Soil organic carbon decomposition from recently added and older sources estimated by $\delta^{13}$C values of CO$_2$ and organic matter

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A B S T R A C T

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.

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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 fractions 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
various C sources, is the admixture of atmospheric CO₂ to soil air resulting in the modification of its δ¹³C value. This strongly limits the application of δ¹³C values of CO₂ for the estimation of CO₂ sources and for the evaluation of their availability for microorganisms (Sée et al., 2004). Removal of atmospheric CO₂ is therefore a prerequisite for analyzing the soil CO₂. 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 CO₂ concentration is used to estimate the y-axis intercept equivalent to the δ¹³C value of pure soil CO₂. The Miller/Tans model is based on the linear regression between the product of CO₂ concentration and its δ¹³C value plotted against the CO₂ concentration. The isotopic composition of pure CO₂ 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 δ¹³C values of soil CO₂ are less variable in comparison to the Keeling plot approach, especially if the measured CO₂ concentration varies across a broad range. As soil CO₂ 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 CO₂ before calculating the contribution of older (C₃-derived) and younger (C₄-derived) sources to CO₂.

The C₃ to C₄ vegetation change approach has commonly been applied to determine either the sources of CO₂ or of SOM, but has very seldom been used to relate isotopic compositions of CO₂ to that of SOM in a way that allows for a quantitative estimation of the availability of SOM for microorganisms (Flessa et al., 2000; Kuzyakov, 2011). We therefore hypothesized that the purified δ¹³C values of CO₂ and the δ¹³C values of SOM after a C₃ to C₄ 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 C₃ vegetation. The main soil properties are presented in Table 1.

A vegetation change in 2009 from C₃ to C₄ crops was used to introduce a distinct δ¹³C 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. Ronaldino) 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 ‘C₃ reference’ for the plots with continuous wheat cropping, ‘C₄/C₄’ for the plots with maize cropping in the first and in the second year of the experiment, and ‘C₄/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 C₃ 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 (C₄/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 mm, 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 (C₃ reference, C₄/C₄ and C₄/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 CO₂ concentration gradient in the upper soil depths, the 10 cm samplers were horizontally inserted after excavating a soil core (0.008 m³). 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 sucked into the vial.

### Table 1

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Depth [m]</th>
<th>pH (CaCl₂)</th>
<th>Bulk density [g cm⁻³]</th>
<th>Corg [g kg⁻¹]</th>
<th>Total N [g kg⁻¹]</th>
<th>C/N</th>
<th>δ¹⁵N [%]</th>
<th>δ¹³C [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ap1</td>
<td>0–0.25</td>
<td>6.0 ± 0.1 a</td>
<td>1.4 ± 0.0 a</td>
<td>12.4 ± 0.4 a</td>
<td>1.3 ± 0.0 a</td>
<td>9.8 a</td>
<td>8.0 ± 0.2 a</td>
<td>−27.4 ± 0.1 a</td>
</tr>
<tr>
<td>Ap2</td>
<td>0.25–0.37</td>
<td>6.2 ± 0.1 a</td>
<td>1.6 ± 0.0 b</td>
<td>6.9 ± 1.2 b</td>
<td>0.8 ± 0.1 b</td>
<td>9.2 a</td>
<td>7.9 ± 0.5 ab</td>
<td>−26.5 ± 0.2 b</td>
</tr>
<tr>
<td>Bt1</td>
<td>0.37–0.65</td>
<td>6.6 ± 0.1 b</td>
<td>1.7 ± 0.0 c</td>
<td>3.3 ± 0.5 c</td>
<td>0.4 ± 0.0 c</td>
<td>8.9 ab</td>
<td>6.4 ± 0.3 bc</td>
<td>−26.1 ± 0.1 bc</td>
</tr>
<tr>
<td>Bt2</td>
<td>&gt;0.65</td>
<td>7.0 ± 0.1 c</td>
<td>1.6 ± 0.0 b</td>
<td>1.8 ± 0.4 a</td>
<td>0.3 ± 0.0 c</td>
<td>6.9 b</td>
<td>5.6 ± 0.6 c</td>
<td>−25.5 ± 0.3 c</td>
</tr>
</tbody>
</table>

* Small quantities of CaCO₃ 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 $^{13}$C value of pure soil CO$_2$ was determined by correcting the measured $^{13}$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., 2008). 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 the 0 °C value for the admixture of atmospheric CO$_2$ based on the Miller/Tans model (Phillips and Gregg, 2001). 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 ($p ≤ 0.001$). 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 $^{13}$C values of the C$_3$ reference at the 40–50 cm and at the 60–70 cm soil depths, ranging from $-30_{\text{‰}}$ to $-24_{\text{‰}}$, did not show a trend over time, the $^{13}$C values of the C$_4$/C$_4$ treatment increased at mid-June and reached values of up to $-16_{\text{‰}}$ and decreased again in August at all depths. The $^{13}$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.
and due to the decomposition of the C4 organics which remained after the first year of maize cropping. At the end of the vegetation period, δ13C values of −27.3% were detected, which were within the δ13C range of the C3 reference. During the vegetation period the δ13C values of CO2 of the C3 reference soil at the depth of 40–50 cm and 60–70 cm were lower compared to the values at the C4/fallow and the C4/C4 treatments. The different dynamics of the δ13C values indicate changes in the contribution of C3 and C4 sources to total CO2.

For all three treatments, the highest δ13C values were found at the 10 cm depth, but with the highest variation for the C3 reference soil. High variation of CO2 concentrations and especially of δ13C values, due to the admixture of atmospheric CO2, confirmed that a correction for the admixture was required to determine the δ13C of pure soil CO2. Using the Miller/Tans model the single CO2 concentration data (shown as means in Fig. 1) was plotted against the δ13C value multiplied by the CO2 concentration (Miller and Tans, 2003) (Fig. 2). The best correlations were found for samples taken from the C3 reference soil because of the highest difference of δ13C values of organics utilized by microorganisms and atmospheric CO2. The slope of the regression line equals the integrated δ13C value of pure soil CO2 without atmospheric CO2. The purified δ13C values of soil CO2 consistently and strongly differed between the three treatments for all depths investigated (Fig. 3A). The lowest δ13C values were calculated for CO2 produced at the C3 reference site. The C4/fallow treatment represented an intermediate stage and the highest δ13C values were detected for the C4/C4 treatment. Correspondingly to the δ13C of SOM (Table 2) the δ13C of CO2 at the C3 reference increased slightly with depth, from about −31.6% at the 10 cm depth to −30.5% at the 60–70 cm depth (Fig. 3A). However, there were no depth gradients for the C4/fallow and the C4/C4 treatments with average δ13C values of approximately −26.1% and −23.4% respectively.

3.2. Contribution of recently added C to total soil CO2 and SOM and relative availability of younger and older C

Based on two component isotopic mixing lines, the contribution of younger C (C4-derived) to soil CO2 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 CO2 at the C4/C4 treatment site was approximately 50% at the 10 cm depth, which was twice as high compared to the C4/fallow treatment. This can be explained by root-derived CO2 (root and rhizomicrobial respiration) in the presence of living roots in the C4/C4 soil.

The contribution of younger C to soil CO2 decreased with depth for both C4 treatments. At the 40–50 cm and the 60–70 cm depth of the C4/C4 treatment, younger C contributed about 43% to soil CO2, while at the C4/fallow treatment only 16% C4-derived C was detected at the 60–70 cm depth. Despite the high contribution of recently added C to soil CO2, even at the 60–70 cm soil depth of the C4/fallow treatment, the bulk SOM was only slightly (<5%) enriched by the C4 signal after one year of maize cropping (Table 2).

By relating the contribution of recently added C to SOM to its contribution to CO2, the availability of younger C relative to older C was estimated. This was done only for the 10 cm depth of the C4/fallow treatment, because of the absence of significant differences in δ13C of SOM below the plough horizon at 40–50 cm between the C3 reference and the C4/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 CO2

The δ13C values of the measured soil CO2 of the C4/C4 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 CO2 with increasing root biomass during the growing season. At the end of the growing season, the δ13C values of CO2 declined again to the initial level due to reduced root-derived respiration.
The $\delta^{13}C$ of CO$_2$ in the C$_4$/fallow soil decreased consistently with time, and converged to the range of the $\delta^{13}C$ 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 $-27^\circ_{oo}$ to $-19^\circ_{oo}$ (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$_3$/C$_4$ and to the C$_4$/fallow treatment, which might have led to bigger differences in microbial activity and hence in C decomposition.

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![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.](image1)

![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 ($P < 0.05$).](image2)
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 C3 reference increased at the end of the vegetation period due to a higher admixture of atmospheric CO2 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 CO2. 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 CO2 because the isotopic composition of atmospheric CO2 in the vicinity of the soil surface was not measured. The use of the atmospheric $\delta^{13}$C value of $-8\%$, and a CO2 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 CO2 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 CO2

The isotopic signature of pure soil CO2, without admixing of atmospheric CO2, 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 CO2 concentration ranges, such as soil CO2, 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 CO2 (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 CO2 and atmospheric CO2 (Pataki et al., 2003). During the sampling period, the contribution of the two components may change, but the isotopic composition of the single components does not. In our experiment, besides the $\delta^{13}$C value of the former C3 vegetation and that of the atmospheric CO2 mixed into the soil, a third source, with a C4 isotopic signal, was added to soil by maize cropping. Since the contribution of the C3 signal varied spatially and temporally, e.g. with changing root biomass and soil moisture (Fig. 1), the $\delta^{13}$C of soil CO2 did not remain constant. Nevertheless, both C3- and C4-derived CO2 were accompanied by high CO2 concentrations, whereas atmospheric CO2, strongly enriched in $^{13}$C compared to C3–CO2, had constantly lower concentrations. This enabled us to distinguish between soil CO2 and atmospheric air mixed into the soil.

Additionally, the second component of the mixing model, atmospheric CO2, may be a source of uncertainty since its $\delta^{13}$C values can also differ spatially and temporally. The atmospheric CO2 in the vicinity of the soil surface did not reflect the isotopic composition of the bulk atmospheric CO2 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 CO2 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 C4 source, reasonable results can nonetheless be obtained by applying the Miller/Tans model, mainly because of the broad CO2 concentration range and the vicinity of our data to the $\delta^{13}$C value and concentration of CO2 from soil CO2. 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 CO2

The application of a two source isotopic mixing model for partitioning total soil CO2 into younger (C4-derived) and older (C3-derived) sources required the $\delta^{13}$C values of both endmembers, i.e. (1) of the CO2 from the decomposition of SOM from a C3 bare fallow (C3-derived), and (2) of pure root and/or rhizosphere respiration (C4-derived). As a C3 endmember for the mixing models (Eq. (1)) the isotopic composition of CO2 from the C3-reference site, obtained from the Miller/Tans models was used. This allowed accounting for $^{13}$C fractionation between roots and soil CO2. However, the $^{13}$C fractionation between wheat roots and soil CO2 under wheat not only comprised $^{13}$C fractionation between SOM and SOM-derived CO2, but also $^{13}$C fractionation by root-derived respiration, i.e. between $^{13}$C of roots and that of root-derived CO2. The latter may have lead to a slightly biased contribution of younger and older C to total CO2 since $^{13}$C fractionation by root-derived respiration should not have been accounted for in the C3 endmember because of the absence of wheat roots at the C4/fallow and at the C4/C4 treatment. To overcome this problem, the $^{13}$C of CO2 from a bare fallow with long term C3 history can be used as the C3 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 C4 endmember when partitioning CO2 (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 CO2 since $^{13}$C fractionation in the C4 endmember (maize roots) was not accounted for. To overcome this problem root-derived CO2 measured in hydrocultures can be used.

<table>
<thead>
<tr>
<th>Depth [cm]</th>
<th>$\delta^{13}$C [%]</th>
<th>Contribution of recently added C to total SOM [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C3 reference</td>
<td>C4/fallow</td>
</tr>
<tr>
<td>10</td>
<td>$-27.0 \pm 0.2 \text{ aA}$</td>
<td>$-26.3 \pm 0.1 \text{ aB}$</td>
</tr>
<tr>
<td>40–50</td>
<td>$-26.2 \pm 0.1 \text{ bA}$</td>
<td>$-26.0 \pm 0.2 \text{ bB}$</td>
</tr>
<tr>
<td>60–70</td>
<td>$-25.3 \pm 0.1 \text{ bB}$</td>
<td>$-25.7 \pm 0.1 \text{ bB}$</td>
</tr>
</tbody>
</table>
as C4 endmember. However, this was not applicable in the present experiment.

An estimated 50% of CO₂ released at the 10 cm depth of the C4/fallow treatment was derived from recently added C (C₄-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 CO₂ signature increased. Maize roots, and consequently rhizodeposition, are concentrated near the soil surface (Amos and Walters, 2006). The contribution of root-derived CO₂ under maize can account for up to 45% of total soil CO₂ (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 C₄-derived CO₂ for the C₄/fallow treatment in comparison to the C₄/fallow treatment.

4.4. Relative availability of younger and older C

A high contribution of younger C to CO₂ was detected for the C₄/fallow as well as for the C₃/C₄ 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 CO₂. A discussion by Kuzyakov (2011) showed that the C₄-to-C₃ ratio of SOM slowly increases and reaches saturation while it rises exponentially in CO₂ (outlined in Fig. 5 by Kuzyakov, 2011). Therefore, even a few decades after the C₄/C₃ vegetation change there will still be a high proportion of C₃-derived C in SOM, but a low contribution to the CO₂ efflux (Kuzyakov, 2011). This reflects the availability of recent and old SOM pools and allows to calculate the relative availability of C₄- and C₃-derived C by relating the C₄- to C₃ ratio of CO₂ to that of SOM. Since root-derived CO₂ played a crucial role for the C₄/C₃ treatment, we only calculated the relative availability for the 10 cm depth of the C₄/fallow treatment (Fig. 4). While the ratio of C₄-to-C₃ at the 10 cm soil depth was 0.05 in SOM, it was about 0.5 in CO₂ (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 CO₂ 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 CO₂ and the estimation of pure soil CO₂ — the Miller/Tans model was successfully applied and provided a clear δ¹³C signature of the soil CO₂. In the second step we estimated the contributions of younger (C₄-derived) and older (C₃-derived) carbon to CO₂ and to SOM. In the last step, we compared the contributions of older and younger sources to CO₂ 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 CO₂ was about 27% at a soil depth of 10 cm within the C₄/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.

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