ELSEVIER

Soil Biology & Biochemistry 40 (2008) 625–637

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Root-derived carbon in soil respiration and microbial biomass determined by ^{14}C and ^{13}C

M. Werth[a,*], Y. Kuzyakov[b]

[a]*Department of Systematic Botany and Ecology, University of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany*
[b]*Department of Agroecosystem Research, University of Bayreuth, 95440 Bayreuth, Germany*

Received 13 March 2007; received in revised form 11 September 2007; accepted 19 September 2007
Available online 16 October 2007

Abstract

Two approaches to quantitatively estimating root-derived carbon in soil CO_2 efflux and in microbial biomass were compared under controlled conditions. In the ^{14}C labelling approach, maize (*Zea mays*) was pulse labelled and the tracer was chased in plant and soil compartments. Root-derived carbon in CO_2 efflux and in microbial biomass was estimated based on a linear relationship between the plant shoots and the below-ground compartment. Since the maize plants were grown on C_3 soil, in a second approach the differences in ^{13}C natural abundance between C_3 and C_4 plants were used to calculate root-derived carbon in the CO_2 efflux and in the microbial biomass. The root-derived carbon in the total CO_2 efflux was between 69% and 94% using the ^{14}C labelling approach and between 86% and 94% in the natural ^{13}C labelling approach. At a ^{13}C fractionation measured to be 5.2‰ between soil organic matter (SOM) and CO_2, the root-derived contribution to CO_2 ranged from 70% to 88% and was much closer to the results of the ^{14}C labelling approach. Root-derived contributions to the microbial biomass carbon ranged from 2% to 9% using ^{14}C labelling and from 16% to 36% using natural ^{13}C labelling. At a 3.2‰ ^{13}C fractionation between SOM and microbial biomass, both labelling approaches yielded an equal contribution of root-derived C in the microbial biomass. Both approaches may therefore be used to partition CO_2 efflux and to quantify the C sources of microbial biomass. However, the assumed ^{13}C fractionation strongly affects the contributions of individual C sources.

Keywords: ^{14}C pulse labelling; ^{13}C natural abundance; Isotopic fractionation; Rhizosphere; Soil organic matter; Soil respiration

1. Introduction

Carbon dioxide (CO_2) efflux from soils is an important component of the global carbon (C) cycle and related to global climatic change because increasing amounts of CO_2 in the atmosphere promote the greenhouse effect. Small changes in the turnover intensity of soil organic matter (SOM) could significantly alter the CO_2 concentration in the atmosphere: the amount of C in SOM approximately doubles the amount of C in the atmosphere (Grace, 2004). These small variations in the decomposition intensity of SOM cannot be determined directly by measuring organic C contents because changes in soil organic C are very small during short periods (e.g. 1–3% during a single vegetative growth season). Alternatively, measuring CO_2 efflux from soil is commonly used to investigate short-term SOM turnover. This method is sensitive enough to detect small and actual changes, especially for recently altered ecosystems (Kuzyakov and Cheng, 2001). Most soils, however, are covered with vegetation, which also contributes to the CO_2 efflux from soil. Therefore, CO_2 efflux from planted soil consists of SOM- and root-derived CO_2. The latter can be further subdivided into root respiration and rhizomicrobial respiration of rhizodeposits (exudates, lysates, etc.). This separation is exceptionally difficult, since plant roots and rhizosphere microorganisms use the same C source, i.e. plant assimilates. It is much easier to separate CO_2 from microbial decomposition of SOM and root-derived CO_2, i.e. the sum of root respiration and respiration of rhizosphere microorganisms consuming rhizodeposits. Since root-derived CO_2 is not part of soil C loss, partitioning the total CO_2 efflux from soil is very important to identify individual sinks or sources of CO_2.

*Corresponding author. Tel.: +49 731 50 22693; fax +49 731 50 22720.
E-mail address: martin.werth@uni-ulm.de (M. Werth).

0038-0717/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2007.09.022

CO_2 derived from SOM decomposition and that derived from the roots can be partitioned and quantified by isotopic labelling of plants with ^{13}C or ^{14}C isotopes and tracing the label in root-derived CO_2 (Warembourg and Paul, 1977; Andrews et al., 1999). Besides artificial labelling techniques, the difference in the natural abundances of ^{13}C in C_3 plants ($-35‰ \leqslant \delta^{13}C \leqslant -20‰$) and in C_4 plants ($-15‰ \leqslant \delta^{13}C \leqslant -7‰$) can also be used as a natural C tracer (Cheng, 1996; Qian et al., 1997; Rochette and Flanagan, 1997; Ekblad and Högberg, 2001; Kuzyakov and Cheng, 2001). The difference between the labelled fraction and the total CO_2 efflux represents CO_2 from SOM decomposition. Non-isotopic methods to separate root- from SOM-derived CO_2, such as a combination of trenching and excised-root methods, have also been used (Kelting et al., 1998; Chen et al., 2006). The results vary strongly depending on plants, soils and environmental and experimental conditions. By *in situ* ^{14}C labelling of Canadian prairie grass, Warembourg and Paul (1977) found low contributions (19%) of root-derived CO_2 to the total CO_2 efflux from soil. On the other hand, under controlled conditions, Chen et al. (2006) reported very high contributions of root-derived CO_2, with values of up to 99% in a ryegrass (*Lolium perenne* L.) rhizosphere. Various studies under controlled conditions have found results within this range (Robinson and Scrimgeour, 1995; Qian et al., 1997; Kuzyakov and Cheng, 2001; Lin et al., 2001), with an average contribution of $59 \pm 23\%$ root-derived CO_2. The broad variability of these results indicates that there is urgent need to find a reproducible standard method including a protocol for standardized soil preparation, plant age and growing conditions and analytical procedures.

The turnover of SOM and rhizodeposits is caused by the soil microbial biomass, which derives its energy from oxidising soil organic C. Both the plant residue C and the rhizodeposits pass through the soil microbial biomass at least once as they are transferred from one C pool to another and finally mineralised to CO_2 (Ryan and Aravena, 1994). In a system of C_3–C_4–vegetation change, active microorganisms can be identified by high contributions of the C_4 source to their $\delta^{13}C$ signature, since this is an indicator of food uptake from recently assimilated C. Alternatively, active rhizosphere microorganisms can be determined by the ^{14}C tracer after labelling of plants followed by a rhizodeposition of this tracer. Root-derived C—i.e. C_4-derived C—ranges for instance from 9% to 52% in the soil microbial biomass after 110 days of maize growth (Liang et al., 2002). Several other studies using ^{13}C or ^{14}C labelling techniques have found contributions of root-derived C within this range (Merckx et al., 1987; Ryan and Aravena, 1994; Angers et al., 1995; Bruulsema and Duxbury, 1996; Qian and Doran, 1996; Rochette et al., 1999; Gregorich et al., 2000).

Fractionations between the substrate, the microbial biomass, and the microbially respired CO_2 have not always been considered in earlier studies or it was assumed that the fractionation is not significantly different from zero (Cheng, 1996; Ekblad and Högberg, 2000; Ekblad et al., 2002). However, control treatments without plants allow these fractionations to be determined. Several studies have considered ^{13}C fractionations between the substrate, the microbial biomass, and the CO_2 (Mary et al., 1992; Schweizer et al., 1999; Šantrůčková et al., 2000; Fernandez and Cadisch, 2003; Kristiansen et al., 2004). In order to identify the impact of isotopic fractionation on root-derived C contributions, we used the natural ^{13}C labelling technique with and without consideration of ^{13}C fractionation.

Determining root-derived contributions to below-ground C pools using the ^{14}C pulse labelling technique and the natural ^{13}C labelling technique has often led to different, sometimes contrasting results. This is because both methods are based on different assumptions, their sensitivity strongly differs and the distributions of the tracer could vary. The ^{14}C pulse labelling technique allows the distribution of recently assimilated C at specific plant development stages to be determined, but the partitioning of the tracer into plant and soil pools has to be completed on the sampling date. The distribution of plant-derived C to below-ground pools can only be determined for the whole growth period by repeated labelling pulses. In contrast, natural ^{13}C labelling is equivalent to a continuous labelling approach, which does not focus on recently assimilated carbon but on the total plant-derived carbon in plant and soil pools, i.e. sampling can be done at any time. On short time scales, however, both methods should produce similar results. It is unclear whether differences between the two methods reflect differences in plants, soils, experimental conditions, etc. or whether they are methodological artefacts. This calls for applying both methods under exactly the same experimental conditions, preferably in the same experiment.

The objective of this study was to determine the contributions of maize-root-derived carbon to the CO_2 efflux from soil and to the soil microbial biomass. Two approaches were compared: (a) the ^{14}C pulse labelling approach and (b) the natural ^{13}C labelling technique. In the former, maize plants were artificially labelled with $^{14}CO_2$ and the tracer was chased in plant and soil pools. The amount of total root-derived C in CO_2 or microbial biomass was then calculated with a linear function according to Kuzyakov et al. (1999). In the natural ^{13}C labelling technique (Balesdent and Mariotti, 1996), ^{13}C natural abundance was used by growing maize as a C_4 plant on a soil developed solely under C_3 vegetation ('C_3 soil'). Hence, four specific $\delta^{13}C$ values were used in mass balances to determine the contributions of root-derived C to CO_2 efflux and microbial biomass.

2. Materials and methods

2.1. Experimental set-up

Maize plants (*Zea mays* L.) were grown under controlled laboratory conditions in 20 pots filled with a loamy Haplic Luvisol from loess with C_3 vegetation history (*Lolium*

perenne L.), collected from the University of Hohenheim's research farm 'Heidfeldhof' in Stuttgart, Germany. The maize seeds (cv. Tassilo) were germinated on wet filter paper. One day after germination the seedlings were transferred to 250 ml polycarbonate filtration devices (SM16510/11, Sartorius, Göttingen, Germany) filled with 400 g of the C_3 soil ($pH(CaCl_2) = 6.0$), one plant per container (Fig. 1). A control treatment with one unplanted pot per sampling date was established, which was treated exactly in the same way as the planted treatment. One day before the start of CO_2 trapping, the holes in the pots around the plant shoots were sealed with a 1-cm-thick silicone rubber layer (TACOSIL 145, Thauer & Co., Dresden, Germany) between roots and shoots, and the seal was tested for air leaks. Trapping of CO_2 from soil air started on day 9 after germination in a closed system for each plant (or control treatment). Air was pumped through every single pot from bottom to top by a membrane pump (Type 113, Rietschle Thomas, Memmingen, Germany; pumping rate 100 ml min^{-1}), which was connected to the pot by a polyvinyl chloride (PVC) tube (Fig. 1). Another PVC tube was connected to the top outlet of the filter device and to a CO_2 trapping tube filled with 20 ml 1 M sodium hydroxide (NaOH) solution. The output of the trapping tube was connected to the input of the membrane pump. Therefore, the air containing CO_2 evolved from soil respiration circulated in a closed system. Firstly, the air was pumped through the pot, with any CO_2 from total soil respiration being trapped in NaOH solution. Secondly, the remaining CO_2-free air coming from the NaOH trapping tube was pumped back through the pot. Thus, the air cycling was closed and was done continuously by the membrane pump. This completely prevented CO_2 losses and contamination with air CO_2.

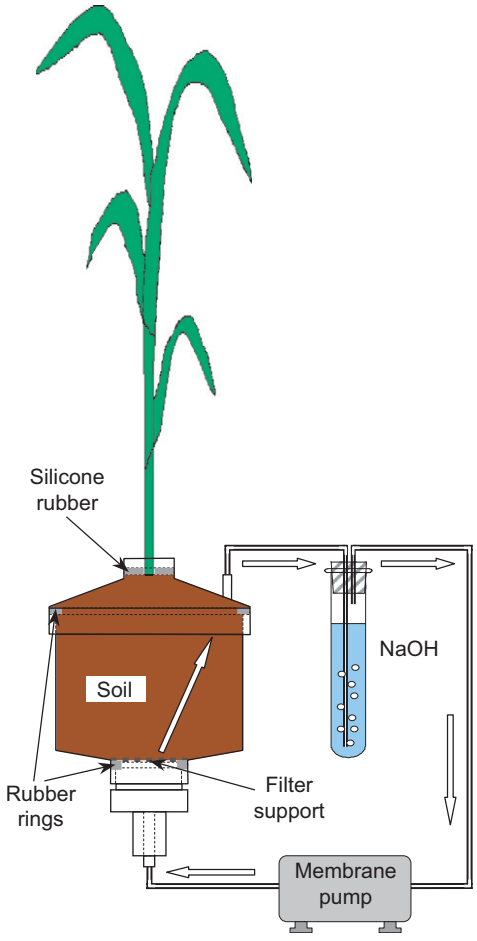

Fig. 1. Experimental set-up for trapping of below-ground CO_2 in NaOH solution (redrawn from Werth et al., 2006). White arrows show airflow.

The soil moisture was maintained at about 25% of the gravimetrical water content throughout the experiment by controlling the pots' weights after the first water addition. On days 9, 15, 21, 27 and 33 after germination, a full fertilizer (5 kg nitrate-N ha^{-1}, 0.4 kg monophosphate-P ha^{-1}, 10 kg K^+ ha^{-1}; see Werth and Kuzyakov 2005 for further details) was added with the water to the soil from 1–5 times depending on the date of sampling of the pots.

2.2. ^{14}C pulse labelling

On day 9 after germination, the 20 maize plants were labelled for the first time. All sealed pots with plants were placed into a Plexiglas chamber ($0.5 \times 0.5 \times 0.6\ m^3$) for the labelling procedure described in detail by Cheng et al. (1993). Briefly, the chamber was connected by tubing with a flask containing 2.0 ml 1 mM $Na_2^{14}CO_3$ solution to which 5 ml 9 M H_2SO_4 was added to produce $^{14}CO_2$. The plants were labelled during 2.5 h in the $^{14}CO_2$ atmosphere. Usually, about 30 min of labelling time are required for C_4 plants to reach the CO_2 compensation point (Kuzyakov and Cheng, 2004). A longer time period was used in our experiment to increase the ^{14}C incorporation into plant biomass. Before opening the labelling chamber, the chamber air was pumped through 1 M NaOH solution to remove unassimilated $^{14}CO_2$. Activities of unassimilated $^{14}CO_2$ and of the ^{14}C residue in the $Na_2^{14}CO_3$ source were subtracted from the total ^{14}C present in the flask prior to labelling in order to calculate the total ^{14}C input activity. The latter was divided by the number of plants in the labelling chamber, yielding an input activity of 246.7 kBq per plant. After labelling, the chamber was opened and the trapping of CO_2 evolved by root respiration was started. The same labelling procedure was repeated on days 15, 21, 27 and 33 with a total of 16, 12, 8 and 4 plants in the chamber, respectively. The ^{14}C input activity was adjusted by the reduced numbers of plants in the labelling chamber (0.1 ml 1 mM $Na_2^{14}CO_3$ solution per plant).

2.3. Sampling and analyses

One week after labelling, soil and plants were destructively sampled in four replicates (i.e. one replicate for the control treatment) on days 16, 22, 28, 34 and 40 after germination. At harvest, each shoot was cut at the base, the lid of the pot was opened and each root–soil column pulled out of the pot. Bulk soil was sampled by cutting a small

wedge into the soil column from the edge towards the centre. We then loosened the soil column from the edge and discarded the soil falling down. The soil still adhering to the roots was collected as the inner rhizosphere fraction and was used later on for microbial biomass $\delta^{13}C$ analyses. The moist soil samples were immediately frozen until preparation for microbial biomass. The roots were carefully washed with deionised water to remove soil particles. Shoots and roots were dried at 40 °C. CO_2 trapped in NaOH was sampled on the harvest days and additionally once to twice between two harvest days.

To estimate total CO_2 efflux, the CO_2 trapped in NaOH solution was precipitated with 0.5 M barium chloride ($BaCl_2$) solution and then the NaOH was titrated with 0.2 M hydrochloric acid (HCl) against phenolphthalein indicator (Zibilske, 1994). Soil microbial biomass was determined by the chloroform fumigation extraction method (modified after Vance et al., 1987): roots were removed from the unfrozen soil by handpicking, and 10 g of soil were extracted with 40 ml of 0.05 M potassium sulphate (K_2SO_4) solution. Another 10 g of soil were firstly fumigated with chloroform for 24 h and then extracted in the same way. The K_2SO_4 and soil mixtures were shaken for 1 h at 200 rev min^{-1}, centrifuged at 3000 rev min^{-1} for 10 min, and then filtrated through a ceramic vacuum filter. The extracts were frozen until analyses for total C concentrations were done with a Dimatoc-100 TOC/TIC analyser (Dimatec, Essen, Germany). The microbial biomass C concentration was calculated from these results using a k_{EC} value of 0.45 (Wu et al., 1990) and is presented in percent of dry soil. The soil water content was determined in another 10 g of soil, which was dried at 105 °C. These soil samples and the plant samples were ground with a ball mill before analysis. The C concentration in shoots, roots, and soil was measured with a Euro EA C/N analyser (EuroVector, Milan, Italy).

The ^{14}C activity of $^{14}CO_2$ trapped in NaOH solution was measured in 2 ml aliquots added to 4 ml scintillation cocktail Rotiszint Eco Plus (Carl Roth, Karlsruhe, Germany) after decay of chemiluminescence. ^{14}C activity was measured using a Wallac 1411 Liquid Scintillation Counter (Wallac Oy, Turku, Finland). The ^{14}C counting efficiency was about 85% and the ^{14}C activity measurement error did not exceed 2%. The absolute ^{14}C activity was standardised by addition of NaOH solution as quencher to the scintillation cocktail and using the spectrum of an external standard (SQP(E) method). ^{14}C in solid samples (dried shoots, roots, and soil) was measured on the liquid scintillation counter after combustion of 200 mg of plant samples or 1 g of soil samples within an oxidizer unit (Model 307, Canberra Packard Ltd., Meriden, USA), absorption of the ^{14}C in Carbo-Sorb E (Perkin-Elmer, Inc., Boston, USA), and addition of the scintillation cocktail Permafluor E^+ (Perkin-Elmer, Inc.).

A Thermo Finnigan MAT Delta plus Advantage isotope ratio mass spectrometer (IRMS from Thermo Electron Corporation, Waltham, USA) was coupled to the C/N analyser to measure $\delta^{13}C$ values in shoots, roots and soil. Since only solid samples could be analysed by the IRMS unit, the CO_2 and microbial biomass samples had to be specifically prepared. Any CO_2 trapped as sodium carbonate (Na_2CO_3) in 5 ml of NaOH was precipitated with 5 ml of 0.5 M strontium chloride ($SrCl_2$) aqueous solution. To prevent fractionation in this step, carbonate was completely precipitated to a maximum of 2.6×10^{-5}% of the total CO_2-C absorbed by NaOH remaining in the solution. The maximum residue in the NaOH solution was calculated according to the $SrCO_3$ solubility product. The NaOH solutions containing the $SrCO_3$ precipitants were then centrifuged three times at 3000 rev min^{-1} for 10 min and washed in between with deionised and degassed water to remove NaOH and to reach a pH of 7. Keeping the tubes opened for washing as briefly as possible prevented contamination by atmospheric CO_2 during sample preparation. After washing, the remaining water was removed from the vials and the $SrCO_3$ was dried at 105 °C. The $SrCO_3$ was analysed on the IRMS for $\delta^{13}C$ values. For the microbial biomass, an aliquot of the K_2SO_4 samples was pipetted directly into tin capsules and dried at 60 °C prior to IRMS analyses.

2.4. Calculations

The ^{14}C activity found in a certain compartment (a_{ct}, i.e. shoot, root, soil, CO_2 or microbial biomass) was related to the total ^{14}C recovery after sampling, i.e. the sum of ^{14}C activity in shoots (a_{shoot}), roots (a_{root}), soil (a_{soil}) and CO_2 (a_{CO_2}), and was termed $^{14}C_{ct}$ (data are shown in Fig. 3):

$$^{14}C_{ct} = \frac{a_{ct}}{a_{shoot} + a_{root} + a_{soil} + a_{CO_2}} \times 100\%. \quad (1)$$

Root-derived C in CO_2 and microbial biomass were calculated based on ^{14}C activity in the plant shoots ($^{14}C_{shoot}$); the total amount of carbon in the shoots (C_{shoot}) and the ^{14}C activity in CO_2 ($^{14}C_{CO_2}$) and microbial biomass ($^{14}C_{MB}$) according to Kuzyakov et al. (1999) (data are shown in Table 1 and Fig. 3):

$$C_{maize\text{-}ct} = C_{shoot} \times \frac{^{14}C_{ct}}{^{14}C_{shoot}}, \quad (2)$$

where $C_{maize-ct}$ is the amount of maize-derived C in a compartment (CO_2 or microbial biomass) and $^{14}C_{ct}$ is the ^{14}C activity in that compartment related to the total recovery (Eq. (1)). The ^{14}C activity and the amount of C in the shoots were chosen as a reference because these can be measured more accurately in the shoots compared with the roots, where adhering soil particles increase the root mass in all replicates. The amount of maize-derived carbon ($C_{maize-ct}$) was then related to the amount of total carbon in a compartment ($C_{total-ct}$) and was termed $f_{maize-ct}$:

$$f_{maize\text{-}ct} = \frac{C_{maize\text{-}ct}}{C_{total\text{-}ct}} \times 100\%. \quad (3)$$

Table 1
Carbon in shoots, roots, bulk soil, cumulative CO_2 efflux and microbial biomass on five sampling dates of maize grown on C_3 soil (means ± SD, $n = 4$) and of unplanted C_3 soil (means ±SD, $1 \leqslant n \leqslant 2$).

Days of maize growth	Shoots C (mg)	Roots C (mg)	Soil C (mg)	CO_2 C (mg)	Microbial biomass C (mg)
Maize on C_3 soil					
16	265±12	86±11	5886±211	111±5	125±13
22	451±31	108±11	5831±198	181±17	80±13
28	634±46	153±7	5697±944	232±11	98±9
34	872±97	198±23	5846±190	286±11	84±11
40	1329±66	228±6	5659±205	359±9	83±4
Unplanted C_3 soil					
16	n.a.	n.a.	5972	63	122
22	n.a.	n.a.	5930	72	81
28	n.a.	n.a.	6003	80	68
34	n.a.	n.a.	5857±3	113±11	74
40	n.a.	n.a.	5651±175	121±5	83

n.a.: not applicable.
CO_2 was trapped for 7, 14, 21, 28 and 35 days on sampling days 16, 22, 28, 34 and 40, respectively

A mass balance equation was used to determine the $\delta^{13}C$ value of microbial biomass ($\delta^{13}C_{MB}$):

$$\delta^{13}C_{MB} = \frac{\delta^{13}C_{fum} \times C_{fum} - \delta^{13}C_{nf} \times C_{nf}}{C_{fum} - C_{nf}}, \quad (4)$$

where $\delta^{13}C_{fum}$ and $\delta^{13}C_{nf}$ are the $\delta^{13}C$ values of the fumigated and non-fumigated samples, respectively, and C_{fum} and C_{nf} are the amounts of C in the fumigated and non-fumigated K_2SO_4 samples, respectively.

In the beginning of every CO_2 trapping, a small volume of atmospheric CO_2 was present in the closed system, especially in the soil pore space and in the trapping tube above the NaOH solution. We eliminated this atmospheric CO_2 from the measured $\delta^{13}C$ value using a mass balance equation

$$\delta^{13}C_{CO_2} = \frac{\delta^{13}C_{total} \times C_{total} - \delta^{13}C_{air} \times C_{air}}{C_{total} - C_{air}}, \quad (5)$$

where $\delta^{13}C_{CO_2}$ is the corrected $\delta^{13}C$ value of soil air without atmospheric air, $\delta^{13}C_{total}$ is the measured $\delta^{13}C$ value of CO_2, $\delta^{13}C_{air}$ is the $\delta^{13}C$ value of ambient air (–7.8‰, see Boutton, 1991), C_{total} is the amount of CO_2-C trapped in NaOH, and C_{air} is the amount of C in the soil pore space and the trapping tube in our closed system (0.024 mg C) calculated from a CO_2 concentration of 345 mg kg^{-1} (Boutton, 1991) and the volume of air in the system.

After having calculated the $\delta^{13}C$ of microbial biomass (Eq. (4)) and the corrected $\delta^{13}C$ of total CO_2 efflux (Eq. (5)), it became possible to calculate the contributions of the C_4 plant source C to below-ground CO_2 ($f_{C_4-CO_2}$) and to microbial biomass (f_{C_4-MB}):

$$f_{C_4\text{-}CO_2} = \frac{\delta^{13}C_{CO_2} - \delta^{13}C_{SOM}}{\delta^{13}C_{maize} - \delta^{13}C_{SOM}} \times 100\%, \quad (6)$$

$$f_{C_4\text{-}MB} = \frac{\delta^{13}C_{MB} - \delta^{13}C_{SOM}}{\delta^{13}C_{maize} - \delta^{13}C_{SOM}} \times 100\%, \quad (7)$$

where $\delta^{13}C_{maize}$ is the $\delta^{13}C$ value of maize roots and $\delta^{13}C_{SOM}$ is the $\delta^{13}C$ value of SOM from unplanted soil (data are shown in Table 2).

Isotopic fractionations were considered between SOM and SOM-derived CO_2, between SOM and microbial biomass, and between rhizodeposits and microbial biomass. The fractionation between maize rhizodeposits and microbial biomass was assumed to be the same as the fractionation between SOM and microbial biomass. Since the $\delta^{13}C$ value of root-derived CO_2 is dominated by the $\delta^{13}C$ value of CO_2 from root respiration, we assumed no ^{13}C fractionation between root-derived C and CO_2 according to Werth and Kuzyakov (2006). Considering these fractionations, $\delta^{13}C_{SOM}$ in Eq. (6) was replaced by $\delta^{13}C_{SOM-CO_2}$, $\delta^{13}C_{SOM}$ in Eq. (7) was replaced by $\delta^{13}C_{SOM-MB}$, and $\delta^{13}C_{maize}$ in Eq. (7) was replaced by $\delta^{13}C_{maize-MB}$:

$$\delta^{13}C_{SOM\text{-}CO_2} = \delta^{13}C_{SOM} + \varepsilon_{SOM\text{-}CO_2}, \quad (8)$$

$$\delta^{13}C_{SOM\text{-}MB} = \delta^{13}C_{SOM} + \varepsilon_{SOM\text{-}MB}, \quad (9)$$

$$\delta^{13}C_{maize\text{-}MB} = \delta^{13}C_{maize} + \varepsilon_{SOM\text{-}MB}, \quad (10)$$

where ε_{SOM-CO_2} and ε_{SOM-MB} are ^{13}C isotopic fractionations as absolute values in ‰ between SOM and CO_2 and between SOM and microbial biomass from unplanted soil, respectively.

Standard deviations (SD) were calculated as a variability parameter for all our results. We used a one-way analysis of variance (ANOVA) to identify differences between $\delta^{13}C$ values of various below-ground carbon pools, between ^{14}C recoveries at the five sampling dates in a certain pool, between maize-derived CO_2 contributions calculated by ^{14}C or ^{13}C tracers at a certain sampling date, and between maize-derived C contributions to the microbial biomass calculated by ^{14}C or ^{13}C tracers at a certain sampling date. A Fisher LSD test was used as *post hoc* test to identify individual differences. Where variances were not equal,

Table 2
$\delta^{13}C$ values of shoots, roots, bulk soil, CO_2 efflux and microbial biomass on five sampling dates of maize grown on C_3 soil (means ± SD, $n = 4$) and of unplanted C_3 soil (means ± SD, $1 \leqslant n \leqslant 2$)

Days of maize growth	$\delta^{13}C$ (‰)				
	Shoots	Roots	Soil	CO_2	Microbial biomass
Maize on C_3 soil					
16	−15.2 ± 0.1	−14.9 ± 0.2	−26.7 ± 0.4	−16.9 ± 0.0	−24.6 ± 0.9
22	−15.8 ± 0.1	−15.7 ± 0.3	−26.9 ± 0.1	−16.7 ± 0.5	−25.1 ± 0.2
28	−16.2 ± 0.1	−16.0 ± 0.1	−26.9 ± 0.1	−17.9 ± 0.2	−23.4 ± 0.3
34	−16.0 ± 0.1	−16.1 ± 0.2	−26.5 ± 0.4	−16.8 ± 0.3	−23.0 ± 0.5
40	−16.2 ± 0.1	−16.2 ± 0.2	−26.7 ± 0.1	−16.7 ± 0.8	−22.5 ± 0.7
Unplanted C_3 soil					
16	n.a.	n.a.	−26.8	−18.6	n.d.
22	n.a.	n.a.	−27.0	−21.9	−23.0
28	n.a.	n.a.	−27.3	−21.2	−23.1
34	n.a.	n.a.	−26.9 ± 0.1	−21.7	−24.9 ± 0.6
40	n.a.	n.a.	−26.8 ± 0.1	−22.6	−24.2

n.a. - not applicable.
n.d. - not determined.

a Studentised maximum modulus test had to be applied as *post hoc* test. Statistics were calculated with the SPSS 10.0 package.

3. Results

3.1. Plant and soil carbon pools

Between days 16 and 34 the amount of C in the maize shoots increased linearly by 33.4 mg C d^{-1} (Fig. 2a). Continuing this linear trend would lead to 1057 mg C on average in the shoots on day 40, but the actual amount of C was about 300 mg higher on the last sampling date (Table 1). Hence, the shoot biomass was no longer increasing linearly between days 34 and 40 (but rather exponentially). The maize roots grew linearly and gained 142 mg C within the whole sampling period of 24 days (Fig. 2b). Such a linear increase of shoot and root biomass is a prerequisite for calculating the root-derived C contributions to the microbial biomass and the CO_2 efflux by Eq. (3).

The amount of C in the soil planted with maize was constant during the whole growth period, averaging 5784 mg C (Table 1). Although the roots were growing and increasing amounts of rhizodeposits should have been supplied, the amount of C in the microbial biomass was also constant at 86 mg C on average from days 22–40 (Table 1). On the first sampling date, however, the amount of C in the microbial biomass was significantly higher ($P < 0.05$) compared with the following dates. Significant differences could not be tested between the maize soil and the unplanted soil because only one to two soil samples per date were available for the unplanted soil. Since total C of the unplanted soil was always within the standard deviation of the related sample in the planted soil, no significant difference between the two treatments can be assumed. A similar relationship between planted and unplanted treatments was found for the amounts of C in the microbial biomass. The cumulative CO_2 efflux from the planted soil increased linearly from 8.5 mg C d^{-1} between days 22 and 28 to 12.3 mg C d^{-1} between days 34 and 40 (Table 1). In contrast, the control pots without plants showed a reduced rate of increase (from 1.4 mg C d^{-1} minimum between days 34 and 40 to 5.4 mg C d^{-1} maximum between days 24 and 34).

3.2. ^{14}C activities

The mean ^{14}C activities recovered from the inputs per plant from sampling dates 16–40 were: 62.1 ± 17.8%, 47.8 ± 9.7%, 37.7 ± 4.8%, 35.7 ± 5.8% and 36.0 ± 2.6% (Table 3). After every additional ^{14}C pulse, the total radioactivity, however, increased in all pools. Most ^{14}C was allocated to the maize shoots. A maximum of 9 kBq ^{14}C was translocated into the soil at the end of the experiment. The ^{14}C activity in the soil microbial biomass made up about one-third at maximum of the ^{14}C activity in the soil. The loss of ^{14}C label by shoot respiration increased from one-third to two-third of the input until the end of the experiment. The partitioning of ^{14}C activity into the five different pools in relation to the total recovery was constant throughout the experiment (Fig. 3). It amounted on average to 67.1 ± 1.6% for shoots, 10.2 ± 0.8% for roots, 1.9 ± 0.6% for the soil, 20.7 ± 1.4% for the CO_2 efflux, and 0.5 ± 0.3% for the microbial biomass (the latter not shown in Fig. 3). Only on the first sampling there was significantly more ^{14}C in the soil than on the other sampling dates ($P < 0.05$).

3.3. $\delta^{13}C$ values

Between days 16 and 40, the $\delta^{13}C$ values of maize shoots and roots decreased significantly ($P < 0.001$), by 1.0‰ for the shoots and by 1.3‰ for the roots (Table 2). The $\delta^{13}C$ of

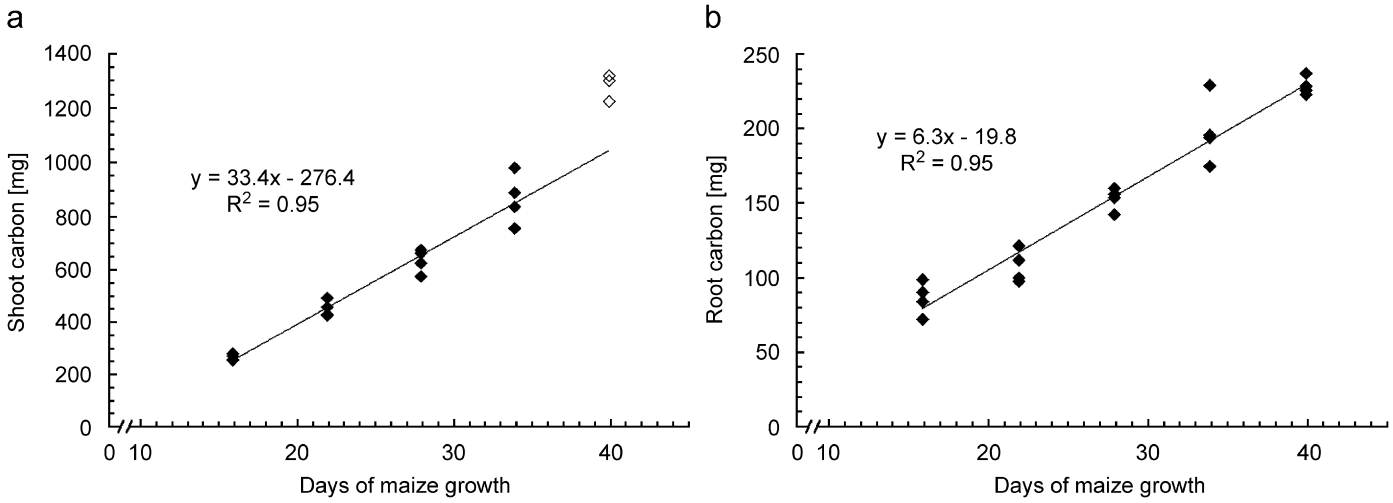

Fig. 2. Linear regressions of amounts of carbon (closed diamonds) in (a) maize shoots and (b) maize roots towards time of maize growth ($n = 4$). The amounts of carbon on day 40 in the shoots (open diamonds) are not included in the linear function.

Table 3
Average ^{14}C activity ($n = 4$) per plant container in plant and soil pools after repeated labelling of maize shoots in a $^{14}CO_2$ atmosphere

	Days of maize growth				
	16	22	28	34	40
Days of ^{14}C pulse labelling	9	9, 15	9, 15, 21	9, 15, 21, 27	9, 15, 21, 27, 33
^{14}C activity (kBq)					
Total input	246.4	493.1	739.7	986.4	1233.0
Shoots	102.3±44.8	163.9±46.5	182.7±25.1	238.7±39.9	302.4±31.0
Roots	16.0±3.5	23.8±2.2	29.2±2.8	36.9±6.1	39.5±3.4
Soil	4.1±0.6	4.1±0.3	3.4±0.6	6.6±2.2	8.8±1.2
CO_2	30.6±19.5	43.9±7.7	63.2±8.5	70.0±19.0	93.5±1.8
Sum of recovery	153.1±43.9	235.5±47.9	278.6±35.5	352.1±57.4	444.1±32.4
Microbial biomass	1.3±0.3	0.5±0.2	0.9±0.2	2.1±0.6	2.0±0.6
Loss by shoot respiration	93.4±43.9	257.5±47.9	461.2±35.5	634.2±57.4	788.9±32.4

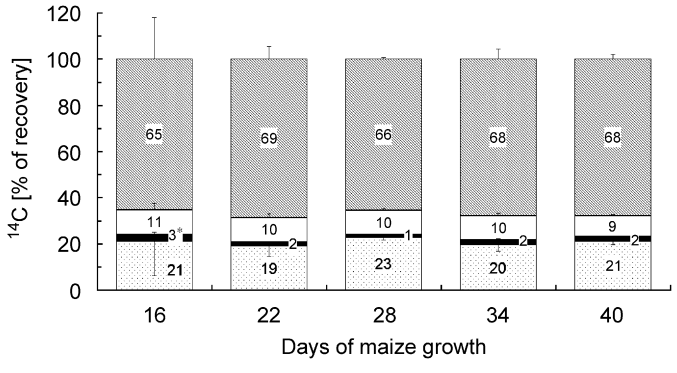

Fig. 3. Partitioning of ^{14}C activity into maize shoots (hatched shading), roots (white shading), bulk soil (black shading), and CO_2 efflux (dotted shading). Shoots were consecutively pulse-labelled, 7 days before the harvest date indicated on the figure (total of 1–5 pulses). Values are means ($n = 4$) with standard deviations shown to one side of the bars only. Significant differences between sampling dates within one type of pool are labelled as *, i.e. $P < 0.05$.

the total CO_2 efflux from planted soil (–16.9‰ on average over time) was, by 1‰, significantly more negative ($P < 0.05$) than the $\delta^{13}C$ of the roots. Nevertheless, $\delta^{13}C$ values of roots and CO_2 were very close. The $\delta^{13}C$ values of CO_2 presented in Table 2 were corrected by Eq. (5) for small amounts of air CO_2 remaining in the soil pores and in the trapping tube. This correction made the $\delta^{13}C$ values of below-ground CO_2 slightly more negative compared with uncorrected data, but this difference was less than 0.02‰. The $\delta^{13}C$ of SOM was constant and amounted to –26.8‰. Until day 40, the $\delta^{13}C$ of microbial biomass increased from –24.6‰ to –22.5‰; the mean value was –23.7‰, which was significantly more positive than the mean $\delta^{13}C$ value of SOM ($P < 0.001$).

The average $\delta^{13}C$ of SOM in unplanted soil (–27.0‰) was the same as in planted soil (Table 2). In the total CO_2 efflux of unplanted soil, the mean $\delta^{13}C$ between days 22 and 40 was –21.8‰. The mean $\delta^{13}C$ of microbial biomass

between days 22 and 40 was intermediate between these two values (–23.8‰). Consequently, there was a ^{13}C fractionation of about 3.2‰ between organic matter in unplanted soil and microbial biomass (ε_{SOM-MB}, $P<0.001$), and of 2.0‰ between microbial biomass and microbially respired CO_2 ($P<0.05$). The fractionation ε_{SOM-CO_2} between SOM and microbial CO_2 was 5.2‰ ($P<0.001$).

3.4. Contributions of maize roots to CO_2 efflux and microbial biomass

The comparisons between the two different methods for calculating the contributions of root-derived CO_2 to total CO_2 efflux showed the following results (Fig. 4): First, no significant difference was found on day 16 between these contributions as calculated by the ^{14}C approach and the ^{13}C approach with and without fractionation. Second, only the ^{13}C approach without fractionation yielded significantly more root-derived CO_2 (91% on day 22, 94% on day 34) versus the ^{14}C approach ($P<0.05$). Consideration of ^{13}C fractionation between SOM and CO_2 led to equal percentages of root-derived CO_2 on those days. Third, the results on day 28 from both ^{13}C methods were significantly smaller (without ^{13}C fractionation $P<0.05$, with ^{13}C fractionation $P<0.001$) than the 94% calculated by the ^{14}C approach. Fourth, the root-derived CO_2 contribution based on the ^{14}C method exceeded the 100% level by 16% on the last sampling day. The result from the ^{13}C method without fractionation was below 100%, but not significantly different from the ^{14}C result. Considering the ^{13}C fractionation led to a significantly smaller root-derived CO_2 contribution (91%) than the ^{14}C approach ($P<0.05$).

The contributions of root-derived C to microbial biomass C calculated by the two methods increased with the age of the maize (Fig. 5). Using ^{14}C labelling, this

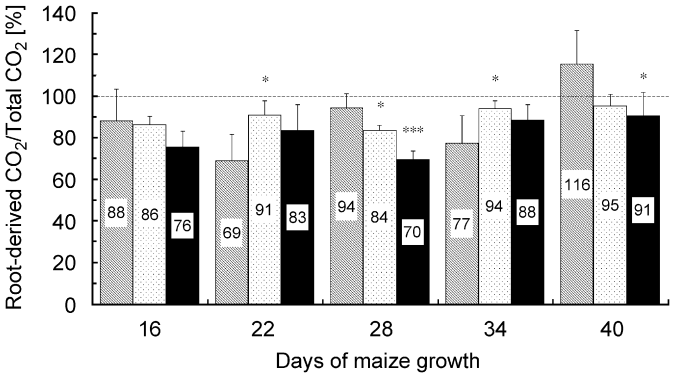

Fig. 4. Contributions of root-derived CO_2 to total CO_2 efflux from a C_3 soil planted with maize. Methods used to calculate the root-derived CO_2 contributions are: the ^{14}C labelling technique (hatched shading), the natural ^{13}C labelling technique (dotted shading), and the natural ^{13}C labelling technique with a fractionation of 5.2‰ between SOM and CO_2 (black shading). Error bars show standard deviations ($n=4$). On each day, significant differences are shown for the natural ^{13}C labelling technique with and without fractionation when compared with the ^{14}C labelling technique (*$P<0.05$, ***$P<0.001$).

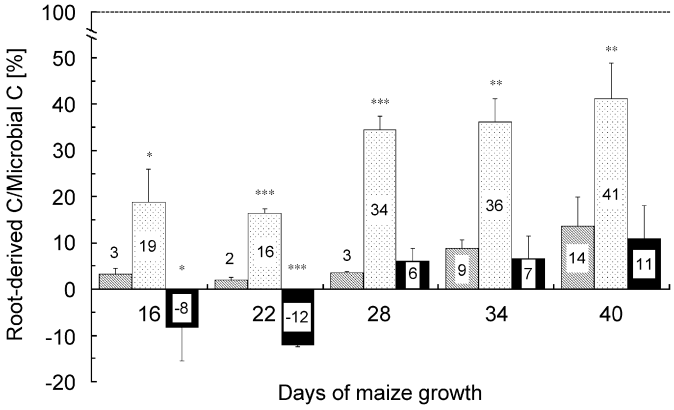

Fig. 5. Contributions of root-derived carbon to total microbial biomass C from a C_3 soil planted with maize. Methods used to calculate the root-derived carbon contributions are: the ^{14}C labelling technique (hatched shading), the natural ^{13}C labelling technique (dotted shading), and the natural ^{13}C labelling technique with a fractionation of 3.2‰ between SOM and microbial biomass and between rhizodeposits and microbial biomass (black shading). Error bars show standard deviations ($n=4$). On each day, significant differences are shown for the natural ^{13}C labelling technique with and without fractionation when compared with the ^{14}C labelling technique (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

increase was significant on day 34 ($P<0.05$). On the first two sampling dates, the ^{13}C approach without fractionation yielded significantly higher values—up to eight times as high—than the ^{14}C approach. Incorporating ^{13}C fractionation on those 2 days led to negative values, which were significantly different to the ^{14}C approach. On the last three sampling dates, the contribution calculated by the ^{13}C approach without fractionation was from 3–11 times as high as the contribution calculated by the ^{14}C approach. There was no significant difference to the ^{14}C method when ^{13}C fractionation was considered on the last three sampling dates.

4. Discussion

4.1. Comparison of the ^{14}C pulse labelling and natural ^{13}C labelling approaches to estimate maize-root-derived carbon contributions

Both methods showed similar contributions to total CO_2 efflux from the maize rhizosphere when ^{13}C fractionations between SOM and CO_2 were considered (Fig. 4). In a previous publication on maize grown on C_3 soil (Werth et al., 2006), we concluded that the natural ^{13}C labelling technique overestimated root respiration by comparing observed CO_2 partitioning into three sources with literature results. By validating the ^{13}C results with ^{14}C results, we now determined that the root-derived carbon was between 69% and 94% of below-ground CO_2 efflux. Thus, the results from Werth et al. (2006) were correct rather than an overestimation. Under controlled conditions, other comparable studies report root-derived C values of about 75% for 38-day-old spring wheat (*Triticum aestivum* L.)

(Kuzyakov and Cheng, 2001) or between 35% and 41% for 42-day-old ryegrass (*Lolium perenne* L.) (Chen et al., 2006). Our values therefore correspond with the upper range of these studies. The broad range of root-derived CO_2 already mentioned in the introduction reveals, however, that CO_2 efflux partitioning very much depends on the observed plants, their growth stage, controlled or field conditions, soil preparation, etc. This problem always has to be considered when comparisons between different studies are made.

The exceeding of 100% of the total CO_2 efflux on day 40 using the ^{14}C labelling technique reflects non-linear plant growth up to that day (Table 1, Fig. 2). The linear model of Kuzyakov et al. (1999) to calculate root-derived C amounts from ^{14}C activity was not applicable on day 40. Had the plants grown linearly up to day 40, the root-derived contribution would have been 91%—another comparable result to ^{13}C labelling including fractionation. Hence, 116% on day 40 (Fig. 4) is an error and should not be compared with the ^{13}C method.

The airflow in our tubing system enabled us not only to trap CO_2, it also assured against leakage of CO_2 out of the system or into the PVC tube's wall. We chose PVC tubes since they generally are airtight. Small errors in our CO_2 budget could have arisen, however, by a minimum of diffusion through the walls (either into or out of the tube). If these errors were present, they would affect both methods in the total amount of CO_2, i.e. ^{14}C pulse labelling in Eq. (3) and natural ^{13}C labelling in Eq. (5).

On the last three sampling dates, the ^{14}C and ^{13}C techniques showed similar results for the root-derived C in the microbial biomass C (when ^{13}C fractionation between SOM and microbial biomass was considered; Fig. 5). Without fractionation, values were significantly different to the ^{14}C approach on all sampling dates. Hence, like for the CO_2 efflux, fractionations should be considered. Root-derived C contributions to total microbial biomass C ranging from 1% to 11% after 42 days of maize growth (Qian and Doran, 1996), from 8% to 10% within one growth period (Rochette et al., 1999), or up to 23% after a single year of maize growth (Bruulsema and Duxbury, 1996) confirm our findings of 2–11% with ^{13}C fractionation (Fig. 5). Our previous publication showed that—without fractionation between the substrate and the CO_2 in calculating the $\delta^{13}C$ value of microbial CO_2—about 37% of the microbial biomass in the rhizosphere was active, i.e. feeding on a C_4 source (Werth et al., 2006). In that study, also assuming a 5‰ fractionation in the microbial substrate respiration when calculating the $\delta^{13}C$ of microbial respiration would reduce the amount of C_4-derived C in the microbial biomass to 9%. This closely corresponds with the 7–11% root-derived C in the microbial biomass on the last two sampling dates of the present study.

Soil samples for microbial biomass extraction were prepared by hand-picking roots. Small amounts of fine roots could still have been present in the samples resulting in destruction of their cell membranes after chloroform fumigation and a contribution of the cell content to the microbial biomass extract. Consequently, total carbon in microbial biomass, ^{14}C activity, and C_4 plant contribution to the $\delta^{13}C$ value could have been overestimated. This source of error could be overcome by a more complex preparation of soil microbial biomass samples, e.g. by pre-extraction with K_2SO_4, wet sieving or centrifuging (Mueller et al., 1992). The same error, however, has to be considered in all the other studies compared with the present experiment.

To be consistent in the assumption of linear shoot growth (used when calculating CO_2 efflux partitioning), we also consider such growth up to day 40 when calculating root-derived carbon in the microbial biomass by the ^{14}C technique: this assumption yields a decrease from 14% to 9%. This result would be closer to the root-derived C contribution calculated by the ^{13}C approach with fractionation. Hence, due to non-linear plant growth, the ^{14}C approach should not be used between days 34 and 40. On days 16 and 22, ^{13}C with fractionation yielded negative values. This reflects the high average fractionation (3.2‰) between SOM and microbial biomass, leading to more positive $\delta^{13}C$ values of SOM than of microbial biomass. Using the maximum possible fractionation ε_{SOM-MB} in the maize treatment (2.2‰ on day 16 and 1.9‰ on day 22), the contribution of root-derived C to the microbial biomass on days 16 and 22 would be zero. This result would again closely reflect the ^{14}C result. It is therefore important to determine the actual fractionation on every sampling day—and not an average fractionation over time—with an appropriate number of replicates and consequently include time changes of the fractionation.

While both methods—^{14}C pulse labelling and natural ^{13}C labelling—worked sufficiently in the determination of root-derived C in the CO_2 efflux from soil, this was much more problematic with the microbial biomass. Although chloroform fumigation–extraction has become a standard method in soil biology, the absence of plant effect on microbial biomass size (Table 1) and $\delta^{13}C$ (Table 2) makes an estimation of root-derived C to microbial biomass very uncertain using this coarse method. As a substitute, molecular methods like biomarkers could be used in combination with isotopic tracer methods to identify C_4-derived C contributions to certain community parts of the soil microbial biomass ($\delta^{13}C$ of phospholipid fatty acids (PLFA)), or to determine plant-derived or microbial residues (individual sugars) (reviewed by Glaser, 2005).

4.2. Advantages and limitations of the ^{14}C pulse labelling approach

General advantages and limitations of the ^{14}C method are presented in Table 4. The method of transforming ^{14}C activity into amounts of C in particular pools has been used before (Kuzyakov et al., 1999, 2001, 2003; Kuzyakov

Table 4
Advantages and limitations of ^{14}C pulse labelling and natural ^{13}C labelling techniques for estimating the contribution of root-derived C to the total CO_2 efflux from soil and to the soil microbial biomass

^{14}C pulse labelling	Natural ^{13}C labelling
Advantages	
• High sensitivity of the contribution of plant-derived C to CO_2 and to microbial biomass	• Continuous labelling of plants and soil pools
• Information on distribution of assimilated C in individual stages of plant development	• No labelling equipment required
• Allows estimating the incorporation of plant C into pools with low and very low turnover rates	• No radioactivity precautions necessary
• One or many pulses are possibly easy to handle	• Easy usage under laboratory and field conditions
• Cheap purchase costs and individual analyses	
Limitations	
• Uncompleted distribution of labelled C between plant organs and below-ground pools if sampling is done too early after the labelling	• Very low sensitivity of the contribution of plant-derived C to CO_2 and to microbial biomass
• Recalculation of total rhizodeposition is suitable only for linear growth periods	• Only incorporation of plant-derived C into pools with high turnover rates during one vegetation period is possible
• Provides only distribution of recently assimilated C at specific development stages of plants	• Applicable only on pure C_3 or C_4 soil
• Both non-recent and recent assimilates can be traced if labelling pulses are repeated	• Contamination with air CO_2 possible
• No recalculation of distribution to whole growth period	• High variation of C in CO_2 or microbial biomass possible
• Radioactivity hazards	• Results are strongly affected by ^{13}C fractionation
• Laborious labelling sessions with chambers required	• Results are strongly affected by preferential isotope utilisation
	• Expensive purchase costs and individual analyses

and Cheng, 2001). A similar method that also considered small increases of plant biomass between individual labelling pulses was suggested by Remus et al. (2006). Our calculation method, however, allows only a rough estimate of the C that passed through each flow because the parameters of Eq. (2) are not constant during plant development. This method can be used only after completed ^{14}C distribution in the plant. For grasses and cereals, this completion takes 5 days after assimilation (Domanski et al., 2001). In accordance with that study, the distribution of the ^{14}C tracer between the plant–soil compartments was completed on all sampling days in our study, since there were no significant differences between sampling days (Fig. 3). Hence, the equation was applicable in our study. Due to non-linear carbon assimilation after day 34, however, the linear equation can no longer be applied at the end of the growth period.

Another limitation of the ^{14}C method is that pulse labelling can effectively track inputs derived from recent assimilates, but that this input is unlikely to constitute the most abundant source of substrate to microbial communities in the rhizosphere (Thornton et al., 2004). Non-recent assimilates, i.e. more complex organic forms, will be exuded much later than recent assimilates; they will also be processed at different rates and most likely by different microorganisms. This problem, however, can be overcome by a series of ^{14}C labelling pulses as used in our study or by continuous labelling techniques, like natural ^{13}C labelling. The triplication of root-derived C in the microbial biomass on day 34 (Fig. 5) indicates that both recent and non-recent assimilates contribute to the root-derived C in the microbial biomass from that day onwards.

4.3. Advantages and limitations of the natural ^{13}C labelling approach

In contrast to the artificial ^{14}C labelling technique, the natural ^{13}C labelling approach corresponds to continuous labelling of plants and plant-derived soil pools (Table 4). A major limitation is that four assumptions are involved concerning ^{13}C isotopic effects during root- and SOM-derived respiration and during utilization of rhizodeposits and SOM by the microbial biomass:

(1) The $\delta^{13}C$ isotope signature of root-derived CO_2 is the same as the $\delta^{13}C$ value of the roots.
(2) The $\delta^{13}C$ isotope signature of SOM-derived CO_2 equals the $\delta^{13}C$ value of SOM.
(3) The $\delta^{13}C$ isotope signature of root-derived microbial biomass corresponds to the $\delta^{13}C$ value of roots and rhizodeposits.
(4) The $\delta^{13}C$ isotope signature of SOM-derived microbial biomass is equal to the $\delta^{13}C$ value of SOM.

According to Werth and Kuzyakov (2006) we can only accept the first assumption, since the $\delta^{13}C$ value of root-derived CO_2 is dominated by the $\delta^{13}C$ value of CO_2 from root respiration. Our unplanted control treatment refutes the last three assumptions. We had to consider mean ^{13}C fractionations of 3.2‰ between SOM and microbial

biomass and of 5.2‰ between SOM and microbially respired CO_2. Henn and Chapela (2000) have shown that the ^{13}C fractionation differs during decomposition of C_3- and C_4-derived sucrose by three specific fungi. However, we assumed the fractionation between maize rhizodeposits and microbial biomass in Eq. (7) and (10) to be equal to the fractionation between SOM and microbial biomass ($\varepsilon_{SOM-MB} = 3.2‰$). In line with earlier studies (Balesdent and Mariotti, 1996; Boutton, 1996; Bol et al., 2003), we accepted this assumption because we had no direct measure to determine the actual fractionation between rhizodeposits and the microbial biomass. This determination is a future challenge, requiring that rhizodeposits be decomposed by exactly the same microbial community as developed in our C_4 plant containers. In the present study, however, we assumed equal fractionations for C_3- and C_4-derived substrates because the root-derived contributions calculated with and without ^{13}C fractionation for the C_4 substrate were not significantly different. In Fig. 5, the latter would read −11%, −17%, 8%, 9% and 16% on sampling dates 16–40, respectively.

The fractionations between SOM and microbial biomass and between SOM and CO_2 do not only include isotopic effects *per se*. They also include preferential utilisation of substrates with different biological availability and different $\delta^{13}C$ values. The first fractionation step leading to a ^{13}C-enriched microbial biomass compared with SOM can be explained by isotope discrimination during biosynthesis of new microbial biomass (Potthoff et al., 2003). Compared with SOM, water-soluble organic compounds with a heavier isotopic composition are preferentially used by soil microorganisms (Henn and Chapela, 2000; Pelz et al., 2005). The second fractionation step yields more ^{13}C-enriched microbial CO_2 compared with the microbial biomass and the substrate. Usually, CO_2 from microbial respiration is ^{13}C-depleted compared with the feeding substrate (Blair et al., 1985; Mary et al., 1992; Potthoff et al., 2003). Opposite results, i.e. ^{13}C enrichment of CO_2 versus source, can be explained by a selective use of ^{13}C-enriched SOM compounds by microorganisms (Ågren et al., 1996; Werth et al., 2006). This selection was more pronounced than the ^{13}C depletion effect of the metabolism itself (Šantrůčková et al., 2000), resulting in ^{13}C-enriched CO_2. The $\delta^{13}C$ value of CO_2 changes during increasing decomposition of plant residues by −5‰ to +2‰ compared with the $\delta^{13}C$ value of the original substrate (Hamer et al., 2004). This requires considering both average fractionations on a single sampling date and their changes during a study. Total fractionation between SOM and microbial biomass and between SOM and CO_2—including kinetic fractionation and preferential utilisation—is important when using ^{13}C natural abundance methods. Compared with other pools like plants or bulk soil, the $\delta^{13}C$ values of CO_2 and microbial biomass often have the highest variation, which will affect the accuracy of below-ground carbon partitioning. Hence, fractionations should be determined with a large number of replicates to ensure exact calculations of plant-derived C to the CO_2 efflux and to the soil microbial biomass. Otherwise, problems could occur like the negative root-derived C contributions to the microbial biomass reported in this study.

5. Conclusions

The ^{14}C pulse labelling technique and the natural ^{13}C labelling technique yielded similar contributions of root-derived C to the CO_2 efflux from soil, when ^{13}C fractionation in the latter approach was considered between SOM and CO_2. Both methods also yielded similar contributions of root-derived C to the microbial biomass when ^{13}C fractionation between SOM and microbial biomass was considered. This calls for accounting for ^{13}C fractionation in calculations of maize-derived C contributions. Rhizodeposition and root-derived CO_2 efflux should only be estimated by the ^{14}C labelling method when plant biomass increases linearly.

Acknowledgements

The German Research Foundation (DFG) supported this study. The authors thank I. Subbotina and G. Bermejo-Dominguez for their practical support during the experiment, Dr. V. Cercasov for usage of the scintillation counter and Dr. W. Armbruster and E. Dachtler for the IRMS analyses.

References

Ågren, G.I., Bosatta, E., Balesdent, J., 1996. Isotope discrimination during decomposition of organic matter: a theoretical analysis. Soil Science Society of America Journal 60, 1121–1126.

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

Angers, D.A., Voroney, R.P., Côté, D., 1995. Dynamics of soil organic matter and corn residues affected by tillage practices. Soil Science Society of America Journal 59, 1311–1315.

Balesdent, J., Mariotti, A., 1996. Measurement of soil organic matter turnover using ^{13}C natural abundance. In: Boutton, T.W., Yamasaki, S.I. (Eds.), Mass Spectrometry of Soils. Marcel Dekker, New York, USA, pp. 83–111.

Blair, N., Leu, A., Munoz, E., Olsen, J., Kwong, E., Des Marais, D., 1985. Carbon isotope fractionation in heterotrophic microbial metabolism. Applied and Environmental Microbiology 50, 996–1001.

Bol, R., Moering, J., Kuzyakov, Y., Amelung, W., 2003. Quantification of priming and CO_2 respiration sources following slurry-C incorporation into two grassland soils with different C content. Rapid Communications in Mass Spectrometry 17, 1–6.

Boutton, T.W., 1991. Stable carbon isotope ratios of natural materials: II. atmospheric, terrestrial, marine, and freshwater environments. In: Coleman, D.C., Fry, B. (Eds.), Carbon Isotope Techniques. Isotopic Techniques in Plant, Soil, and Aquatic Biology. Academic Press, Inc., San Diego, pp. 173–185.

Boutton, T.W., 1996. Stable carbon isotope ratios of soil organic matter and their use as indicators of vegetation and climate change. In: Boutton, T.W., Yamasaki, S.I. (Eds.), Mass Spectrometry of Soils. Marcel Dekker, New York, pp. 47–82.

Bruulsema, T.W., Duxbury, J.M., 1996. Simultaneous measurement of soil microbial nitrogen, carbon, and carbon isotope ratio. Soil Science Society of America Journal 60, 1787–1791.

Chen, C.R., Condron, L.M., Xu, Z.H., Davis, M.R., Sherlock, R.R., 2006. Root, rhizosphere and root-free respiration in soils under grassland and forest plants. European Journal of Soil Science 57, 58–66.

Cheng, W., 1996. Measurement of rhizosphere respiration and organic matter decomposition using natural ^{13}C. Plant and Soil 183, 263–268.

Cheng, W., Coleman, D.C., Carroll, C.R., Hoffman, C.A., 1993. In situ measurement of root respiration and soluble C concentrations in the rhizosphere. Soil Biology & Biochemistry 25, 1189–1196.

Domanski, G., Kuzyakov, Y., Siniakina, S.V., Stahr, K., 2001. Carbon flows in the rhizosphere of ryegrass (*Lolium perenne*). Journal of Plant Nutrition and Soil Science 164, 381–387.

Ekblad, A., Högberg, P., 2000. Analysis of $\delta^{13}C$ of CO_2 distinguishes between microbial respiration of added C_4-sucrose and other soil respiration in a C_3-ecosystem. Plant and Soil 219, 197–209.

Ekblad, A., Högberg, P., 2001. Natural abundance of ^{13}C in CO_2 respired from forest soils reveals speed of link between tree photosynthesis and root respiration. Oecologia 127, 305–308.

Ekblad, A., Nyberg, G., Högberg, P., 2002. ^{13}C-discrimination during microbial respiration of added C_3-, C_4- and ^{13}C-labelled sugars to a C_3-forest soil. Oecologia 131, 245–249.

Fernandez, I., Cadisch, G., 2003. Discrimination against C-13 during degradation of simple and complex substrates by two white rot fungi. Rapid Communications in Mass Spectrometry 17, 2614–2620.

Glaser, B., 2005. Compound-specific stable-isotope ^{13}C analysis in soil science. Journal of Plant Nutrition and Soil Science 168, 633–648.

Grace, J., 2004. Understanding and managing the global carbon cycle. Journal of Ecology 92, 189–202.

Gregorich, E.G., Liang, B.C., Drury, C.F., Mackenzie, A.F., McGill, W.B., 2000. Elucidation of the source and turnover of water soluble and microbial biomass carbon in agricultural soils. Soil Biology & Biochemistry 32, 581–587.

Hamer, U., Dalhus, W., Marschner, B., Schulte, U., Gleixner, G., 2004. Isotopic ^{13}C fractionation during the mineralisation of organic substrates. In: Hamer, U. (Ed.) Priming effects of Dissolved Organic Substrates on the Mineralisation of Lignin, Peat, Soil Organic Matter and Black Carbon determined with ^{14}C and ^{13}C Isotope Techniques, Ph.D. Thesis Ruhr-Universität Bochum, Germany, pp. 129–150.

Henn, M.R., Chapela, I.H., 2000. Differential C isotope discrimination by fungi during decomposition of C_3- and C_4-derived sucrose. Applied and Environmental Microbiology 66, 4180–4186.

Kelting, D.L., Burger, J.A., Edwards, G.S., 1998. Estimating root respiration, microbial respiration in the rhizosphere, and root-free soil respiration in forest soils. Soil Biology & Biochemistry 30, 961–968.

Kristiansen, S.M., Brandt, M., Hansen, E.M., Magid, J., Christensen, B.T., 2004. ^{13}C signature of CO_2 evolved from incubated maize residues and maize-derived sheep faeces. Soil Biology & Biochemistry 36, 99–105.

Kuzyakov, Y., Cheng, W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology & Biochemistry 33, 1915–1925.

Kuzyakov, Y., Cheng, W., 2004. Photosynthesis controls of CO_2 efflux from maize rhizosphere. Plant and Soil 263, 85–99.

Kuzyakov, Y., Kretzschmar, A., Stahr, K., 1999. Contribution of *Lolium perenne* rhizodeposition to carbon turnover of pasture soil. Plant and Soil 213, 127–136.

Kuzyakov, Y., Ehrensberger, H., Stahr, K., 2001. Carbon partitioning and below-ground translocation by *Lolium perenne*. Soil Biology & Biochemistry 33, 61–74.

Kuzyakov, Y., Raskatov, A., Kaupenjohann, M., 2003. Turnover and distribution of root exudates of *Zea mays*. Plant and Soil 254, 317–327.

Liang, B.C., Wang, X.L., Ma, B.L., 2002. Maize root-induced change in soil organic carbon pools. Soil Science Society of America Journal 66, 845–847.

Lin, G., Rygiewicz, P.T., Ehleringer, J.R., Johnson, M.G., Tingey, D.T., 2001. Time-dependent responses of soil CO_2 efflux components to elevated atmospheric [CO_2] and temperature in experimental forest mesocosms. Plant and Soil 229, 259–270.

Mary, B., Mariotti, A., Morel, J.L., 1992. Use of ^{13}C variations at natural abundance for studying the biodegradation of root mucilage, roots and glucose in soil. Soil Biology & Biochemistry 24, 1065–1072.

Merckx, R., Dijkstra, A., den Hartog, A., van Veen, J.A., 1987. Production of root-derived material and associated microbial growth in soil at different nutrient levels. Biology and Fertility of Soils 5, 126–132.

Mueller, T., Joergensen, R.G., Meyer, B., 1992. Estimation of soil microbial biomass C in the presence of living roots by fumigation–extraction. Soil Biology & Biochemistry 24, 179–181.

Pelz, O., Abraham, W.-R., Saurer, M., Siegwolf, R., Zeyer, J., 2005. Microbial assimilation of plant-derived carbon in soil traced by isotope analysis. Biology and Fertility of Soils 41, 153–162.

Potthoff, M., Loftfield, N., Buegger, F., Wick, B., John, B., Jörgensen, R.G., Flessa, H., 2003. The determination of $\delta^{13}C$ in soil microbial biomass using fumigation–extraction. Soil Biology & Biochemistry 35, 947–954.

Qian, J.H., Doran, J.W., 1996. Available carbon released from crop roots during growth as determined by carbon-13 natural abundance. Soil Science Society of America Journal 60, 828–831.

Qian, J.H., Doran, J.W., Walters, D.T., 1997. Maize plant contributions to root zone available carbon and microbial transformations of nitrogen. Soil Biology & Biochemistry 29, 1451–1462.

Remus, R., Augustin, J., Hüve, K., Plugge, J., 2006. Dynamik des Eintrages und der Umsetzung von Assimilat-Kohlenstoff im Boden bei Roggen und Mais: Modellierung der C-Flüsse nach ^{14}C-Pulsbegasung. In: Merbach, W., Gans, W., Augustin, J. (Eds.), Reaktionen und Stoffflüsse im wurzelnahen Raum—16. Borkheider Seminar zur Ökophysiologie des Wurzelraumes. Beiträge aus der Hallenser Pflanzenernährungsforschung, No. 11. Verlag Grauer, Beuren, Stuttgart, pp. 40–48.

Robinson, D., Scrimgeour, C.M., 1995. The contribution of plant C to soil CO_2 measured using $\delta^{13}C$. Soil Biology & Biochemistry 27, 1653–1656.

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

Rochette, P., Angers, D.A., Flanagan, L.B., 1999. Maize residue decomposition measurement using soil surface carbon dioxide fluxes and natural abundance of carbon-13. Soil Science Society of America Journal 63, 1385–1396.

Ryan, M.C., Aravena, R., 1994. Combining ^{13}C natural abundance and fumigation–extraction methods to investigate soil microbial biomass turnover. Soil Biology & Biochemistry 26, 1583–1585.

Šantrůčková, H., Bird, M.I., Frouz, J., Šustr, V., Tajovský, K., 2000. Natural abundance of ^{13}C in leaf litter as related to feeding activity of soil invertebrates and microbial mineralisation. Soil Biology & Biochemistry 32, 1793–1797.

Schweizer, M., Fear, J., Cadisch, G., 1999. Isotopic (^{13}C) fractionation during plant residue decomposition and its implications for soil organic matter studies. Rapid Communications in Mass Spectrometry 13, 1284–1290.

Thornton, B., Paterson, E., Midwood, A.J., Sim, A., Pratt, S.M., 2004. Contribution of current carbon assimilation in supplying root exudates of *Lolium perenne* measured using steady-state ^{13}C labelling. Physiologia Plantarum 120, 434–441.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19, 703–707.

Warembourg, F.R., Paul, E.A., 1977. Seasonal transfers of assimilated ^{14}C in grassland: plant production and turnover, soil and plant respiration. Soil Biology & Biochemistry 9, 295–301.

Werth, M., Kuzyakov, Y., 2005. Below-ground partitioning (^{14}C) and isotopic fractionation ($\delta^{13}C$) of carbon recently assimilated by maize. Isotopes in Environmental and Health Studies 41, 237–248.

Werth, M., Kuzyakov, Y., 2006. Assimilate partitioning affects ^{13}C fractionation of recently assimilated carbon in maize. Plant and Soil 284, 311–325.

Werth, M., Subbotina, I., Kuzyakov, Y., 2006. Three-source partitioning of CO_2 efflux from soil planted with maize by ^{13}C natural abundance fails due to inactive microbial biomass. Soil Biology & Biochemistry 38, 2772–2781.

Wu, J., Jörgensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass-C by fumigation–extraction—an automated procedure. Soil Biology & Biochemistry 22, 1167–1169.

Zibilske, L.M., 1994. Carbon mineralization. In: Weaver, R.W., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., Tabatabai, A., Wollum, A. (Eds.), Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties. Soil Science Society of America Book Series, vol. 5, Soil Sci. Soc. Am., Inc., Madison, pp. 835–864.