Three-source partitioning of CO_2 efflux from maize field soil by ^{13}C natural abundance

Martin Werth[1]* and **Yakov Kuzyakov**[2]

[1] Institute of Systematic Botany and Ecology, University of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany
[2] Department of Agroecosystem Research, University of Bayreuth, 95440 Bayreuth, Germany

Abstract

A natural-^{13}C-labeling approach—formerly observed under controlled conditions—was tested in the field to partition total soil CO_2 efflux into root respiration, rhizomicrobial respiration, and soil organic matter (SOM) decomposition. Different results were expected in the field due to different climate, site, and microbial properties in contrast to the laboratory. Within this isotopic method, maize was planted on soil with C_3-vegetation history and the total CO_2 efflux from soil was subdivided by isotopic mass balance. The C_4-derived C in soil microbial biomass was also determined. Additionally, in a root-exclusion approach, root- and SOM-derived CO_2 were determined by the total CO_2 effluxes from maize (*Zea mays* L.) and bare-fallow plots. In both approaches, maize-derived CO_2 contributed 22% to 35% to the total CO_2 efflux during the growth period, which was comparable to other field studies. In our laboratory study, this CO_2 fraction was tripled due to different climate, soil, and sampling conditions. In the natural-^{13}C-labeling approach, rhizomicrobial respiration was low compared to other studies, which was related to a low amount of C_4-derived microbial biomass. At the end of the growth period, however, 64% root respiration and 36% rhizomicrobial respiration in relation to total root-derived CO_2 were calculated when considering high isotopic fractionations between SOM, microbial biomass, and CO_2. This relationship was closer to the 50% : 50% partitioning described in the literature than without fractionation (23% root respiration, 77% rhizomicrobial respiration). Fractionation processes of ^{13}C must be taken into account when calculating CO_2 partitioning in soil. Both methods—natural ^{13}C labeling and root exclusion—showed the same partitioning results when ^{13}C isotopic fractionation during microbial respiration was considered and may therefore be used to separate plant- and SOM-derived CO_2 sources.

Key words: CO_2 efflux / rhizomicrobial respiration / root respiration / SOM decomposition / ^{13}C isotopic fractionation / ^{13}C natural abundance

Accepted May 12, 2008

1 Introduction

Partitioning the total CO_2 efflux from soil is very important in order to identify individual C sinks or sources. This CO_2 efflux can be separated into five components (*Kuzyakov*, 2006): (1) root respiration, *i.e.*, respiration of assimilates by roots of autotrophic plants, (2) rhizomicrobial respiration, *i.e.,* respiration of rhizodeposits (exudates, lysates, mucilages, *etc.*) by heterotrophic microorganisms in the rhizosphere, (3) decomposition of dead plant residues by heterotrophic microorganisms, (4) priming effect, *i.e.,* plant-induced additional (or limited) decomposition of soil organic matter (SOM) by heterotrophic microorganisms, and (5) decomposition of SOM by heterotrophic microorganisms. In the absence of plant residues and assuming a low contribution of priming effects in fertilized agricultural soils (*Cheng* and *Coleman*, 1990; *Paterson* and *Sim*, 1999, 2000), the three main components of CO_2 efflux will be (1) root respiration, (2) rhizomicrobial respiration, and (3) SOM decomposition. The sum of CO_2 from root respiration and rhizomicrobial respiration is termed "root-derived CO_2" and the related process "root-derived respiration" (RDR).

Carbon dioxide derived from SOM decomposition (SOMD) and that derived from the roots can be quantified by isotopic labeling of plants with ^{13}C or ^{14}C isotopes and tracing the label in root-derived CO_2 (*e.g., Andrews* et al., 1999; *Warembourg* and *Paul*, 1977). The difference between this labeled fraction and the total CO_2 efflux represents SOMD. Nonisotopic methods to separate root- from SOM-derived CO_2, such as a combination of trenching and excised-root methods, have also been used (*Chen* et al., 2006; *Kelting* et al., 1998). The results vary strongly depending on plants, soils, and environmental and experimental conditions. By *in situ* ^{14}C labeling 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 to the total CO_2 efflux, with values of up to 99% in a ryegrass (*Lolium perenne* L.) rhizosphere. Various studies with grass species have found results within this range of root-derived CO_2 (Tab. 1).

It is very difficult to further differentiate between the CO_2 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 a major challenge in quantifying rhizo-

* Correspondence: Dr. M. Werth; e-mail: martin.werth@uni-ulm.de

Table 1: Contributions of root-derived respiration (RDR) and SOM decomposition (SOMD) to total CO_2 efflux and of root respiration (RR) and rhizomicrobial respiration (RMR) to root-derived respiration from various laboratory and field studies with grass species (means ± standard deviations).

Site	CO_2 partitioning [%]		References
CO_2 efflux	**RDR**	**SOMD**	
Laboratory	70 ± 27	30 ± 27	*Chen* et al. (2006), *Robinson* and *Scrimgeour* (1995), *Qian* et al. (1997), *Kuzyakov* and *Cheng* (2001), *Midwood* et al. (2006)
Field	36 ± 15	64 ± 15	*Warembourg* and *Paul* (1977), *Gloser* and *Tesa* (1978), *Rochette* and *Flanagan* (1997), *Rochette* et al. (1999b), *Raich* and *Mora* (2005)
Total	53 ± 27	47 ± 27	see above
RDR	**RR**	**RMR**	
Laboratory	49 ± 10	51 ± 10	*Johansson* (1992), *Cheng* et al. (1993), *Kuzyakov* et al. (1999, 2001), *Kuzyakov* (2002b), *Kuzyakov* and *Domanski* (2002), *Sapronov* and *Kuzyakov* (2004), *Chen* et al. (2006)
Field	56 ± 7	44 ± 8	*Craine* et al. (1999), *Larionova* et al. (2006)
Total	50 ± 10	50 ± 10	see above

sphere C flows. Separation is important to quantify C sources for SOM and for rhizosphere microorganisms, to identify respiration of autotrophic and heterotrophic organisms, and to calculate C turnover by rhizosphere microorganisms (*Kuzyakov*, 2004). Recent studies on various grass species reveal that root-derived CO_2 consists of nearly equal RR and RMR contributions (Tab. 1).

Some attempts to separate root and rhizomicrobial respiration were tested with various success (reviewed by *Kuzyakov* and *Larionova*, 2005). Most of those studies were conducted under controlled conditions. The methods were based on pulse labeling of plants in a $^{14}CO_2$ atmosphere and tracing the $^{14}CO_2$ dynamics (*Kuzyakov* and *Domanski*, 2002; *Kuzyakov* et al., 1999, 2001), isotopic dilution (*Cheng* et al., 1993), or various treatments with ^{14}C-labeled plants and rhizodeposits (*Johansson*, 1992; *Swinnen*, 1994). The only field studies that attempted to separate root and rhizomicrobial respiration were based on trenching and excised-roots (*Kelting* et al., 1998), on shading and clipping and excised-roots (*Craine* et al., 1999), or on root-exclusion and component-integration (*Larionova* et al., 2006) methods. All these approaches very strongly disturb the soil and/or the roots, making the relevance of these results questionable.

In the present study, the CO_2 efflux from soil was partitioned into two sources, *i.e.,* plant- and SOM-derived pools, by means of natural ^{13}C labeling and root exclusion. A further partitioning of CO_2 into three sources, *i.e.,* root respiration, rhizomicrobial respiration, and SOM decomposition, was achieved by natural ^{13}C labeling.

The natura-^{13}C-labeling approach was recently suggested by *Kuzyakov* (2004, 2005) and was practically tested under controlled conditions by *Werth* et al. (2006). The method is based on the natural-^{13}C-labeling 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, 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 (*Kuzyakov*, 2004, 2005). Contributions of root-derived CO_2 (RDR) and of SOM-derived CO_2 (SOMD) were compared to the results of the root-exclusion approach. Total CO_2 effluxes from maize (*Zea mays*) and bare-fallow plots were considered to estimate root- and SOM-derived CO_2 in the root-exclusion approach used in our study. The same type of root-exclusion technique was used earlier, *e.g.,* by *Rochette* et al. (1999b).

The main differences between the two approaches are: (1) in the natural-^{13}C-labeling approach, the $\delta^{13}C$ values of CO_2 and microbial biomass are measured on the maize plot only and (2) in the root-exclusion approach, total CO_2 effluxes are measured from two different plots (maize and bare fallow). Hence, the natural-^{13}C-labeling approach has some disadvantages—the two most important are isotopic fractionation and activity of rhizosphere microorganisms. Natural ^{13}C labeling involves two assumptions concerning ^{13}C isotopic effects during root and microbial respiration (*Kuzyakov*, 2004, 2005):

(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 CO_2 respired by microorganisms corresponds to the $\delta^{13}C$ value of microbial biomass.

The first assumption has been accepted by *Werth* et al. (2006), since ^{13}C depletion of root-derived CO_2 ranges from 0.2‰ in sand (*Cheng*, 1996) to 0.7‰ in nutrient-rich hydroculture (*Werth* and *Kuzyakov*, 2006) compared to the $\delta^{13}C$ value of the roots. The second assumption, however, cannot be accepted and hence, changes of $\delta^{13}C$ (*i.e.,* "fractionations") between microbial biomass and microbial CO_2 have to be considered (*Werth* et al., 2006). Additionally, isotopic fractionations between substrate (SOM or exudates) and microbial biomass are important and have to be determined.

Only a minor part of microbial biomass is metabolically active in soil (*Stenström* et al., 2001), *i.e.,* consuming rhizodeposits from plants. Some studies have reported this active fraction between 6% and 23% of total microbial biomass after a single growing season (*Bruulsema* and *Duxbury*, 1996; *Qian* and *Doran*, 1996; *Rochette* et al., 1999a). Hence, activity *versus* dormancy of soil microorganisms is crucial when calculating RMR using the natural-^{13}C-labeling technique, since the method of *Kuzyakov* (2004, 2005) uses the δ^{13}C value of microbial biomass itself as a substitute for the δ^{13}C value of microbial CO_2 (the sum of RMR and SOMD). It is not possible to separate microbial CO_2 directly. In order to determine the C_4-derived C-consuming active microorganisms contributing to the δ^{13}C of total microbial biomass, we used a calculated δ^{13}C value by mass balance of C_4- and C_3-source contributions and back-calculated the CO_2-efflux partitioning obtained by the natural-^{13}C-labeling technique including isotopic fractionations (*Werth* et al., 2006).

Compared to the natural-^{13}C-labeling approach, the root-exclusion method has its own limitations: (1) the water regime and temperature balance may differ considerably between planted and unplanted soil (*Fisher* and *Gosz*, 1986; *Jones* et al., 2004; *Rochette* et al., 1999b; *Ross* et al., 2001), (2) the cycling of nutrients such as N, which affects the C cycle, also varies between vegetated and nonvegetated soil (*Hinsinger* et al., 2005; *Rochette* et al., 1999b), and (3) the decomposition of SOM and other plant residues is dependent on both the physical effects (water regime and temperature balance) of vegetation on soil and the direct biological effects of living roots (reviewed by *Cheng* and *Kuzyakov*, 2005; *Dormaar*, 1990; *Kuzyakov*, 2002a, b; *Paterson*, 2003). Consequently, exudation from maize roots could either increase (or decrease) SOM decomposition by priming effects (*Kuzyakov* et al., 2000). Since we calculated SOM decomposition only from the bare-fallow plot, priming effects cannot be considered in the root-exclusion approach, but by direct comparison of SOM-derived CO_2 from bare-fallow and maize plots.

In both approaches, seasonal effects in CO_2-efflux partitioning could occur. In the root-exclusion approach, site properties like soil moisture content, soil temperature, or soil C : N ratio may change in planted and bare-fallow plots in space and also in time. In the natural-^{13}C-labeling approach, isotopic fractionation and δ^{13}C values could differ during a growing season especially in plant parts, soil microbial biomass, and CO_2 (*Pate* and *Arthur*, 1998; *Scartazza* et al., 2004).

The objective of this study was to test the natural-^{13}C-labeling approach under field conditions in order to separate SOM- and root-derived CO_2, and to further split up the latter into root and rhizomicrobial respiration. This method was not successful under laboratory conditions, since fewer microorganisms were consuming C_4 than were consuming C_3 source (*Werth* et al., 2006). We therefore hypothesize:

(1) In the field, the root system can develop during a whole growth period and there is no limitation of rhizosphere development by plant-container size. Hence, the active microbial community feeding on maize rhizodeposits could be much larger than in the laboratory.

(2) This larger rhizosphere and also natural climate and site conditions (*e.g.,* soil moisture and temperature) in contrast to controlled laboratory conditions could have changed the CO_2-partitioning results in contrast to the short laboratory period.

(3) Accounting for ^{13}C fractionation between substrates and products could increase the amount of rhizomicrobial respiration.

(4) We expected that natural ^{13}C labeling rather than root exclusion could better reproduce root- and SOM-derived CO_2 pools of former field studies (Tab. 1), since in natural ^{13}C labeling, all samples were taken from the same plot, whereas root exclusion depended on two different plots.

(5) Finally, CO_2 partitioning could be affected by seasonal climate differences.

2 Materials and methods

2.1 Experimental set-up

In May 2004, a maize (*Zea mays* L., cv. Tassilo) plot (10 m^2, seed distance: 30 cm within rows, 75 cm between rows) was established on a loamy Haplic Luvisol from loess with C_3-vegetation history (grasses with dominance of *Lolium perenne* L.), located on the University of Hohenheim's research farm "Heidfeldhof" in Stuttgart, Germany (48°42′50″ N, 9°11′21″ E). No C_4 plants have ever been grown on this plot before, which was crucial to the use of the natural-^{13}C-labeling technique, since it works only in the first year of C_3–C_4 vegetation change. Nine steel collars were installed on the plot, each collar 10 cm away from a maize shoot. The steel collars (11 cm ∅, 10 cm height) were inserted 5 cm deep into the soil to trap CO_2 from the soil. Additional nine steel collars were established on a bare-fallow plot located next to the maize plot. Both plots were fertilized with 202 kg N ha^{-1}, equally provided as NH_4^+ and NO_3^-. The plots were kept free of weeds by the pesticide dimetheanid–P (900 g ha^{-1}) and by manual weeding.

2.2 Sampling and analyses

On day 39 (July 6, 2004) after germination of the maize, a glass dish filled with 20 mL 1 *M* NaOH solution was placed into every collar of the maize and bare-fallow plots, and the collars were sealed with a plastic lid. The CO_2 efflux from the soil was trapped for 7 d in NaOH, then the NaOH was collected from the traps, and the glass dishes were rinsed with 20 mL deionized water, which was mixed with the NaOH samples. The trapping and sampling procedure was repeated at day 117 of maize growth (September 22, 2004).

On the first day of each CO_2-trapping period, soil samples were taken from 0 to 5 cm depth next to the steel collars. The moist soil samples were immediately frozen until preparation for microbial-biomass analyses. We assume that there is no significant difference between microbial C and N concentrations of field-moist and frozen soil, which was approved by

Stenberg et al. (1998). The maize was harvested in mid-October, when the plants were 146 d old. The roots were carefully washed with deionized water to remove adhering soil particles. Shoots and roots were dried at 40°C.

To estimate the total CO_2 efflux, the CO_2 trapped in NaOH solution was precipitated with 0.5 *M* $BaCl_2$ solution, and then the NaOH was titrated with 0.2 *M* HCl against phenolphthalein indicator (*Zibilske*, 1994). Soil microbial biomass was determined by the chloroform-fumigation extraction method (*Vance* et al., 1987), in which the typical extractant concentration of 0.5 *M* K_2SO_4 solution was reduced to 0.05 *M* in order to increase the ratio of extracted C and N to salt to facilitate analysis by direct combustion prior to mass-spectrometer analyses. *Bruulsema* and *Duxbury* (1996) have shown that the same extraction-efficiency factors (k_{EC} and k_{EN}) can be used in this modified method compared to the original method. Aggregates of the unfrozen soil were destroyed with tweezers, and roots were carefully removed from the sample by handpicking. An amount of 10 g of soil was extracted with 40 mL of the 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 on a horizontal shaker, centrifuged at 1449 *g* for 10 min, and then filtrated through a ceramic vacuum filter. The extracts were frozen until analyses for total C and N concentrations were done with a Dimatoc-100 TOC/TIC analyzer (Dimatec, Germany). Microbial biomass C and N concentrations were calculated from these results 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 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 an aliquot of the leaf or root samples were ground with a ball mill before analysis. The total C and N content in leaves, roots, and soil was measured with a Euro EA C/N analyzer (EuroVector, Italy).

A Thermo Finnigan MAT Delta plus Advantage isotope-ratio mass spectrometer (IRMS) was coupled to this C/N analyzer to measure $\delta^{13}C$ values in shoots, roots, and soil. Since the IRMS unit could analyze only solid samples, the CO_2 and microbial biomass samples had to be specifically prepared. Any CO_2 trapped as Na_2CO_3 in an aliquot of 4 mL of NaOH was precipitated with 5 mL of 0.5 *M* $SrCl_2$ aqueous solution. The NaOH solutions containing the $SrCO_3$ precipitants were then centrifuged three times at 1449 *g* for 10 min and washed in between with deionized and degassed water to remove NaOH and to reach a pH of 7.0. After washing, the remaining water was removed from the vials and the $SrCO_3$ was dried at 105°C. The $SrCO_3$ was analyzed using 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 (according to *Brant* et al., 2006).

2.3 Calculations

In the root-exclusion approach, root- and SOM-derived CO_2 were separated by measuring the total CO_2 efflux from maize and bare-fallow plots and by subtracting the latter from the former. This difference between the two kinds of plots was taken to be the contribution of root-derived CO_2 to the total CO_2 efflux from the maize plot. The contribution of SOM decomposition on the maize plot was considered to be equal to the total CO_2 efflux from the bare-fallow plot, since no plant residues remained on the latter from the previous crop. Contributions of root-derived respiration (RDR_{re}) and SOM decomposition ($SOMD_{re}$) to the total CO_2 efflux from soil planted with maize in the root exclusion approach were calculated by the following equations:

$$RDR_{re} = \frac{C_m - C_{bf}}{C_m} \cdot 100\%, \quad (1)$$

$$SOMD_{re} = \frac{C_{bf}}{C_m} \cdot 100\%, \quad (2)$$

where C_m and C_{bf} are the amounts of C from maize and bare-fallow plot CO_2 effluxes, respectively.

In the second—the natural ^{13}C labeling—approach, the method proposed by *Kuzyakov* (2004, 2005) was used to separate RR, RMR, and SOMD. The following equations were used to calculate $\delta^{13}C$ values and CO_2-efflux partitioning. A mass-balance equation was used to determine the $\delta^{13}C$ value of microbial biomass ($\delta^{13}C_{MB}$):

$$\delta^{13}C_{MB} = \frac{\delta^{13}C_{fum} \cdot C_{fum} - \delta^{13}C_{nf} \cdot C_{nf}}{C_{fum} - C_{nf}}, \quad (3)$$

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

In the beginning of every CO_2 trapping, there was a small volume of atmospheric CO_2 inside the steel collar. We considered this atmospheric CO_2 in relation to the measured $\delta^{13}C$ value by a mass-balance equation:

$$\delta^{13}C_{CO_2} = \frac{\delta^{13}C_{total} \cdot C_{total} - \delta^{13}C_{air} \cdot C_{air}}{C_{total} - C_{air}}, \quad (4)$$

where $\delta^{13}C_{CO_2}$ 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 (as an approximation –7.8‰ was taken from *Boutton*, 1991), C_{total} is the amount of CO_2-C trapped in NaOH, and C_{air} is the amount of C inside the steel collar (0.08 mg C) calculated from a CO_2 concentration of 345 ppm (*Boutton*, 1991) and the aboveground volume of air inside the steel collar.

After calculating the $\delta^{13}C$ of microbial biomass (by Eq. 3) and the corrected $\delta^{13}C$ of total CO_2 efflux (by Eq. 4), it was possible to calculate belowground CO_2 partitioning. The development of the equations used to calculate the three-sources-CO_2 partitioning is presented in detail by *Kuzyakov* (2004, 2005). The equations for the contributions of SOM decomposition ($SOMD_{nl}$) and rhizomicrobial respiration (RMR_{nl}) used in the ^{13}C-natural-labeling (hence the subscript *nl*) approach are:

$$SOMD_{nl} = \frac{\delta^{13}C_{CO_2} - \delta^{13}C_{Rhiz}}{\delta^{13}C_{SOM} - \delta^{13}C_{Rhiz}} \cdot 100\%, \quad (5)$$

$$RMR_{nl} = \frac{(\delta^{13}C_{MB} - \delta^{13}C_{SOM}) \cdot (\delta^{13}C_{CO_2} - \delta^{13}C_{Rhiz})}{(\delta^{13}C_{Rhiz} - \delta^{13}C_{SOM}) \cdot (\delta^{13}C_{MB} - \delta^{13}C_{Rhiz})} \cdot 100\%, \quad (6)$$

where $\delta^{13}C_{CO_2}$ is the δ^{13}C value of the total CO_2 efflux from planted soil (Eq. 4), $\delta^{13}C_{Rhiz}$ is the δ^{13}C value of C_4 plant roots, $\delta^{13}C_{SOM}$ is the δ^{13}C value of SOM from unplanted soil, and $\delta^{13}C_{MB}$ is the δ^{13}C value of microorganisms from planted soil (Eq. 3). Having calculated these two contributions to the belowground CO_2 efflux, the remaining part would be root respiration (RR_{nl}):

$$RR_{nl} = 100\% - SOMD_{nl} - RMR_{nl} \; . \quad (7)$$

When isotopic fractionation was considered between SOM and CO_2 derived from SOM and between microbial biomass and microbially derived CO_2, $\delta^{13}C_{SOM}$ and $\delta^{13}C_{MB}$ were replaced in Eq. 5 and 6 by $\delta^{13}C_{SOM-CO_2}$ and $\delta^{13}C_{MB-CO_2}$:

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

$$\delta^{13}C_{MB-CO_2} = \delta^{13}C_{MB} + \varepsilon_{MB-CO_2}, \quad (9)$$

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

A calculated δ^{13}C value was used to determine the influence of rhizodeposits- and SOM-consuming microorganisms on δ^{13}C of total microbial biomass according to *Werth* et al. (2006). This δ^{13}C value ($\delta^{13}C_{calcMB}$) was calculated by a mass-balance equation using δ^{13}C values of maize roots ($\delta^{13}C_{Rhiz}$) for rhizodeposits-consuming ($\delta^{13}C_{C_4}$) and δ^{13}C values of SOM ($\delta^{13}C_{SOM}$) from unplanted soil for SOM-consuming ($\delta^{13}C_{C_3}$) portions of microbial biomass including the fractionation ε_{SOM-CO_2} between the substrate and the CO_2:

$$\delta^{13}C_{calcMB} = \frac{\delta^{13}C_{C_4} \cdot C_{C_4} + \delta^{13}C_{C_3} \cdot C_{C_3}}{100\%}, \quad (10)$$

$$\delta^{13}C_{C_4} = \delta^{13}C_{Rhiz} + \varepsilon_{SOM-CO_2}, \quad (11)$$

$$\delta^{13}C_{C_3} = \delta^{13}C_{SOM} + \varepsilon_{SOM-CO_2}, \quad (12)$$

where C_{C_4} and C_{C_3} are C proportions of microbial biomass consuming maize rhizodeposits or SOM, respectively. C_{C_4} was adjusted to match measured results of belowground CO_2 partitioning (see section 3), and C_{C_3} was 100% – C_{C_4}. The fractionation between maize rhizodeposits and CO_2 was assumed to be the same as the fractionation between SOM and CO_2 (compare *Balesdent* and *Mariotti*, 1996; *Bol* et al., 2003; *Boutton*, 1996).

The total CO_2 efflux (in g m^{-2}) from the maize plot was split up into different sources by multiplying with the percentage contributions gained from Eqs. 1, 2, 5, 6, and 7. The means of all results were calculated for maize and bare-fallow plots, and standard deviations (SD) were calculated as a variability parameter. Outliers in the CO_2 trapping were excluded from the calculation of the mean CO_2 efflux (and its SD), which led to a total of six collars (instead of nine). For calculations of mean δ^{13}C values, we used only three replicates, since we sampled three plants randomly. We determined the δ^{13}C values only in the three corresponding soil and microbial-biomass samples. A one-way analysis of variance (ANOVA) was used to identify differences between total C concentrations, total N concentrations, or C : N ratios of various plant or soil pools. The ANOVA was conducted in pairs between plant parts, between sampling dates of an individual plot, or between plots for individual sampling dates. For the δ^{13}C values, a one-way ANOVA was used to find differences between sampling dates of an individual plot, between plots for an individual sampling date, or between carbon pools for an individual sampling date and plot. For the latter, a Fisher-LSD test was used as *post hoc* test to identify differences in δ^{13}C values of individual C-pool pairs. Since variances were not equal for maize-plot C pools on the September sampling, a Studentized-maximum-modulus test had to be used as *post hoc* test here. One-way ANOVA was also used when the contributions to the total CO_2 efflux were compared between the root-exclusion and natural-^{13}C-labeling approaches. Statistics were calculated with the SPSS 10.0 package.

3 Results

3.1 Carbon and nitrogen concentrations, C : N ratios, and soil water content

No significant difference in total C concentration was determined between shoots and roots; it averaged approx. 44% (Tab. 2). The shoots' total N concentration was about twice as high as that in the roots ($p < 0.01$), doubling the C : N ratio in roots *versus* shoots ($p < 0.01$). The total C concentration in the soil (Tab. 2) remained constant between the two sampling dates at 1.2% and 1.1% for the maize and bare-fallow plots, respectively. On the maize plot, the total soil N concentration declined between summer and autumn samplings ($p < 0.001$). This decline significantly increased the soil C : N ratio by 4 between the two sampling dates ($p < 0.001$). In July, the total C concentration in the microbial biomass was approx. 0.008% of soil weight on the maize plot and 0.012% of soil weight on the bare-fallow plot (Tab. 2). This between-plot difference ($p < 0.05$) and a nearly constant N concentration in microbial biomass of 0.003% of soil weight on both plots led to smaller C : N ratios on the maize *versus* bare-fallow plot. Due to the high SD on the bare-fallow plot, this difference was not significant. There was no difference in soil water contents between maize and bare-fallow plots in July (Tab. 2). In September, however, the water content of the bare-fallow plot was significantly higher compared to the maize plot ($p < 0.01$).

3.2 Values of δ^{13}C

In none of three C pools (*i.e.,* SOM, microbial biomass, and soil-derived CO_2) on the maize plot did δ^{13}C values change significantly between sampling dates (Fig. 1a). On the Sep-

Table 2: Total C and N concentrations and C : N ratios of shoots, roots, soil, and microbial biomass and soil water content of maize and bare-fallow plots (means ± SD, $2 \le n \le 9$, n.d. = not determined), based on plant part or soil dry matter. Values followed by the same first letter within columns are not significantly different ($p > 0.05$) between plant parts or between soil and microbial biomass sampling dates of an individual plot. Values followed by the same second letter within columns are not significantly different ($p > 0.05$) between plots for individual sampling dates.

	Sampling date in 2004	**C** [% of dry matter]	**N** [% of dry matter]	**C : N**	**Soil water content** [mass-%]
maize					
shoots	Oct. 22	42.16 ^{a} ± 1.54	2.43 ^{a} ± 0.19	17.4 ^{a} ± 0.8	n.d.
roots	Oct. 22	45.27 ^{a} ± 8.70	1.26 ^{b} ± 0.18	36.0 ^{b} ± 5.3	n.d.
soil					
maize plot	July 7	1.19 ^{b,d} ± 0.05	0.27 ^{c,f} ± 0.01	4.5 ^{c,f} ± 0.4	6.4 ^{a,d} ± 1.8
	Sept. 22	1.09 ^{b,e} ± 0.17	0.13 ^{d,h} ± 0.01	8.3 ^{d,h} ± 0.4	5.9 ^{a,e} ± 1.1
bare-fallow plot	July 7	1.09 ^{c,d} ± 0.09	0.16 ^{e,g} ± 0.01	7.0 ^{e,g} ± 0.9	6.3 ^{b,d} ± 1.0
	Sept. 22	1.13 ^{c,e} ± 0.05	0.14 ^{e,h} ± 0.01	8.0 ^{e,h} ± 0.1	8.7 ^{c,f} ± 1.8
microbial biomass					
maize plot	July 7	0.008 ^{f,h} ± 0.002	0.004 ^{i,j} ± 0.001	3.1 ^{i,j} ± 0.8	n.d.
	Sept. 22	0.011 ^{f,j} ± 0.006	0.003 ^{i} ± 0.002	6.9 ^{i} ± 4.2	n.d.
bare-fallow plot	July 7	0.012 ^{g,i} ± 0.004	0.003 ^{j} ± 0.002	7.4 ^{j} ± 4.2	n.d.
	Sept. 22	0.012 ^{g,j} ± 0.002	n.d.	n.d.	n.d.

tember sampling date, the $\delta^{13}C$ value of microbial biomass on the maize plot was significantly less negative (by 1.5‰) than that of SOM (–25.5‰, $p < 0.05$). On both sampling dates, CO_2 from soil respiration was significantly enriched in ^{13}C (by approx. 5.0‰) compared to microbial biomass ($p < 0.05$). The $\delta^{13}C$ of maize roots (–12.2‰) was less negative than the $\delta^{13}C$ of CO_2 (–18.6‰, $p < 0.05$). Maize roots were significantly more enriched in ^{13}C than leaves (–13.2‰, $p < 0.01$).

On the bare-fallow plot, the $\delta^{13}C$ value of CO_2 from soil respiration was less negative in September than in July (by 3.8‰, $p < 0.001$, Fig. 1b). Only the $\delta^{13}C$ values of SOM and CO_2 differed significantly (by 1.6‰) on the first sampling ($p < 0.01$). In July, the difference between microbial biomass and CO_2 was only 0.7‰, which was not significant. On the second sampling, all $\delta^{13}C$ values were significantly different. Soil organic matter was depleted by 1.6‰ ($p < 0.01$), and CO_2 was enriched by 3.7‰ ($p < 0.001$) compared to microbial biomass ($\delta^{13}C = -24.0$).

The $\delta^{13}C$ values of SOM and microbial biomass did not differ between the two plots. The CO_2 efflux from soil, however, was significantly enriched in ^{13}C on the maize *versus* bare-fallow plot ($p < 0.05$).

3.3 CO_2-efflux partitioning

The comparison of total CO_2 effluxes from maize and bare-fallow plots revealed that the value from the maize plot was derived 71% to 78% from SOM decomposition and 29% to 22% from roots (Fig. 2a). This increase of the SOMD contribution was not significant between the two sampling dates.

In contrast to the root-exclusion approach, the contribution of SOMD to the total CO_2 efflux estimated by the natural-^{13}C-

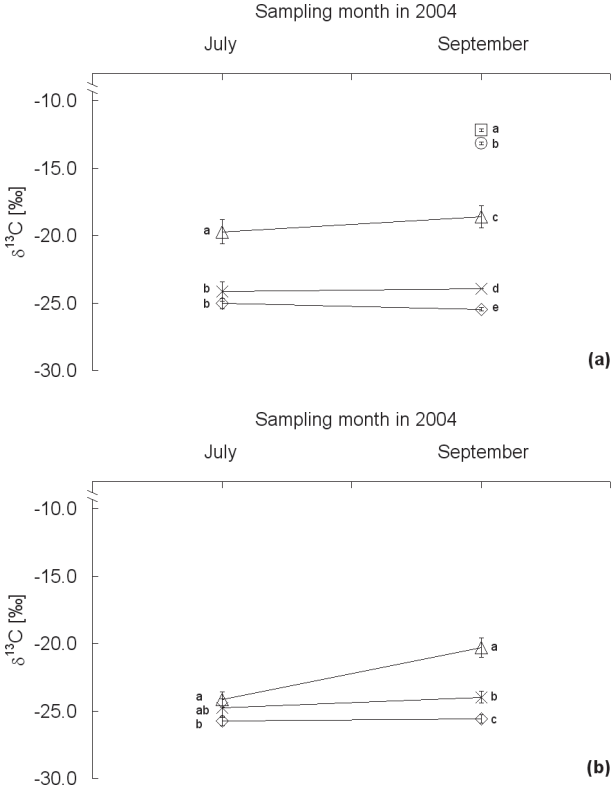

Figure 1: $\delta^{13}C$ values of C-pools from (a) maize and (b) bare-fallow plots; C pools are maize leaves (○), maize roots (□), soil organic matter (◇), total CO_2 efflux (△), and microbial biomass (×); error bars show standard deviation ($n = 3$). Values followed by the same letter within one sampling month are not significantly different ($p > 0.05$).

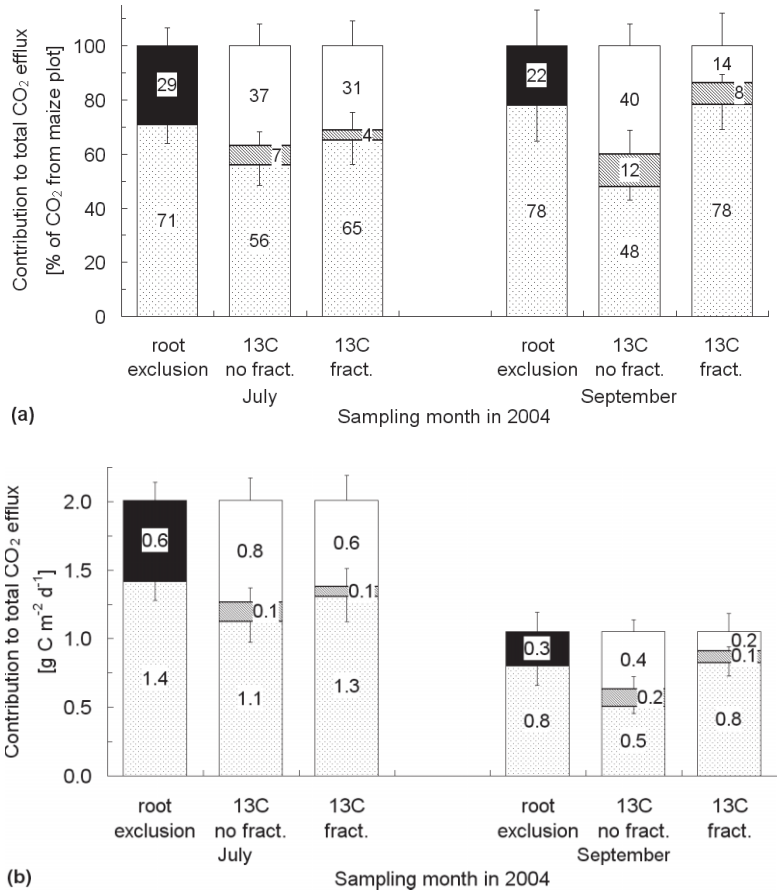

Figure 2: Carbon dioxide–efflux partitioning of the maize plot calculated by the root-exclusion (n = 6) and natural-^{13}C-labeling approaches (n = 3) without and with ^{13}C fractionation between microbial biomass and CO_2 (1‰ in July, 4‰ in September) and between SOM and CO_2 (2‰ in July, 5‰ in September). Results are shown in (a) percentage and (b) absolute values. Contributions are root respiration (no shading), rhizomicrobial respiration (hatched shading), total root-derived respiration (black shading), and soil organic matter (SOM) decomposition (dotted shading). Error bars show standard deviation.

labeling technique decreased between sampling dates from 56% to 48% (Fig. 2a). Between dates, the contributions of RR and RMR increased from 37% to 40% and from 7% to 12%, respectively. None of these changes between sampling dates were significant. ^{13}C fractionations between SOM and CO_2 as well as between microbial biomass and CO_2 were obtained from the respective δ^{13}C values of the bare-fallow plot (Fig. 1b) and were used for further calculations. The fractionations between microbial biomass and CO_2 (ε_{MB-CO_2}) were 1.0‰ and 4.0‰ in July and September, respectively. At both dates, fractionations between SOM and CO_2 (ε_{SOM-CO_2}) were 1.0‰ higher than between microbial biomass and CO_2. Using these values, we calculated belowground CO_2 partitioning for three CO_2 sources (Fig. 2a: right column in each month). In September, we found significant differences ($p < 0.05$) between calculations with and without ^{13}C fractionation (Fig. 2a) for SOMD and for the total root-derived respiration (RDR).

In the comparison of the root-exclusion and the natural-^{13}C-labeling approaches (including ^{13}C fractionation), root-derived CO_2 (and accordingly SOM-derived CO_2) was the same ($p > 0.05$) between approaches in both months, July and September (Fig. 2a). Converted into g C m^{-2} d^{-1}, root-derived CO_2-C was only 0.1 g m^{-2} d^{-1} more in July and even equal in September in the natural-^{13}C-labeling *versus* the root-exclusion approach (Fig. 2b).

3.4 Influence of rhizodeposits-feeding microbial biomass on belowground CO_2 partitioning

We have redrawn the CO_2-efflux partitioning by the natural-^{13}C-labeling technique including fractionations between SOM and CO_2 and between the microbial biomass and CO_2 from Fig. 2a (right column in each month) into Fig. 3 (left column in each month). In order to simulate the influence of rhizodeposits- and SOM-feeding fractions of the microbial biomass on CO_2 partitioning, we used calculated δ^{13}C values for the microbial biomass that considered both fractions (see Eq. 10). Percentages of these fractions in Eq. 10 were adjusted to match the CO_2-partitioning results obtained in this study (Fig. 3: middle column in each month) and literature results (Fig. 3: right column in each month). Values of δ^{13}C for roots (–12.2‰) and for SOM (–25.8‰ in July, –25.6‰ in September) were used to represent δ^{13}C values for rhizodeposits- and SOM-consuming microorganisms, respectively. Fractionations of 2‰ in July and 5‰ in September between the substrates (*i.e.,* roots or SOM) and the CO_2 were included in the calculations.

Maize-derived portions of the total microbial biomass of approx. 5% in July and 6% in September (Fig. 3: middle column in each month) were determined to reflect the results observed in this study (Fig. 3: left column in each month). Hypothetical rhizodeposits-consuming portions of the total microbial biomass of 18% in July and 8% in September (Fig. 3: right column in each month), however, would have

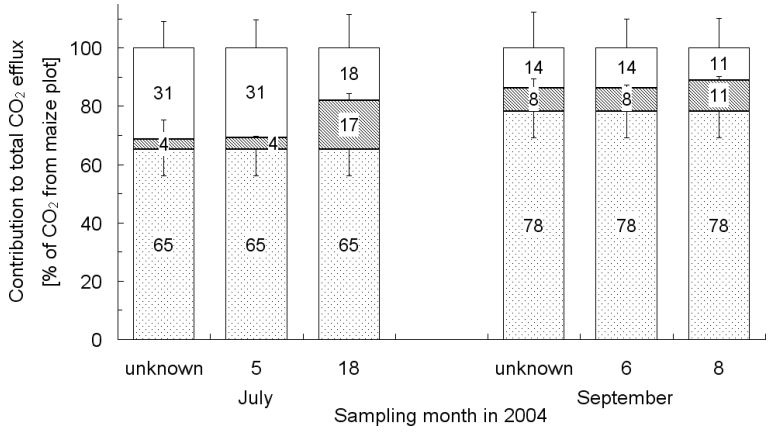

Figure 3: Influence of the C_4-derived portion (in percentage on the x-axis) of total microbial biomass on belowground CO_2 partitioning. In the middle column in each month, the C_4-derived microbial biomass was adjusted to achieve calculated CO_2-partitioning results of this present study (left column, including isotopic fractionation according to Fig. 2). In the right column in each month, the C_4-derived microbial biomass was adjusted to achieve CO_2-partitioning results of literature studies (Tab. 1). Patterns are: contributions of root respiration (no shading), rhizomicrobial respiration (hatched shading), and SOM decomposition (dotted shading) to total CO_2 efflux from a C_3 soil planted with maize; error bars show standard deviation (n = 3).

been necessary to yield a 50% contribution each for RR and RMR related to total RDR as reported in various studies (Tab. 1).

4 Discussion

Two approaches on CO_2-efflux partitioning were used in this field study—natural ^{13}C labeling and root exclusion. In root exclusion, total CO_2 effluxes from maize and bare-fallow soils were used to determine two CO_2 sources, *i.e.,* root- and SOM-derived CO_2. In natural ^{13}C labeling by conversion of a C_3 field to maize, the CO_2 efflux was split up into three sources, *i.e.,* root respiration, rhizomicrobial respiration, and SOM decomposition. Although the rhizosphere was larger in the field than in the laboratory, the active microbial biomass consuming maize rhizodeposits did not increase, which does not support the first hypothesis (*cf.*, section 4.3). In contrast to the laboratory, climate and site properties and the method of CO_2 sampling rather than the larger rhizosphere changed CO_2-efflux partitioning, *i.e.,* mainly increased SOMD and decreased RR. The effect of site properties on CO_2 partitioning in part supported the second hypothesis (*cf.*, section 4.2). In the third hypothesis, it was expected that accounting for ^{13}C fractionation between substrates and products would increase the amount of rhizomicrobial respiration, and this was confirmed (*cf.*, section 4.1). Both approaches—natural ^{13}C labeling and root exclusion—approximated literature results of 36% RDR and 64% SOMD (Tab. 1) discarding the fourth hypothesis (*cf.,* section 4.2). Finally, it was expected that seasonal climatic differences would influence CO_2 partitioning, but this was not supported. Seasonal climatic differences had no significant effect on CO_2 partitioning (*cf.*, section 4.4), and seasonal differences were only found in the ^{13}C fractionation.

4.1 Isotopic fractionation within the natural-^{13}C-labeling approach

Isotopic fractionations between substrate (SOM or exudates), microbial biomass, and microbial CO_2 have to be considered when using the natural-^{13}C-labeling approach. The first fractionation step leading to a ^{13}C-enriched microbial biomass compared to SOM can be explained by isotope discrimination during biosynthesis of new microbial biomass (*Potthoff* et al., 2003). Compared to SOM, water-soluble organic compounds with a heavier isotopic composition are preferentially used by soil microorganisms (*Pelz* et al., 2005). The second fractionation step results in more ^{13}C-enriched microbial CO_2 compared to the microbial biomass and the substrate. 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). Our experimental results showed, therefore, that the shift of C isotope composition of microbial biomass towards increasing $\delta^{13}C$ values was mainly caused by microbial selective utilization (*Piao* et al., 2006). The microbial population uses compounds preferentially, such as cellulose, starch, and protein, that have larger $\delta^{13}C$ values than the average of soil organic C (SOC) (*Bird* et al., 2002). 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 (1‰ in July and 4‰ in September) compared to microbial biomass. Total fractionation between SOM and CO_2 and between microbial biomass and CO_2 including kinetic and biological processes is important, when using the method proposed by *Kuzyakov* (2004, 2005). Fractionations will also be of importance in studies that use similar ^{13}C–natural abundance methods including mass-balance equations with soil or microbial biomass substitutes to soil or microbial 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. We assumed the fractionation between maize rhizodeposits and microbial CO_2 in Eq. 11, however, to be equal to the fractionation between SOM and microbial CO_2 (2‰ in July and 5‰ in September). In line with earlier studies (*Balesdent* and *Mariotti*, 1996; *Bol* et al., 2003; *Boutton*, 1996), we accepted this assumption because we had no direct measure to determine the actual fractionation between rhizodeposits and microbial CO_2. This determination is a future challenge, requiring that rhizodeposits be decomposed by exactly the same microbial community as developed on the maize plot. In the present study, however, we assumed equal fractionations for C_3- and C_4-derived substrates because the root-derived contributions to the microbial biomass calculated with and without ^{13}C fractionation for the C_4 substrate were not significantly different. Omitting C_4-

substrate fractionation, the middle columns in Fig. 3 would also read a rhizodeposits-feeding microbial biomass fraction of 5% in July and 6% in September on the x-axis.

4.2 CO_2-efflux partitioning by the natural-^{13}C-labeling and root-exclusion approaches

Consideration of ^{13}C fractionations led to CO_2-partitioning results that were much closer to the root-exclusion method than those calculated without fractionations (Fig. 2a). Several other studies reported ^{13}C fractionations in this range (1‰–5‰) (*Formánek* and *Ambus*, 2004; *Rochette* et al., 1999a; *Šantrůčková* et al., 2000). In September, CO_2 contributions calculated by the ^{13}C-labeling approach (taking fractionation into account) match the root-exclusion results very well (Fig. 2a). This correspondence, however, might be coincidental since both approaches have their shortcomings.

In other field studies with grass species, the root-derived CO_2 contribution was lower and the SOM-derived CO_2 contribution was higher than in the laboratory (Tab. 1). These results were comparable to our study. This difference between laboratory and field can be explained by different environmental influences (soil moisture, temperature, *etc.*) in experiments under controlled and field conditions. Additionally, in our field either a possible severance of the roots by the CO_2-trap installation, the distance of the CO_2 trap to the shoot, or a combination of both effects could have led to lower contributions of root-derived CO_2 than in the laboratory. On the contrary, the SOMD contribution to the total CO_2 efflux could have been overestimated due to contributions of root-free soil. Hence, further studies should prefer to use CO_2-trapping collars, which encompass the complete rhizosphere. In such studies, it would also be possible to relate CO_2 emissions to the root density under the trapping collar.

In the calculations for CO_2-efflux partitioning (Fig. 2a) without considering isotopic fractionation and in July, when a low fractionation between SOM and CO_2 and between microbial biomass and CO_2 was considered, 4% to 12% RMR were remarkably low compared to the mean of 24% of other studies (*Chen* et al., 2006; *Crow* and *Wieder*, 2005; *Kelting* et al., 1998). Literature results have shown nearly equal contributions of RR and RMR to root-derived CO_2 (Tab. 1). Even the smallest contributions of RMR determined by combining root-exclusion and component-integration methods on a maize and spring barley (*Hordeum vulgare* L.) field amounted approx. 40% of total root-derived CO_2 (*Larionova* et al., 2006) and were between double to four times as high as in our field experiment. In our maize plot, low RMR could result from nonrepresentative soil sampling for microbial-biomass determination. Soil samples were taken 10 cm apart from the stem and from 0 to 5 cm depth. Although these samples definitely contained maize roots, the root density was probably not representative for the maize rhizosphere. In future studies, maize plants should be removed from the soil and samples should be taken directly from the soil adhering to the roots.

Regarding general disadvantages of the root-exclusion approach mentioned in the introduction, it has to be tested under which conditions the approach is reliable. We can assume that both plots—maize and bare fallow—had similar site properties in July. There was no significant difference in soil moisture (Tab. 2) and due to the small size of the plants (approx. 1 m shoot length) and their loose growing (30 cm distance within rows, 75 cm distance between rows), we can also assume no difference in soil temperature. In July, the soil C : N ratio on the maize plot was lower than on the fallow plot (Tab. 2), which implies a higher SOM decomposition on the maize plot. Simply applying the root-exclusion method would then underestimate the CO_2 from SOM decomposition on the maize plot.

In September, C : N ratios were similar on both plots. Soil moisture, however, was significantly higher on the bare-fallow plot. In planted soil, the SOM mineralization may have been lower due to lower soil moisture than in bare soil. Consequently, assuming an equal SOM mineralization on the two plots leads to underestimated root-respiration contribution in the root-exclusion approach in September. As an overall result, CO_2 partitioning of the root-exclusion method was uncertain in July due to differing C : N ratios between plots and in September due to differing soil moisture. These parameters as well as soil temperature should be determined in future studies on CO_2 partitioning in the field when using the root-exclusion method.

Another shortcoming of the root-exclusion method is the disregarding of priming effects in the approach *per se.* The CO_2 efflux from the bare-fallow plot, however, can be used to determine priming effects on the maize plot. Without consideration of ^{13}C fractionation, a negative priming effect has been calculated by a reduction of SOM decomposition from 11.1 to 8.8 g C m^{-2} in July and from 5.6 to 3.5 g C m^{-2} in September (Fig. 2b). This negative priming diminishes or even disappears completely when we account for ^{13}C fractionation. Therefore, exact determination of isotope fractionation is of major importance when calculating priming effects with ^{13}C–natural abundance methods.

4.3 Influence of rhizodeposits-feeding microbial biomass on belowground CO_2 partitioning

A maximum of a 6% C_4-derived microbial biomass contributing to the microbial CO_2 efflux was calculated in this field study (Fig. 3). In a laboratory study with maize grown on the same soil as in the field study, we showed that—without fractionation between the substrate and the CO_2 in the calculation of the $\delta^{13}C$ value of microbial CO_2—approx. 37% of the microbial biomass in the rhizosphere was C_4-derived (*Werth* et al., 2006). Considering also a fractionation in the microbial substrate respiration of 5‰ when calculating the $\delta^{13}C$ of microbial respiration (according to Eq. 10) would reduce the rhizodeposits-consuming microbial biomass in the laboratory study to 9%. This still higher activity compared to the field study can be explained by the controlled conditions (water content, temperature, *etc.*), the small size of the plant-growing containers, and the resulting sampling of rhizosphere soil

closely related to the plants. On the contrary, the lower activity in the field can be explained by suboptimal weather conditions for microbial growth, the longer distance of the soil-sampling location to the center of the root system, a contribution of surface-near dry soil with generally low microbial activity to the soil sample, and a resulting stronger contribution of SOM-feeding microorganisms to the total microbial biomass.

In both experiments, substituting the $\delta^{13}C$ value of microbial CO_2 with the $\delta^{13}C$ value of microbial biomass was problematic. We assumed two C sources for microbial biomass, *i.e.,* C_4- and C_3-derived C. The same sources with the same ratio were expected in soil CO_2. In contrast to our assumption, however, microbial biomass consists of three pools: (1) a large active CO_2-respiring fraction consuming C_4-derived substances, (2) a small active CO_2-respiring fraction consuming C_3-derived substances, and (3) a very large nonrespiring fraction with microorganisms in dormant states. Therefore, microbial CO_2 should consist to a major part of C_4-derived C and to a minor part of C_3-derived C. Due to the large contribution of dormant microorganisms, $\delta^{13}C$ of microbial biomass is not an appropriate substitute for $\delta^{13}C$ of microbial respiration. Besides isotopic fractionation between microbial biomass and CO_2, the discrepancy between active microbial contributions in the biomass itself and in the CO_2 enforces us to disprove the assumption of equal $\delta^{13}C$ values of microbial biomass and CO_2 when using the natural-^{13}C-labeling technique. Consequently, a new method has to be found to determine the activity of the microbial biomass independently from our mass-balance calculations and then to calculate the $\delta^{13}C$ of microbial respiration with the proportion obtained from that method in mass balance Eq. 10. Subsequent calculations of CO_2 partitioning could lead to results much closer to former experiments (Tab. 1).

4.4 Seasonal effect on belowground CO_2 partitioning

The $\delta^{13}C$ values of plant parts were only measured on the autumn sampling. In our 40-days experiment under controlled conditions (*Werth* et al., 2006), we did not find any differences in $\delta^{13}C$ values of plant parts from different sampling dates. During a whole plant-growth period like in this field experiment, however, $\delta^{13}C$ values of different plant tissues could change with plant growth. *Giesemann* et al. (2006) have reported no significant changes in shoots $\delta^{13}C$ values during the growth period of winter barley (*Hordeum vulgare* L.) and winter wheat (*Triticum aestivum* L.). They found, however, a depletion of 1.5‰ in the shoots of sugar beet (*Beta vulgaris* L.) during plant growth. According to this, ^{13}C-enriched roots in our maize plants in July would have a significant impact on the results of CO_2-efflux partitioning. Consequently, in future studies $\delta^{13}C$ values in plant tissues should be measured on each sampling date.

On both plots, the total CO_2 efflux in September was half as high as in July (Fig. 2b). This typical decline in autumn has been reported by several studies under comparable climate conditions (*Amos* et al., 2005; *Han* et al., 2007; *Kutsch* et al., 2001; *Rochette* et al., 1999b). This decline reflects lower air and soil temperatures in September and, thus, lower total respiration of plant roots and soil microorganisms. Low root-respiration rates late in the season can be explained by a decrease of the mass ratio of respiring fine roots to structural, coarse roots as roots age (*Lipp* and *Andersen*, 2003). Whereas absolute soil respiration declined in our field experiment (Fig. 2b), the contribution of SOMD to the total CO_2 efflux increased from 65% to 78% whilst the contribution of RMR increased from 4% to 8% (Fig. 2a). Maize plants were already in the senescence stage in September, *i.e.,* parts of the roots were dying and became decomposed by rhizosphere microorganisms. Consequently, the contribution of SOMD was higher in September than in July due to less root-derived CO_2; the contribution of RMR increased due to reduced root respiration and decomposition of dead root cells by rhizosphere microorganisms. Total RDR in September, however, may have been underestimated due to higher $\delta^{13}C$ values of CO_2 from SOM decomposition than in July. Similar studies have reported an increased $\delta^{13}C$ value of CO_2 from bare-soil respiration late in the season (*Rochette* and *Flanagan*, 1997; *Rochette* et al., 1999b). One interpretation is that this late-season $\delta^{13}C$ increase reflects a reduced soil-respiration rate. The change in weather conditions in early autumn may also have led to a convective transfer of CO_2 downward from the aboveground atmosphere into the soil when the temperature is lower at the soil surface than below (*Rochette* et al., 1999b). Consequently, the higher $\delta^{13}C$ value of CO_2 from bare soil in autumn (Fig. 1b) might be less a result of fractionation than of weather-induced mixing of soil CO_2 and atmospheric CO_2. The fractionations of 4‰ between microbial biomass and CO_2—and of 5‰ between SOM and CO_2—in September would then be overestimations. Lower fractionations would lead to a higher contribution of root-derived CO_2 to the total CO_2 efflux from the maize plot than shown in Fig. 2a. This calls for further clarification as to whether the higher $\delta^{13}C$ value of CO_2 from bare-soil respiration in autumn reflects fractionation or changing weather conditions.

5 Conclusions

In contrast to our assumptions, the active microbial biomass consuming maize rhizodeposits did not increase in the field compared to our former laboratory study. Accordingly, RMR was not considerably higher in the field, which could also be related to surface-near soil sampling from marginal rhizosphere. Due to different climate, site, and sampling conditions in the field, RR decreased and SOMD increased compared to our former laboratory study. Accounting for high ^{13}C fractionation during microbial uptake and respiration in autumn increased the relative amount of RMR in comparison to RR, which approximated to former studies. Therefore, isotopic fractionations in the natural-^{13}C-labeling approach should be further investigated. Considering fractionations also led to equal RDR and SOMD contributions in natural-^{13}C-labeling and root-exclusion approaches. Consequently, both approaches can be used to separate two CO_2 sources. The natural-^{13}C-labeling method, however, remains problematic for the separation of three CO_2 sources due to different $\delta^{13}C$ values of microbial-biomass extracts and microbial respiration.

Acknowledgments

The *German Research Foundation (DFG)* kindly supported this work. The authors would like to thank *H. Stelz* for the field plots maintenance and Dr. *W. Armbruster* and *E. Dachtler* for the IRMS analyses.

Abbreviations

IRMS, isotope-ratio mass spectrometer; RDR, root-derived respiration; RMR, rhizomicrobial respiration; RR, root respiration; SD, standard deviation; SOM, soil organic matter; SOMD, soil organic matter decomposition

References

Amos, B., Arkebauer, T. J., Doran, J. W. (2005): Soil surface fluxes of greenhouse gases in an irrigated maize-based agroecosystem. *Soil Sci. Soc. Am. J.* 69, 387–395.

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 Sci. Soc. Am. J.* 63, 1429–1435.

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

Bird, M., Šantrůčková, H., Lloyd, J., Lawson, E. (2002): The isotopic composition of soil organic carbon on a north-south transect in western Canada. *Eur. J. Soil Sci.* 53, 393–403.

Blair, N., Leu, A., Munoz, E., Olsen, J., Kwong, E., Des Marais, D. (1985): Carbon isotope fractionation in heterotrophic microbial metabolism. *Appl. Environ. Microb.* 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 Commun. Mass Spec.* 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. Academic Press, Inc., San Diego, USA, 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.: Mass Spectrometry of Soils. Marcel Dekker, New York, USA, pp. 47–82.

Brant, J. B., Sulzman, E. W., Myrold, D. D. (2006): Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. *Soil Biol. Biochem.* 38, 2219–2232.

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 Biol. Biochem.* 17, 837–842.

Bruulsema, T. W., Duxbury, J. M. (1996): Simultaneous measurement of soil microbial nitrogen, carbon, and carbon isotope ratio. *Soil Sci. Soc. Am. J.* 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. *Eur. J. Soil Sci.* 57, 58–66.

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

Cheng, W., Coleman, D. C. (1990): Effect of living roots on soil organic matter decomposition. *Soil Biol. Biochem.* 22, 781–787.

Cheng, W., Kuzyakov, Y. (2005): Root effects on soil organic matter decomposition, in Wright, S., Zobel, R. (eds.): Roots and Soil Management: Interactions between Roots and the Soil. Vol. 48. American Society of Agronomy, Madison, USA, pp. 119–143.

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 Biol. Biochem.* 25, 1189–1196.

Craine, J. M., Wedin, D. A., Chapin, F. S. I. (1999): Predominance of ecophysiological controls on soil CO_2 flux in a Minnesota grassland. *Plant Soil* 207, 77–86.

Crow, S. E., Wieder, R. K. (2005): Sources of CO_2 emission from a northern peatland: Root respiration, exudation, and decomposition. *Ecology* 86, 1825–1834.

Dormaar, J. F. (1990): Effect of active roots on the decomposition of soil organic materials. *Biol. Fertil. Soils* 10, 121–126.

Fisher, F. M., Gosz, J. R. (1986): Effects of trenching on soil processes and properties in a New Mexico mixed-conifer forest. *Biol. Fertil. Soils* 2, 35–42.

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 Commun. Mass Spec.* 18, 1–6.

Giesemann, A., Manderscheid, R., Burkart, S., Weigel, H.-J. (2006): Carbon (C) isotopic composition of agricultural crops cultivated in a crop rotation system under FACE (Free Air Carbon Dioxide Enrichment) conditions. Proc. 29th Annual Meeting of the German Association for Stable Isotope Research (GASIR), Oct. 4–6, 2006, Freiberg/Sachsen, Germany, p. 37.

Gloser, J., Tesarová, M. (1978): Litter, soil, and root respiration measurement. An improved compartmental analysis method. *Pedobiologia* 18, 76–81.

Han, G., Zhou, G., Xu, Z., Yang, Y., Liu, J., Shi, K. (2007): Biotic and abiotic factors controlling the spatial and temporal variation of soil respiration in an agricultural ecosystem. *Soil Biol. Biochem.* 39, 418–425.

Henn, M. R., Chapela, I. H. (2000): Differential C isotope discrimination by fungi during decomposition of C_3- and C_4-derived sucrose. *Appl. Environ. Microb.* 66, 4180–4186.

Hinsinger, P., Gobran, G. R., Gregory, P. J., Wenzel, W. W. (2005): Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. *New Phytol.* 168, 293–303.

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

Jones, D. L., Hodge, A., Kuzyakov, Y. (2004): Plant and mycorrhizal regulation of rhizodeposition. *New Phytol.* 163, 459–480.

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 Biol. Biochem.* 30, 961–968.

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

Kutsch, W. L., Staack, A., Wotzel, J., Middelhoff, U., Kappen, L. (2001): Field measurements of root respiration and total soil respiration in an alder forest. *New Phytol.* 150, 157–168.

Kuzyakov, Y. (2002a): Review: Factors affecting rhizosphere priming effects. *J. Plant Nutr. Soil Sci.* 165, 382–396.

Kuzyakov, Y. (2002b): Separating microbial respiration of exudates from root respiration in non-sterile soils: a comparison of four methods. *Soil Biol. Biochem.* 34, 1621–1631.

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

Kuzyakov, Y. (2005): Theoretical background for partitioning of root and rhizomicrobial respiration by $\delta^{13}C$ of microbial biomass. *Eur. J. Soil Biol.* 41, 1–9.

Kuzyakov, Y. (2006): Sources of CO_2 efflux from soil and review of partitioning methods. *Soil Biol. Biochem.* 38, 425–448.

Kuzyakov, Y., Cheng, W. (2001): Photosynthesis controls of rhizosphere respiration and organic matter decomposition. *Soil Biol. Biochem.* 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 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. *J. Plant Nutr. Soil Sci.* 168, 503–520.

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

Kuzyakov, Y., Friedel, J. K., Stahr, K. (2000): Review of mechanisms and quantification of priming effects. *Soil Biol. Biochem.* 32, 1485–1498.

Kuzyakov, Y., Ehrensberger, H., Stahr, K. (2001): Carbon partitioning and below-ground translocation by *Lolium perenne*. *Soil Biol. Biochem.* 33, 61–74.

Larionova, A. A., Sapronov, D., Lopes de Gerenju, V. O., Kuznetsova, L. G., Kudeyarov, V. N. (2006): Contribution of plant root respiration to the CO_2 emission from soil. *Eurasian Soil Sci.* 39, 1127–1135.

Lipp, C. C., Andersen, C. P. (2003): Role of carbohydrate supply in white and brown root respiration of ponderosa pine. *New Phytol.* 160, 523–531.

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 Biol. Biochem.* 24, 1065–1072.

Midwood, A. J., Gebbing, T., Wendler, R., Sommerkorn, M., Hunt, J. E., Millard, P. (2006): Collection and storage of CO_2 for ^{13}C analysis: an application to separate soil CO_2 efflux into root- and soil-derived components. *Rapid Commun. Mass Spec.* 20, 3379–3384.

Pate, J., Arthur, D. (1998): $\delta^{13}C$ analysis of phloem sap carbon: novel means of evaluating seasonal water stress and interpreting carbon isotope signatures of foliage and trunk wood of *Eucalyptus globulus*. *Oecologia* 117, 301–311.

Paterson, E. (2003): Importance of rhizodeposition in the coupling of plant and microbial productivity. *Eur. J. Soil Sci.* 54, 741–750.

Paterson, E., Sim, A. (1999): Rhizodeposition and C-partitioning of *Lolium perenne* in axenic culture affected by nitrogen supply and defoliation. *Plant Soil* 216, 155–164.

Paterson, E., Sim, A. (2000): Effect of nitrogen supply and defoliation on loss of organic compounds from roots of *Festuca rubra*. *J. Exp. Bot.* 51, 1449–1457.

Pelz, O., Abraham, W.-R., Saurer, M., Siegwolf, R., Zeyer, J. (2005): Microbial assimilation of plant-derived carbon in soil traced by isotope analysis. *Biol. Fertil. Soils* 41, 153–162.

Piao, H. C., Zhu, J. M., Liu, G. S., Liu, C. Q., Tao, F. X. (2006): Changes of natural ^{13}C abundance in microbial biomass during litter decomposition. *Appl. Soil Ecol.* 33, 3–9.

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 Biol. Biochem.* 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 Sci. Soc. Am. J.* 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 Biol. Biochem.* 29, 1451–1462.

Raich, J. W., Mora, G. (2005): Estimating root plus rhizosphere contributions to soil respiration in annual croplands. *Soil Sci. Soc. Am. J.* 69, 634–639.

Robinson, D., Scrimgeour, C. M. (1995): The contribution of plant C to soil CO_2 measured using $\delta^{13}C$. *Soil Biol. Biochem.* 27, 1653–1656.

Rochette, P., Flanagan, L. B. (1997): Quantifying rhizosphere respiration in a corn crop under field conditions. *Soil Sci. Soc. Am. J.* 61, 466–474.

Rochette, P., Angers, D. A., Flanagan, L. B. (1999a): Maize residue decomposition measurement using soil surface carbon dioxide fluxes and natural abundance of carbon-13. *Soil Sci. Soc. Am. J.* 63, 1385–1396.

Rochette, P., Flanagan, L. B., Gregorich, E. G. (1999b): Separating soil respiration into plant and soil components using analyses of the natural abundance of carbon-13. *Soil Sci. Soc. Am. J.* 63, 1207–1213.

Ross, D. J., Scott, N. A., Tate, K. R., Rodda, N. J., Townsend, J. A. (2001): Root effects on soil carbon and nitrogen cycling in a *Pinus radiata* D. Don plantation on a coastal sand. *Aust. J. Soil Res.* 39, 1027–1039.

Šantrůčková, H., Bird, M. I., Frouz, J., Šustr, V., Tajovsky, K. (2000): Natural abundance of ^{13}C in leaf litter as related to feeding activity of soil invertebrates and microbial mineralisation. *Soil Biol. Biochem.* 32, 1793–1797.

Sapronov, D., Kuzyakov, Y. (2004): Separation of root and microbial respiration: comparison of three methods. Abstracts EUROSOIL 2004, Sept. 4–12, 2004, University of Freiburg, Freiburg/Br., Germany, p. 354.

Scartazza, A., Mata, C., Matteucci, G., Yakir, D., Moscatello, S., Brugnoli, E. (2004): Comparisons of $\delta^{13}C$ of photosynthetic products and ecosystem respiratory CO_2 and their responses to seasonal climate variability. *Oecologia* 140, 340–351.

Stenberg, B., Johansson, M., Pell, M., Sjödahl-Svensson, K., Stenström, J., Torstensson, L. (1998): Microbial biomass and activities in soil as affected by frozen and cold storage. *Soil Biol. Biochem.* 30, 393–402.

Stenström, J., Svensson, K., Johansson, M. (2001): Reversible transition between active and dormant microbial states in soil. *FEMS Microbiol. Ecol.* 36, 93–104.

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

Vance, E. D., Brookes, P. C., Jenkinson, D. S. (1987): An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 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 Biol. Biochem.* 9, 295–301.

Werth, M., Kuzyakov, Y. (2006): Assimilate partitioning affects ^{13}C fractionation of recently assimilated carbon in maize. *Plant 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 Biol. Biochem.* 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 Biol. Biochem.* 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 Sci. Soc. Am., Inc., Madison, USA, pp. 835–864.

