ELSEVIER

Soil Biology & Biochemistry 38 (2006) 2772–2781

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Three-source partitioning of CO_2 efflux from soil planted with maize by ^{13}C natural abundance fails due to inactive microbial biomass

M. Werth[a,*], I. Subbotina[b], Y. Kuzyakov[a,c]

[a]*Institute of Soil Science and Land Evaluation, University of Hohenheim, Emil-Wolff-Str. 27, 70593 Stuttgart, Germany*
[b]*Department of Soil Science and Agrochemistry, State University of Rostov on Don, 344006, Rostov on Don, Russia*
[c]*Department of Agroecosystem Research, University of Bayreuth, 95440 Bayreuth, Germany*

Received 10 January 2006; received in revised form 12 April 2006; accepted 19 April 2006
Available online 17 May 2006

Abstract

A theoretical approach to the partitioning of carbon dioxide (CO_2) efflux from soil with a C_3 vegetation history planted with maize (*Zea mays*), a C_4 plant, into three sources, root respiration (RR), rhizomicrobial respiration (RMR), and microbial soil organic matter (SOM) decomposition (SOMD), was examined. The $\delta^{13}C$ values of SOM, roots, microbial biomass, and total CO_2 efflux were measured during a 40-day growing period. A three-source isotopic mass balance based on the measured $\delta^{13}C$ values and on assumptions made in other studies showed that RR, RMR, and SOMD amounted to 91%, 4%, and 5%, respectively. Two assumptions were thoroughly examined in a sensitivity analysis: the absence of ^{13}C fractionation and the conformity of $\delta^{13}C$ of microbial CO_2 and that of microbial biomass. This approach strongly overestimated RR and underestimated RMR and microbial SOMD. CO_2 efflux from unplanted soil was enriched in ^{13}C by 2.0‰ compared to microbial biomass. The consideration of this ^{13}C fractionation in the mass balance equation changed the proportions of RR and RMR by only 4% and did not affect SOMD. A calculated $\delta^{13}C$ value of microbial CO_2 by a mass balance equation including active and inactive parts of microbial biomass was used to adjust a hypothetical below-ground CO_2 partitioning to the measured and literature data. The active microbial biomass in the rhizosphere amounted to 37% to achieve an appropriate ratio between RR and RMR compared to measured data. Therefore, the three-source partitioning approach failed due to a low active portion of microbial biomass, which is the main microbial CO_2 source controlling the $\delta^{13}C$ value of total microbial biomass. Since fumigation–extraction reflects total microbial biomass, its $\delta^{13}C$ value was unsuitable to predict $\delta^{13}C$ of released microbial CO_2 after a C_3–C_4 vegetation change. The second adjustment to the CO_2 partitioning results in the literature showed that at least 71% of the active microbial biomass utilizing maize rhizodeposits would be necessary to achieve that proportion between RR and RMR observed by other approaches based on ^{14}C labelling. The method for partitioning total below-ground CO_2 efflux into three sources using a natural ^{13}C labelling technique failed due to the small proportion of active microbial biomass in the rhizosphere. This small active fraction led to a discrepancy between $\delta^{13}C$ values of microbial biomass and of microbially respired CO_2.

Keywords: $\delta^{13}C$; Root respiration; Rhizomicrobial respiration; SOM decomposition; ^{13}C isotopic fractionation; CO_2 efflux from soil; Microbial biomass; ^{13}C natural abundance

1. Introduction

Partitioning the total carbon dioxide (CO_2) efflux from soil is very important in identifying individual sinks or sources of CO_2. Root-derived CO_2 and CO_2 derived from soil organic matter (SOM) decomposition (SOMD) can be quantified by isotopic labelling of plants with ^{13}C or ^{14}C isotopes and tracing the label in root-derived CO_2 (Ekblad and Högberg, 2001; Kuzyakov and Cheng, 2001). The difference between this labelled fraction and total CO_2 efflux represents CO_2 from SOMD. The above studies on below-ground CO_2 from a boreal forest dominated by *Pinus sylvestris* and *Vaccinium myrtillus* (Ekblad and Högberg, 2001) and from soil planted with wheat (*Triticum*

Abbreviations: IRMS, isotope ratio mass spectrometer; RMR, rhizomicrobial respiration; SOM, soil organic matter; RR, root respiration; SOMD, soil organic matter decomposition

*Corresponding author. Tel.: +49 711 459 3684; fax: +49 711 459 3117.
E-mail address: werth@uni-hohenheim.de (M. Werth).

0038-0717/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2006.04.032

aestivum) (Kuzyakov and Cheng, 2001) revealed that about 70% of the CO_2 was derived from rhizosphere respiration and 30% from SOMD. However, these values vary, strongly depending on plants, soils, and environmental conditions. It is exceptionally difficult to further differentiate between CO_2 which is directly derived from root respiration and that derived from mineralization of rhizodeposits (Killham and Yeomans, 2001). This separation of root respiration (RR) and rhizomicrobial respiration (RMR) is one of the greatest challenges in quantifying rhizosphere carbon flows. Separation is important to quantify carbon sources for SOM and for rhizosphere microorganisms, identify respiration of autotrophic and heterotrophic organisms, and calculate carbon turnover by rhizosphere microorganisms (Kuzyakov, 2004).

To date, five adequate methods have been suggested to separate RR and RMR in non-sterile soils:

(1) the isotope dilution method (Cheng et al., 1993), i.e., isotopic dilution of rhizomicrobial $^{14}CO_2$ by addition of unlabelled glucose to the rhizosphere of ^{14}C-labelled plants, where $^{14}CO_2$ from RMR is inversely proportional to the glucose concentration in the rhizosphere, whereas $^{14}CO_2$ from RR is not affected by glucose addition;
(2) the model rhizodeposition technique (Swinnen, 1994), where two variants are used: (a) ^{14}C pulse-labelled plants without model rhizodeposits (RR and RMR) and (b) ^{14}C-labelled model rhizodeposits (glucose or plant extracts) added to soil with unlabelled plants (RMR);
(3) modelling of $^{14}CO_2$ efflux dynamics (Kuzyakov et al., 1999, 2001; Kuzyakov and Domanski, 2002), where a mathematical model is used to split up the curve of $^{14}CO_2$ efflux from soil with ^{14}C-labelled plants into RR and RMR by temporal delay of rhizomicrobial $^{14}CO_2$ compared to $^{14}CO_2$ from root respiration;
(4) the exudate elution procedure (Kuzyakov and Siniakina, 2001), based on the rapid elution of ^{14}C-labelled exudates from soil before microorganisms utilize them;
(5) the difference method between root-derived $^{14}CO_2$ and rhizomicrobial $^{14}CO_2$ (Johansson, 1992), where root-derived $^{14}CO_2$ evolved from the rhizosphere of plants continuously labelled in a $^{14}CO_2$ atmosphere (RR and RMR) is compared with $^{14}CO_2$ evolved by decomposition of uniformly ^{14}C-labelled rhizodeposits (RMR) obtained from the same plants.

These methods, their basic assumptions, as well as possible error sources have been described in detail earlier (Kuzyakov, 2002; Kuzyakov and Larionova, 2005). The first four methods are based on pulse labelling of shoots in a $^{14}CO_2$ atmosphere and subsequent monitoring of $^{14}CO_2$ efflux from the soil. However, the basic assumptions and principles of these methods, as well as the results observed in the original papers, all differ from one another. The comparison of the first four methods in a single experiment under equal conditions showed that $^{14}CO_2$ efflux from ryegrass (*Lolium perenne*) rhizosphere grown on a loamy haplic luvisol consisted of 40–50% RR and 50–60% RMR (Kuzyakov, 2002). The comparison showed that the isotope dilution method (Cheng et al., 1993) and the method based on modelling $^{14}CO_2$ efflux dynamics (Kuzyakov et al., 1999; Kuzyakov et al., 2001; Kuzyakov and Domanski, 2002) are the most reliable methods, because they showed similar separation results despite mutually exclusive assumptions. In the former method, the ratio of ^{14}C in CO_2 from RR to that derived from RMR is assumed to be constant during the observation, whereas this ratio is variable in the latter method.

Component integration (Edwards and Harris, 1977) and tree girdling (Högberg et al., 2001) are two other methods, which were tested to separate RR and RMR. Their shortcomings, including non-comparable respiration rates of disturbed and undisturbed soil in component integration, or stopping of RR and RMR by tree girdling, are discussed in detail by Kuzyakov (2005). Due to many difficulties and non-testable assumptions, none of the suggested methods is acceptable as a standard procedure for separately estimating RR and RMR. Owing to these uncertainties, new and more reliable approaches are required to separate RR, RMR, and SOM respiration types.

The objective of this study was to verify an approach to a quantitative estimation of (1) RR, (2) RMR, and (3) microbial respiration from SOMD in non-sterile soils. The theoretical approach was recently suggested by Kuzyakov (2004, 2005) and was practically tested here. The method is based on the natural ^{13}C labelling technique (Balesdent and Mariotti, 1996), i.e., ^{13}C natural abundance is used by growing C_4 plants on a soil developed under C_3 vegetation ('C_3 soil') or vice versa. Hence, the $\delta^{13}C$ values of SOM, maize roots, microbial biomass, and total CO_2 efflux from the soil are used to determine the three fractions of CO_2. These contributions of RR, RMR, and SOMD to total soil CO_2 efflux can be calculated according to the isotopic mass balance of microbial biomass and CO_2. This method involves two assumptions concerning ^{13}C isotopic effects during root and microbial respiration:

(1) the $\delta^{13}C$ isotope signature of CO_2 from rhizosphere respiration is the same as the $\delta^{13}C$ value of the roots; and
(2) the $\delta^{13}C$ isotope signature of CO_2 respired by microorganisms corresponds to the $\delta^{13}C$ value of microbial biomass.

A verification and discussion of these assumptions is provided here.

2. Materials and methods

2.1. Experimental set-up

Twenty maize plants (*Zea mays* L.) were grown under controlled laboratory conditions on a loamy haplic luvisol

from loess with C_3 vegetation history (*L. 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, Germany) filled with 400 g of the C_3 soil, 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 silicone rubber (TACOSIL 145, Thauer & Co., 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). Air was pumped through every single pot from bottom to top by a membrane pump (Type 113, Rietschle Thomas, Germany; pumping rate 100 ml min^{-1}), which was connected to the pot by a tube (Fig. 1). Another tube was connected to the top outlet of the filter device and to a CO_2 trapping tube filled with 20 ml of 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 that 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 the NaOH solution. Secondly, the resulting 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.

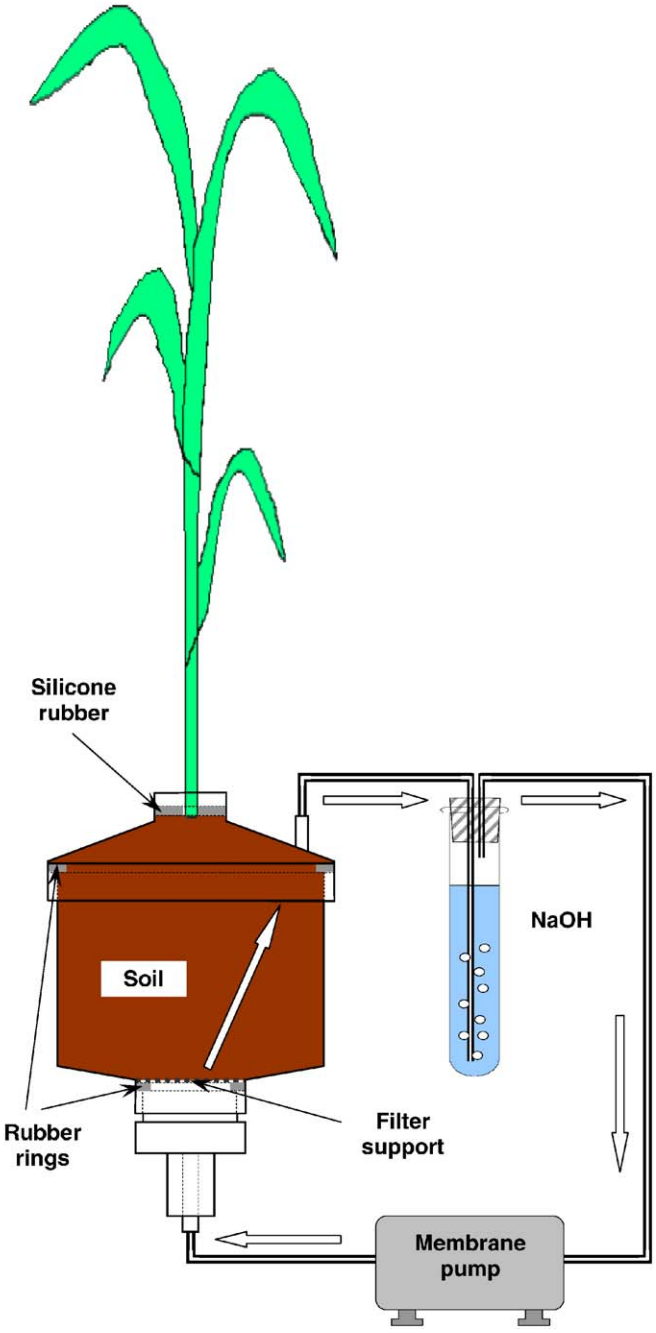

Fig. 1. Experimental set-up for trapping of below-ground CO_2 in NaOH solution. White arrows show air flow.

The soil moisture was maintained gravimetrically at about 25% of the water-holding capacity 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 water to the soil, from one to five times, depending on the date of sampling of the pots.

2.2. Sampling and analyses

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. The soil was divided into bulk soil, rhizosphere, and non-rhizosphere soil. 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 to gain the non-rhizosphere fraction. The soil adhering to the roots was collected as the rhizosphere fraction. Only the results of the rhizosphere fraction are presented here. The moist soil samples were immediately frozen until preparation for microbial biomass was started. 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 or twice between two harvest days.

To estimate total CO_2 efflux, the CO_2 trapped in NaOH solution was precipitated with a 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 was extracted with 40 ml of 0.05 M potassium sulphate (K_2SO_4) solution. Another 10 g of soil was first 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 rpm, centrifuged at 3000 rpm for 10 min, and then filtered through a ceramic vacuum filter. The extracts were frozen until analyses for total carbon (C) and nitrogen (N) concentrations by using a Dimatoc-100 TOC/TIC analyser (Dimatec, Germany). Microbial biomass C and N concentrations were calculated from these results by using a k_{EC} value of 0.45 (Wu et al., 1990) and a k_{EN} value of 0.54 (Brookes et al., 1985) and are presented in per cent of 1 g of dry soil. The soil water content was determined in another 10 g of soil that was dried at 105 °C. These soil samples and the plant samples were ground with a ball mill before analysis. The C and N concentrations in shoots, roots, and soil were measured with a Euro EA C/N analyser (EuroVector, Italy).

A Thermo Finnigan MAT Delta plus Advantage isotope ratio mass spectrometer (IRMS) was coupled to this 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 the 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 rpm for 10 min and washed in between with deionised and degassed water to remove NaOH and to achieve pH 7. Keeping the tubes open for washing for as short a time 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. Drying the K_2SO_4 extracts in tin capsules prevented volatilization of unstable compounds and additional ^{13}C fractionation, which is typical for freeze drying.

2.3. Calculations

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

$$\delta^{13}C_{MO} = \frac{\delta^{13}C_{fum}C_{fum} - \delta^{13}C_{extr}C_{extr}}{C_{fum} - C_{extr}}, \quad (1)$$

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

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

$$\delta^{13}C_{corrected} = \frac{\delta^{13}C_{total}C_{total} - \delta^{13}C_{air}C_{air}}{C_{total} - C_{air}}, \quad (2)$$

where $\delta^{13}C_{corrected}$ is the $\delta^{13}C$ 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 calculating the $\delta^{13}C$ of microbial biomass (Eq. (1)) and the corrected $\delta^{13}C$ of total CO_2 efflux (Eq. (2)), it was possible to calculate below-ground CO_2 partitioning. The development of the equations used to calculate below-ground CO_2 partitioning is presented in detail by Kuzyakov (2004). The equations for SOMD and RMR are:

$$SOMD = \frac{\delta^{CO_2} - \delta_4^{Rhiz}}{\delta_3^{SOM} - \delta_4^{Rhiz}}, \quad (3)$$

$$RMR = \frac{(\delta^{MO} - \delta_3^{SOM})(\delta^{CO_2} - \delta_4^{Rhiz})}{(\delta_4^{Rhiz} - \delta_3^{SOM})(\delta^{MO} - \delta_4^{Rhiz})}, \quad (4)$$

where δ^{CO_2} is the $\delta^{13}C$ value of the total CO_2 efflux from planted soil, δ_4^{Rhiz} is the $\delta^{13}C$ value of C_4 plant roots, δ_3^{SOM} is the $\delta^{13}C$ value of SOM from unplanted soil, and δ^{MO} is the $\delta^{13}C$ value of microorganisms from planted soil. Having calculated these two contributions to the below-ground CO_2 efflux, the remaining part would be RR:

$$RR = 1 - SOMD - RMR. \quad (5)$$

A calculated $\delta^{13}C$ value was used to determine the influence of active and inactive microbial biomass fractions on $\delta^{13}C$ of total microbial biomass. This $\delta^{13}C$ value ($\delta^{13}C_{total}$) was calculated by a mass balance equation using $\delta^{13}C$ values of maize roots for active ($\delta^{13}C_{active}$) and $\delta^{13}C$ values of SOM from unplanted soil for inactive ($\delta^{13}C_{inactive}$) portions of microbial biomass:

$$\delta^{13}C_{total} = \frac{\delta^{13}C_{active}C_{active} + \delta^{13}C_{inactive}C_{inactive}}{C_{total}}, \quad (6)$$

where C_{active}, $C_{inactive}$, and C_{total} are amounts of C in active, inactive, and total microbial biomass fractions, respectively. C_{total} was considered as 100%, C_{active} was adjusted to match measured results of below-ground CO_2 partitioning (see Section 3), and $C_{inactive}$ was C_{total}−C_{active}.

Standard deviations (SD) were calculated as a variability parameter for all our results. We used a one-way analysis of variance to identify differences between $\delta^{13}C$ values of various below-ground CO_2 sources. The effect of ^{13}C

fractionation by microbial respiration on below-ground CO_2 partitioning results was examined by a sensitivity analysis, according to Kuzyakov (2005). $\delta^{13}C$ of microbial CO_2 was increased stepwise in this sensitivity analysis from 1‰ to 5‰ compared to microbial biomass.

3. Results

3.1. C and N concentrations, C/N ratio, and cumulative CO_2 efflux from soil

The C concentration in plant parts was constant during the entire experiment and averaged about 43% and 33% for shoots and roots, respectively (Table 1). The low C concentration in roots can be explained by mineral soil particles remaining on roots after washing. Between days 16 and 40, the total N concentration in the shoots decreased by 2.1% (Table 1). The N concentration in the shoots was about twice as that in the roots. The N concentrations in both shoots and roots were expected to decrease because the plants grew and the amount of fertilization was held constant but not increased. Consequently, on day 40, the C/N ratio increased to 30 in the shoots and 50 in the roots (Table 1). C and N concentrations in the soil (Table 1) remained constant at 1.4% and 0.2%, respectively. The soil C/N ratio was 9 on all sampling days. The C concentration in the microbial biomass was only slightly increased on day 16, then remaining at about 0.022% of soil dry matter on the following dates (Table 1). The N concentration in microbial biomass was also stable during the whole experiment. The C/N ratio of the microbial biomass was 2 units higher compared to that of the bulk soil.

The cumulative CO_2 efflux from the planted soil increased linearly by 10.7 mg C day^{-1} (Fig. 2). In contrast, the control pots without plants showed a reduced rate of increase (2.7 mg C day^{-1}). As a first approximation of separate rhizosphere respiration and SOMD, the latter curve could be considered as CO_2 derived from SOMD (up to 34% of total CO_2 efflux from planted soil). The difference between the two curves would then be rhizosphere respiration, which amounted up to 66% of total CO_2 efflux from planted soil. This difference approach

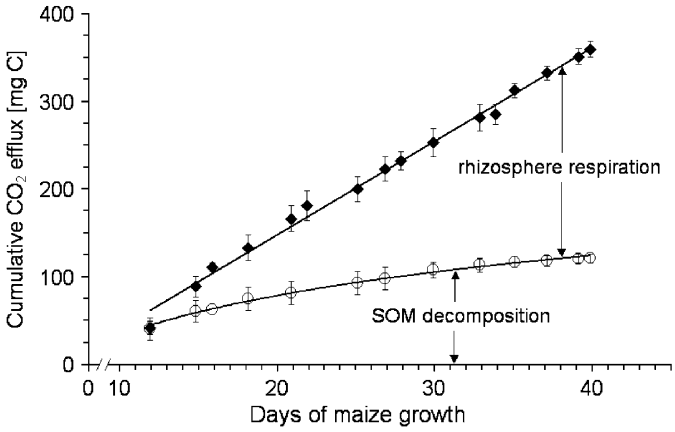

Fig. 2. Cumulative CO_2 efflux from C_3 soil with maize (♦) and without plants (○); error bars show standard deviation ($1 \leqslant n \leqslant 20$, dependent on sampling date).

Table 1
Carbon and nitrogen concentrations and C/N ratios of shoots, roots, soil, and microbial biomass on five sampling dates of maize grown on C_3 soil (mean ± SD, $n = 4$), based on plant part or soil dry matter

	Days of maize growth	C (% of dry matter)	N (% of dry matter)	C/N
Shoots	16	42.0 ± 2.3	3.6 ± 0.4	11.6 ± 0.6
	22	42.5 ± 1.1	2.7 ± 0.3	15.9 ± 1.4
	28	42.6 ± 2.0	2.1 ± 0.1	20.3 ± 0.5
	34	41.4 ± 2.5	1.7 ± 0.2	24.0 ± 2.4
	40	45.0 ± 1.7	1.5 ± 0.1	30.0 ± 2.0
Roots	16	33.1 ± 2.4	1.8 ± 0.2	18.7 ± 1.1
	22	32.9 ± 2.8	1.1 ± 0.1	30.0 ± 1.3
	28	31.8 ± 1.2	0.9 ± 0.0	35.2 ± 2.1
	34	32.5 ± 1.6	0.8 ± 0.0	41.8 ± 1.8
	40	32.8 ± 2.0	0.7 ± 0.1	50.2 ± 6.0
Soil	16	1.5 ± 0.1	0.2 ± 0.0	9.0 ± 0.4
	22	1.5 ± 0.0	0.2 ± 0.0	9.0 ± 0.1
	28	1.4 ± 0.2	0.2 ± 0.0	9.1 ± 0.8
	34	1.5 ± 0.0	0.2 ± 0.0	9.1 ± 0.3
	40	1.4 ± 0.1	0.2 ± 0.0	9.0 ± 0.2
Microbial biomass	16	0.031 ± 0.003	0.002 ± 0.001	11.3 ± 0.9
	22	0.020 ± 0.003	0.002 ± 0.000	10.7 ± 0.1
	28	0.024 ± 0.002	0.002 ± 0.000	11.2 ± 0.7
	34	0.021 ± 0.003	0.002 ± 0.000	11.2 ± 1.2
	40	0.021 ± 0.001	0.002 ± 0.000	11.1 ± 2.0

between planted and unplanted soil neglects interactions between enhanced microbial activity by rhizodeposition and SOMD. Thus, it is only a rough estimate of C flows in the rhizosphere.

3.2. $\delta^{13}C$ values and CO_2 efflux partitioning

Between days 16 and 40, the $\delta^{13}C$ of maize roots slightly decreased, averaging −15.8‰ (Fig. 3a). The $\delta^{13}C$ of the total CO_2 efflux from planted soil (−17.0‰) was significantly more negative ($P<0.05$), by 1‰, compared to $\delta^{13}C$ of the roots. Nevertheless, $\delta^{13}C$ values of roots and CO_2 were very similar. This similarity indicates a high contribution of RR to the total CO_2 efflux from the soil. The $\delta^{13}C$ values of CO_2 presented in Fig. 3a were corrected by Eq. (2) 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 to those of 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 $\delta^{13}C$ of SOM ($P<0.001$).

The $\delta^{13}C$ of SOM in unplanted soil (−27.0‰) was the same as that in planted soil (Fig. 3b). 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 ($P<0.001$), and of 2.0‰ between microbial biomass and microbially respired CO_2 ($P<0.05$). The fractionation between SOM and microbial CO_2 was 5.2‰ ($P<0.001$).

We calculated contributions of RR, RMR, and SOMD to total CO_2 efflux from the $\delta^{13}C$ values in Fig. 3 using Eqs. (3)–(5) (Fig. 4), which are based on the approach of Kuzyakov (2004). The contributions of RR to total CO_2 efflux were very dominant, with a maximum of 91% on days 34 and 40. RMR was maximally only 9% and SOMD doubled this value at maximum.

The portions of RR and RMR in rhizosphere respiration reported in other studies were about 50% each. In our experiment, there was a strong shift towards RR. Potential reasons for this shift are (1) the above-mentioned difference in $\delta^{13}C$ between microbial biomass and microbial CO_2 and (2) the discrepancy between the small active fraction of microbial biomass that feeds on rhizodeposits and the large fraction of microbially derived CO_2 from active microbial biomass. Both reasons are important, because we used $\delta^{13}C$ from microbial biomass to calculate microbially derived CO_2, assuming no fractionation between microbial biomass and microbial CO_2 (see assumption 2). The former case would have yielded underestimated contributions of microbial and rhizomicrobial CO_2 to total CO_2 efflux due to more negative $\delta^{13}C$ values of microbial biomass compared to microbial and rhizomicrobial CO_2. In the latter case, $\delta^{13}C$ of microbial biomass would have been mainly influenced by dormant microorganisms, which had fed formerly on SOM with C_3 signature, leading to a $\delta^{13}C$ value close to that of C_3 soil. However, the $\delta^{13}C$ of rhizomicrobially respired CO_2 would have been mainly controlled by active microorganisms in the rhizosphere, which fed on rhizodeposits, leading to a $\delta^{13}C$ value close to that of C_4 plants. These influences on the contributions of RR, RMR, and SOMD will be presented in the following two sections.

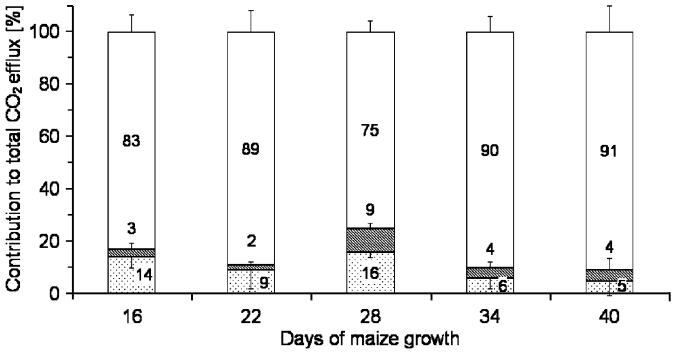

Fig. 4. Contributions of root respiration (no shading), rhizomicrobial respiration (hatched shading), and SOMD (dotted shading) to total CO_2 efflux from a C_3 soil planted with maize; error bars show standard deviation ($n=4$).

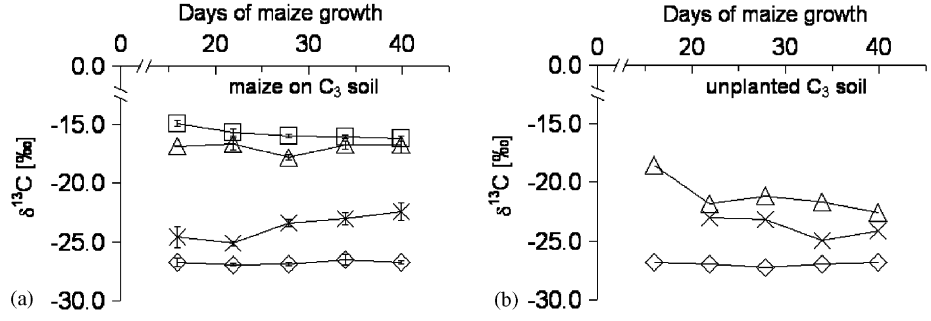

Fig. 3. $\delta^{13}C$ values of carbon pools in (a) maize grown for 40 days on a C_3 soil and (b) C_3 soil without plants. Carbon pools are maize roots (□), soil organic matter (◇), total CO_2 efflux (△), and microbial biomass (×); error bars in (a) show standard deviation ($n=4$); no error bars in (b) ($n=1$).

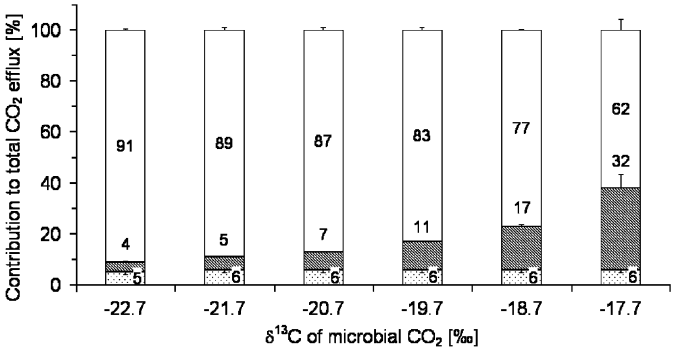

Fig. 5. Sensitivity analysis of ^{13}C fractionation between microbial biomass ($\delta^{13}C = -22.7‰$) and microbial CO_2 ($\delta^{13}C = -22.7‰ + 0$ to 5‰) on contributions of root respiration (no shading), rhizomicrobial respiration (hatched shading), and SOMD (dotted shading) to total CO_2 efflux from a C_3 soil planted with maize; mean CO_2 efflux contributions are built from days 34 and 40; error bars show standard deviation ($n = 2$).

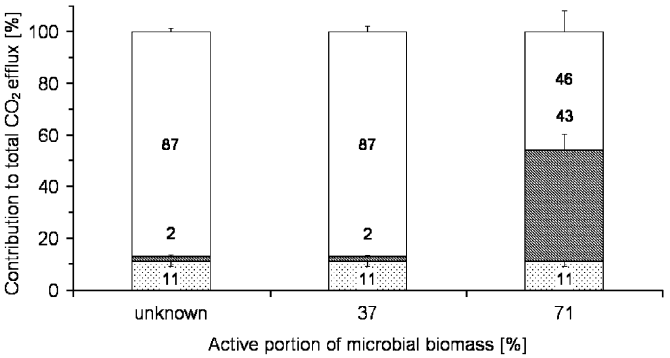

Fig. 6. Influence of active portion of microbial biomass on below-ground CO_2 partitioning. In the middle column, the active portion was adjusted to 37% of total microbial biomass to achieve calculated CO_2 partitioning results of this present study for a mean of days 34 and 40 (left column). In the right column, the active portion of microbial biomass was adjusted to 71% of total microbial biomass to achieve CO_2 partitioning results of literature studies (see Section 1). Patterns are: contributions of root respiration (no shading), rhizomicrobial respiration (hatched shading), and SOMD (dotted shading) to total CO_2 efflux from a C_3 soil planted with maize; error bars show standard deviation ($n = 2$).

3.3. Sensitivity analysis of changing ^{13}C fractionation on below-ground CO_2 partitioning

A sensitivity analysis was conducted to determine the effect of ^{13}C fractionation by microbial respiration on a mean of the CO_2 partitioning results from days 34 and 40. The $\delta^{13}C$ of microbial CO_2 was increased stepwise from 1‰ to 5‰ (Fig. 5). A maximum ^{13}C fractionation of 5‰ compared to $\delta^{13}C$ of microbial biomass increased RMR up to 32% and decreased RR down to 62% of total CO_2 efflux. The contribution of microbial SOMD was not affected by ^{13}C fractionation during microbial respiration. To determine the latter, $\delta^{13}C$ values of CO_2 efflux and microbial biomass from unplanted soil were monitored from day 10 to 40 (Fig. 3b). The difference between these $\delta^{13}C$ values showed a mean ^{13}C fractionation of 2.0‰ with a ^{13}C enrichment in the CO_2. Considering this ^{13}C fractionation in mass balance equation (4), the contributions of RR, RMR, and SOMD to total CO_2 efflux amounted to 87%, 7%, and 6%, respectively.

3.4. Effect of active microbial biomass on below-ground CO_2 partitioning

Isotopic ^{13}C fractionations of 2.0‰ between microbial biomass and microbial CO_2 and of 5.2‰ between SOM and SOM-derived CO_2 were accounted for in this approach. Using these fractionations and the $\delta^{13}C$ values from Fig. 3, we calculated the partitioning of CO_2 efflux from soil for a mean of the last two sampling dates (left column in Fig. 6). In order to simulate the influence of active and inactive fractions of the microbial biomass on CO_2 partitioning, we used calculated $\delta^{13}C$ values for the microbial biomass that considered both fractions (Eq. (6)). Percentages of these fractions in Eq. (6) were adjusted to match the CO_2 partitioning results obtained in this study (middle column in Fig. 6) and literature results (right column in Fig. 6). Values of $\delta^{13}C$ for roots (−16.2‰) and for SOM (−26.9‰) were used to represent $\delta^{13}C$ values for active and inactive microbial biomass fractions, respectively.

An active portion of about 37% of total microbial biomass, which feeds on maize rhizodeposits (middle column in Fig. 6), was determined to reflect the results observed in this study (left column in Fig. 6). A hypothetical active portion of 71% of total microbial biomass (right column in Fig. 6), however, would have been necessary to yield a 50% contribution each for RR and RMR related to total rhizosphere respiration as reported in various studies (Cheng et al., 1993; Kuzyakov et al., 2001).

4. Discussion

4.1. Evaluation of the natural ^{13}C labelling technique for below-ground CO_2 partitioning

On the last two sampling days (34 and 40 after germination), the $\delta^{13}C$ values and the partitioning of the below-ground CO_2 efflux showed that the plant–soil systems had stabilized (Figs. 3a and 4). RR was strongly overestimated by the examined approach of Kuzyakov (2004). RMR and SOMD were both remarkably underestimated. Two indications pointing to the incorrect estimate of CO_2 partitioning by using the ^{13}C labelling technique were found:

(1) The results of the cumulative CO_2 efflux of planted and unplanted soil show much lower portions of rhizosphere respiration (66%) and higher portions of SOMD (34%), which fit very well with literature results (Ekblad and Högberg, 2001; Kuzyakov and Cheng, 2001).

(2) From the literature reviewed in Section 1, we have calculated that RR and RMR each contribute equally (50%) to rhizosphere respiration.

On the basis of these two considerations, the results obtained by the natural ^{13}C labelling technique in this study cannot be accepted.

4.2. Verification of assumptions on ^{13}C fractionation

The below-ground CO_2 partitioning results change slightly if we consider the two assumptions from Section 1. The first assumption—equal $\delta^{13}C$ values of roots and rhizosphere respiration—has been used in most rhizosphere CO_2 studies to date (Cerling et al., 1991; Amundson et al., 1998; Fu and Cheng, 2002). The study by Cheng (1996), in which winter wheat was grown on C-free vermiculite and on a vermiculite–sand mixture, proves this assumption. Even if fractionation occurs in this process, it should be very small, and root-respired CO_2 should be only about 0.7‰ depleted in ^{13}C compared to roots (Werth and Kuzyakov, 2005). Hence, the first assumption has to be accepted.

The second assumption—equal $\delta^{13}C$ values of microbial biomass and microbial CO_2—was checked in the literature: we found ^{13}C fractionations not only between microbial biomass and CO_2 but also between microbial biomass and SOM and between SOM and CO_2. The results vary strongly for the first fractionation between microbial biomass and CO_2. According to Šantrůčková et al. (2000a), $\delta^{13}C$ values of CO_2 respired from 21 Australian soils with C_3 and C_4 vegetation were depleted on average by +2.2‰ compared to microbial biomass. For individual soils, the $\delta^{13}C$ difference between microbial biomass and respired CO_2 varied between +0.1‰ and +5.7‰. Our results, however, showed a ^{13}C enrichment of CO_2 by 2.0‰ compared to microbial biomass (Fig. 3b). This contradiction needs to be discussed relative to the second and third fractionation.

For the second fractionation between microbial biomass and SOM, we observed $\delta^{13}C$ values on average about 3.2‰ higher in the microbial biomass compared to SOM in unplanted soil samples. Results of recent studies confirm this fractionation (Ryan et al., 1995; Šantrůčková et al., 2000a; Potthoff et al., 2003). Isotope discrimination during biosynthesis of new microbial biomass and the heavier isotopic composition of organic compounds preferentially used by soil microorganisms explain this ^{13}C enrichment in microbial biomass (Potthoff et al., 2003).

The third fractionation between SOM as the substrate and microbial CO_2 as the product is the sum of the first and the second fractionation. Usually, CO_2 from microbial respiration is ^{13}C depleted compared to the feeding substrate (Blair et al., 1985; Mary et al., 1992; Potthoff et al., 2003). In a study by Šantrůčková et al. (2000b), the difference between $\delta^{13}C$ of SOM and that of respired CO_2 varied between +0.5‰ and −1.7‰. Formánek and Ambus (2004) reported a ^{13}C enrichment of respired CO_2 compared to SOM between 3.6‰ and 5‰. These results imply a ^{13}C enrichment of CO_2 compared to the substrate in most cases. Such an enrichment agrees with our results from unplanted soil (Fig. 3b) and indicates that only a ^{13}C-enriched fraction of the total organic C was used in these mineralization processes. This isotope effect associated with the selective use of organic compounds was more pronounced than the ^{13}C depletion effect of the metabolism itself (Šantrůčková et al., 2000b). The use of this ^{13}C-enriched SOM fraction leads to a more rapid loss of ^{13}C than ^{12}C during decomposition and therefore depletes the ^{13}C in the remaining material (Benner et al., 1987; Ågren et al., 1996). These results led us to use a 5.2‰ fractionation between SOM and CO_2 in considering the effects of active microbial biomass on below-ground CO_2 partitioning.

Fractionations in the CO_2 sampling and sample preparation can be excluded, because we eliminated the influence of atmospheric CO_2 by calculating corrected $\delta^{13}C$ values according to Eq. (2) and because we completely precipitated the CO_2 by $SrCl_2$ solution. Consequently, our second assumption cannot be accepted because ^{13}C isotopic fractionations between microbial biomass and microbial CO_2 and between SOM and CO_2 from its decomposition remain the two most important sources of error in below-ground CO_2 partitioning. These two fractionations have to be measured under experimental conditions.

The enormous impact of ^{13}C fractionation between microbial biomass and CO_2 on RMR is evident in the 3–5‰ fractionation range in our sensitivity analysis (Fig. 5). Nevertheless, even with 5‰ fractionation, the results of former studies (50% RR and RMR each in relation to rhizosphere respiration) and the results of our cumulative CO_2 efflux from planted and unplanted soil (66% rhizosphere respiration, 34% SOMD) could not be achieved by the tested isotopic approach. Due to a shift in the $\delta^{13}C$ value of microbial respiration with an increase in fractionation towards the $\delta^{13}C$ value of the maize roots, the impact of this fractionation on SOMD was visible only at the second decimal place.

4.3. Influence of active microbial biomass on below-ground CO_2 partitioning

Since only a minor part of microbial biomass is metabolically active in soil (Stenström et al., 2001), we examined the effect of active microbial biomass on below-ground CO_2 partitioning. In both cases—the one matching our measured results and the other one matching literature results—the active microbial biomass fraction (37–71% of total microbial biomass) is rather high (Fig. 6). Especially for this short 40-day period, other studies showed much lower maximum values of 6–23% (Bruulsema and Duxbury, 1996; Qian and Doran, 1996; Rochette et al., 1999). Thus, 37% active microbial biomass gives a very high estimate to explain our results, and 71% is only a

theoretical value to approximate our data to literature data. Thus, a very high active microbial fraction would be necessary to match the literature results (50% RR and 50% RMR contribution to rhizosphere respiration), an absolutely unrealistic value in a real ecosystem. Our calculations assumed that active microbial biomass feeds solely on rhizodeposits. Clearly, some microorganisms also feed on SOM. Our calculated active fraction would therefore be slightly larger when including the latter microorganisms. The inactive microbial biomass fraction would be correspondingly smaller.

Besides the large contribution of inactive SOM-feeding organisms to the microbial biomass, Bruulsema and Duxbury (1996) assumed that the chloroform fumigation method solubilizes a substantial fraction of less active non-microbial soil organic C. Consequently, the natural ^{13}C labelling method fails due to (1) a low active microbial biomass fraction and/or (2) chloroform-soluble non-living organic material.

5. Conclusions

The isotopic mass balance from soil planted with maize was insufficient to accurately partition total CO_2 efflux into three CO_2 sources: RR, RMR, and SOMD. The method strongly overestimated RR and underestimated RMR and SOMD. The main problem of the approach was the strong discrepancy between $\delta^{13}C$ values of CO_2 respired by microbial biomass and of the microbial biomass itself, indicating that only a small portion of active microorganisms utilized maize rhizodeposits. Besides this discrepancy, isotopic fractionation during SOMD and microbial biomass respiration should be estimated in separate experiments with unplanted soil; the results should be considered in all calculations.

Mathematically changing the portion of active microbial biomass showed that this microbial biomass is mainly responsible for altered RR and RMR portions. To attain the partitioning results of other studies, the portion of active microbial biomass would have to be at least 71%. We conclude that the three-sources CO_2 partitioning approach using a natural ^{13}C labelling technique failed and do not recommend its use in future studies.

Acknowledgements

The German Research Foundation (DFG) supported this study. The authors would also like to thank 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.

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

Balesdent, J., Mariotti, A., 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, pp. 83–111.

Benner, M.H., Hendrix, P.F., Coleman, D.C., 1987. Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. Nature 329.

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.

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. Academic Press, San Diego, pp. 173–185.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17, 837–842.

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.

Cerling, T.E., Solomon, D.K., Quade, J., Bowman, J.R., 1991. On the isotopic composition of carbon in soil carbon dioxide. Geochimica et Cosmochimica Acta 55, 3404–3405.

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 and Biochemistry 25, 1189–1196.

Edwards, N.T., Harris, W.F., 1977. Carbon cycling in a mixed deciduous forest floor. Ecology 58, 431–437.

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.

Formánek, P., Ambus, P., 2004. Assessing the use of $\delta^{13}C$ natural abundance in separation of root and microbial respiration in a Danish beech (*Fagus sylvatica* L.) forest. Rapid Communications in Mass Spectrometry 18, 1–6.

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

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

Johansson, G., 1992. Release of organic C from growing roots of meadow fescue (*Festuca pratensis* L.). Soil Biology and Biochemistry 24, 427–433.

Killham, K., Yeomans, C., 2001. Rhizosphere carbon flow measurement and implications: from isotopes to reporter genes. Plant and Soil 232, 91–96.

Kuzyakov, Y., 2002. Separating microbial respiration of exudates from root respiration in non-sterile soils: a comparison of four methods. Soil Biology and Biochemistry 34, 1621–1631.

Kuzyakov, Y., 2004. Separation of root and rhizomicrobial respiration by natural ^{13}C abundance: theoretical approach, advantages, and difficulties. Eurasian Soil Science 37, S79–S84.

Kuzyakov, Y., 2005. Theoretical background for partitioning of root and rhizomicrobial respiration by $\delta^{13}C$ of microbial biomass. European Journal of Soil Biology 41, 1–9.

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

Kuzyakov, Y., Domanski, G., 2002. Model for rhizodeposition and CO_2 efflux from planted soil and its validation by ^{14}C pulse labelling of ryegrass. Plant and Soil 239, 87–102.

Kuzyakov, Y., Larionova, A.A., 2005. Root and rhizomicrobial respiration: a review of approaches to estimate respiration by autotrophic and

heterotrophic organisms in soil. Journal of Plant Nutrition and Soil Science 168, 503–520.

Kuzyakov, Y., Siniakina, S.V., 2001. A novel method for separating root-derived organic compounds from root respiration in non-sterilized soils. Journal of Plant Nutrition and Soil Science 164, 511–517.

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 and Biochemistry 33, 61–74.

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 and Biochemistry 24, 1065–1072.

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 and 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.

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., Gillham, R.W., 1995. The use of ^{13}C natural abundance to investigate the turnover of the microbial biomass and active fractions of soil organic matter under two tillage treatments. In: Lal, R., Kimble, J., Levine, E., Stewart, B.A. (Eds.), Soils and Global Change. CRC Press, Boca Raton, pp. 351–360.

Šantrůčková, H., Bird, M.I., Lloyd, J., 2000a. Microbial processes and carbon-isotope fractionation in tropical and temperate grassland soils. Functional Ecology 14, 108–114.

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

Stenström, J., Svensson, K., Johansson, M., 2001. Reversible transition between active and dormant microbial states in soil. FEMS Microbiology Ecology 36, 93–104.

Swinnen, J., 1994. Evaluation of the use of a model rhizodeposition technique to separate root and microbial respiration in soil. Plant and Soil 165, 89–101.

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

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.

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 and 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, Madison, pp. 835–864.