyakov, Y., 2008. Root-derived carbon in soil respiration and microbial biomass determined by ^{14}C and ^{13}C. Soil Biology & Biochemistry 40, 625–637.
Werth, M., Kuzyakov, Y., 2009. Three-source partitioning of CO_2 efflux from maize field soil by ^{13}C natural abundance. Journal of Plant Nutrition and Soil Science 172, 487–499.
Zobitz, J.M., Keener, J.P., Schnyder, H., Bowling, D.R., 2006. Sensitivity analysis and quantification of uncertainty for isotopic mixing relationships in carbon cycle research. Agricultural and Forest Meteorology 13, 56–75.