



# Photosynthesis controls of rhizosphere respiration and organic matter decomposition

Y. Kuzyakov<sup>a,b,\*</sup>, W. Cheng<sup>a</sup>

<sup>a</sup>Environmental Studies, University of California—Santa Cruz, Santa Cruz, CA 95064, USA

<sup>b</sup>Institute of Soil Science and Land Evaluation, University of Hohenheim, Emil-Wolff-Straße 27, D-70599 Stuttgart, Germany

Received 26 October 2000; received in revised form 20 April 2001; accepted 1 June 2001

---

## Abstract

The effects of shading wheat plants on rhizosphere respiration and rhizosphere priming of soil organic matter decomposition were investigated by using a natural abundance <sup>13</sup>C tracer method and <sup>14</sup>C pulse labeling simultaneously. Seven days with strongly reduced photosynthesis (12/60 h day/night period) resulted in only half of the total CO<sub>2</sub> efflux from soil compared to the treatment with a 12/12 h day/night period. The CO<sub>2</sub> efflux from unplanted soil amounted to only 12 and 20% of the total CO<sub>2</sub> efflux from the soil with non-shaded and shaded plants, respectively. On average 75% of total CO<sub>2</sub> efflux from the planted soil with prolonged night periods was root-derived. Rhizosphere respiration was tightly coupled with plant photosynthetic activity. Any factor affecting photosynthesis, or substrate supply to roots and rhizosphere microorganisms, is an important determinant of root-derived CO<sub>2</sub> efflux, and thereby, total CO<sub>2</sub> efflux from soils. Clear diurnal dynamics of the total CO<sub>2</sub> efflux intensity indicate the existence of an endogenous control mechanism of rhizosphere respiration. The light-on events after prolonged dark periods lead to strong increases of root-derived and therefore of total CO<sub>2</sub> efflux from soil. After <sup>14</sup>C pulse labeling, two maxima of the root-derived <sup>14</sup>CO<sub>2</sub> efflux were measured (6 and 24 h). This result demonstrated the diurnal dynamics of the rhizosphere respiration of recently-assimilated C in both the normal light conditions and shaded plants as well. The total amount of root-derived C respired in the rhizosphere was 17.3 and 20.6% of the total assimilated C for non-shaded and shaded plants, respectively. Both methods used, <sup>13</sup>C natural abundance and <sup>14</sup>C pulse labeling, gave similar estimates of root-derived CO<sub>2</sub> during the whole observation period: 1.80 ± 0.27 and 1.67 ± 0.37 mg C kg<sup>-1</sup> h<sup>-1</sup> (±SD), respectively. Both tracer methods show that the cultivation of wheat led to the increasing decomposition intensity of soil organic matter (priming effect). Additionally, <sup>13</sup>C natural abundance allows tracing of the dynamics of the priming effect depending on the light-on events. © 2001 Elsevier Science Ltd. All rights reserved.

**Keywords:** C turnover; Diurnal dynamics; Priming effect; Rhizodeposition; Rhizosphere; <sup>13</sup>C natural abundance; <sup>14</sup>C pulse labeling; Wheat; Organic matter decomposition; Photosynthesis

---

## 1. Introduction

Carbon dioxide efflux from soils is an important component of the global C cycle and connected with global climatic change, because of the greenhouse effect contributed to by the increasing atmospheric CO<sub>2</sub> concentration. A small alteration in the turnover intensity of soil organic matter (SOM) could lead to a large change of CO<sub>2</sub> concentration in the atmosphere because the amount of C in SOM is twice as large as that in the atmosphere. These small variations in the decomposition intensity of SOM cannot be measured directly, according to the C<sub>org</sub> content in the soil, because of the high variability of SOM content (20–40%) and the

very small relative C<sub>org</sub> changes during short periods (for example 1–3% during a single vegetative growth season). Measuring CO<sub>2</sub> efflux from soil is commonly used to investigate short-term SOM turnover. This method is sensitive enough to detect small and actual changes, especially for recently altered ecosystems. However, most soils are covered with vegetation, which also contributes to the CO<sub>2</sub> efflux from soil. Therefore CO<sub>2</sub> efflux from planted soil is masked by root-derived CO<sub>2</sub>. Root-derived CO<sub>2</sub> comes from root respiration and rhizomicrobial respiration of exudates and dead roots, also called rhizosphere respiration. Root-derived CO<sub>2</sub> is thought to comprise 40–60% of total CO<sub>2</sub> flux (Raich and Schlesinger, 1992). Root-derived CO<sub>2</sub> is not part of soil C loss, and must be separated from the total CO<sub>2</sub> efflux in studies of soil C sequestration or loss.

Different isotope methods have been used to separate rhizosphere respiration from soil-derived CO<sub>2</sub> effluxes,

---

\* Corresponding author. Tel.: +49-711-459-3669; fax: +49-711-459-4071.

E-mail address: kuzyakov@uni-hohenheim.de (Y. Kuzyakov).

such as continuous (Johnen and Sauerbeck, 1977; Whipps, 1987; Meharg, 1994) or pulse (Warembourg and Billes, 1979; Meharg and Killham, 1990; Cheng et al., 1993; Swinnen et al., 1994; Kuzyakov et al., 1999, 2001; Nguyen et al., 1999) labeling with  $^{13}\text{C}$  or  $^{14}\text{C}$ , and  $^{13}\text{C}$  tracing using the natural difference in  $^{13}\text{C}$  abundance between C3 and C4 plants (Cheng, 1996; Qian et al., 1997; Rochette and Flanagan, 1998). The advantages and limitations of these methods were reviewed by Kuzyakov and Domanski (2000). Using these C tracer techniques, it has been shown that rhizosphere respiration can contribute from 19% (Warembourg and Paul, 1977) to 80% (Martin and Merckx, 1992) of the total  $\text{CO}_2$  efflux from planted soil. This high variation of the share of root-derived  $\text{CO}_2$  shows that measurement of total soil  $\text{CO}_2$  efflux alone is not sufficient to assess the contribution of soil C to the global atmospheric  $\text{CO}_2$  because the C source of rhizosphere respiration is plant photosynthesis. Any alteration in the environmental factors affecting photoassimilation may be expected to affect exudate release (Hodge et al., 1997) and root respiration and as consequence the total  $\text{CO}_2$  efflux from planted soil. The first objective of our study was to investigate the relationship between plant photosynthesis and rhizosphere respiration using both the  $^{13}\text{C}$  natural abundance tracer method and a  $^{14}\text{C}$  pulse labelling method.

In addition to the direct contribution of roots to total soil  $\text{CO}_2$  efflux, roots can also affect soil microbial activities by exuding C-rich organic substances easily available for microorganisms and by altering the soil physical and chemical environment (i.e. pH, soil structure, water flow), consequently controlling soil-derived  $\text{CO}_2$  efflux. This can lead to either acceleration or retardation of SOM decomposition in the rhizosphere (Helal and Sauerbeck, 1986, 1989; Bottner et al., 1988, 1991; Mary et al., 1993; Swinnen et al., 1995; Cheng, 1996; Kuzyakov et al., 2000). The second objective of our study was to assess the effect of prolonged night-time on root exudates and their influence on SOM decomposition.

Some investigations of  $\text{CO}_2$  efflux from soil under natural conditions have shown diurnal patterns in the efflux rates (Baldocchi et al., 1986; Kim and Verma, 1992). Most investigators have attributed these diurnal fluctuations to diurnal soil temperature changes because soil temperature has repeatedly been shown to be one of the important controlling factors for soil  $\text{CO}_2$  efflux. Plant photosynthesis has rarely been considered as an important controlling factor for the diurnal fluctuation of soil  $\text{CO}_2$  efflux, even though substrate supply for rhizosphere processes is controlled by plant photosynthesis. High transport rates of assimilates from leaves into the roots and then lost by root respiration and exudation of organic substances into the rhizosphere have been reported based on data from laboratory experiments (Biddulph, 1969; Gregory and Atwell, 1991; Cheng et al., 1993; Kuzyakov et al., 1999, 2001). Therefore, the changes of assimilation rates caused by day/night light cycles may potentially control the diurnal dynamics of root-derived  $\text{CO}_2$ . The third objective of our study was to

investigate the diurnal dynamics of root-derived  $\text{CO}_2$  and its possible dependence on light–dark cycles.

## 2. Materials and methods

### 2.1. Soil

The soil used in the experiment was taken from the Ah horizon of natural Kansas tallgrass prairie at the Konza Prairie Long-Term Ecological Research site, Kansas, USA. The soil was a clay loamy Haplic Chernozem. The soil pH was 7.6. The soil contained 2.3%  $\text{C}_{\text{org}}$  and 0.2% N. Vegetation at this site has been dominated by C4 grasses for possibly thousands of years. The  $\delta^{13}\text{C}$  value of the soil was  $-14.85 \pm 0.19$  (SD). By growing wheat (C3) plants in this soil, we used natural  $^{13}\text{C}$  abundance as a tracer to separately measure plant-derived C from soil-derived C (Cheng, 1996).

### 2.2. Plants and growth conditions

Seeds of spring wheat (*Triticum aestivum* L, var. Andy) (a typical C3 plant) were germinated in Petri dishes for 2 days. Five germinated seedlings were transplanted in each pot and grown at 2 cm distance. Each container was filled with 1 kg of air dried soil. Each pot was a polyvinyl chloride (PVC) container with 76 mm dia and 190 mm in height, connected to tubing for air circulation. The plants were grown in a growth chamber at a constant ( $22 \pm 0.5^\circ\text{C}$ ) day and night temperature with light intensity of approximately  $800 \mu\text{mol m}^{-2} \text{s}^{-1}$  at the top of the plant canopy. Before the start of light treatments (day 31 after germination, see below) the plants were grown under 12/12 h day/night periods. The soil water content of each container was controlled gravimetrically and was adjusted daily with deionized water to 80% of the available field capacity. Twenty five days after germination 40 ml of Hoagland nutrient solution (Hoagland and Arnon, 1950) was added daily to each container in addition to watering.

Two day/night settings were investigated simultaneously in this study. The first setting (normal day/night period) had a day-length of 12 h and a night-length of 12 h. The second day/night setting (prolonged night) had a day-length of 12 h and a prolonged night of 60 h (12 h light + 12 h without light + two full days without light). Two of these cycles were investigated.

To compare soil  $\text{CO}_2$  evolution with or without wheat, a treatment of soil without plants was also included.

### 2.3. $^{14}\text{C}$ labeling

A day before labeling, the top of each pot was sealed first with a thin layer of low melting point ( $42^\circ\text{C}$ ) paraffin and then with Silicon paste NG 3170 from Thauer & Co. (Dresden, Germany). The seal was tested for air leaks. Then  $\text{CO}_2$  accumulated during the plant growth was flushed out from the soil column. After sealing,

water was added once daily through the upper tubing for air circulation. Fresh air was added to each container twice daily to compensate for O<sub>2</sub> consumed by soil microorganisms and roots.

The plants were labeled with <sup>14</sup>C<sub>2</sub>O in the morning of day 31 after germination. The <sup>14</sup>C pulse labeling began at the beginning of the first period of prolonged night-time. Sealed pots with plants for labeling were put into Plexiglas chamber as described in detail by Cheng et al. (1993). Briefly, the chamber was connected by tubing with a flask containing 2.5 M H<sub>2</sub>SO<sub>4</sub> in which the Na<sub>2</sub><sup>14</sup>CO<sub>3</sub> solution was added. The total <sup>14</sup>C input activity was 4.625 MBq per pot. The duration of pulse labeling was 30 min. During the labeling the CO<sub>2</sub> concentration in the chamber was monitored by an Infrared Gas analyzer (Model CI-301, CID, Inc.). Shortly before the start of labeling the CO<sub>2</sub> concentration in the chamber was 530 μl l<sup>-1</sup>. CO<sub>2</sub> concentration in the chamber dropped exponentially to 73 μl l<sup>-1</sup> (near compensation point) at the end of the 30 min labeling period. After labeling the atmosphere inside the chamber was pumped out into 5 M NaOH solution to remove unassimilated CO<sub>2</sub>. Then the top of the labeling chamber was removed and CO<sub>2</sub> trapping from the soil–root column began.

#### 2.4. Sample analysis

During the experiment, the CO<sub>2</sub> evolved from the soil was trapped in 20 ml of 0.6 M NaOH solution by a closed continuous air circulation (100 ml min<sup>-1</sup>) with a diaphragm pump. Because of the closed circulation, there were no losses of CO<sub>2</sub> due to incomplete absorption by NaOH solution. The NaOH trap was changed every 6 h during the observation period. The CO<sub>2</sub> trapped in NaOH was analyzed for total C content, <sup>14</sup>C activity and δ<sup>13</sup>C value. The total C content was measured with 1/10 dilution on an automatic analyzer (Shimadzu TOC-5050A) using NaHCO<sub>3</sub> as standards. The <sup>14</sup>C activity was measured in 1-ml aliquots of NaOH with 3.5 ml of the scintillation cocktail EcoLite<sup>+</sup> (ICN) after the decay of chemiluminescence by a liquid scintillation counter (Beckmann 6500 LS) using a standard <sup>14</sup>C quenching library.

For preparation of samples for <sup>13</sup>C analysis 1 ml of 2 m SrCl<sub>2</sub> was added to the remaining NaOH trapping solution to form a precipitate of SrCO<sub>3</sub>. The SrCO<sub>3</sub> precipitate was carefully washed ten times with deionized water. Washed SrCO<sub>3</sub> was dried by 60°C and 5 mg of dried SrCO<sub>3</sub> together with 10 mg of V<sub>2</sub>O<sub>5</sub> as catalyst were analyzed for δ<sup>13</sup>C value. The δ<sup>13</sup>C value of SrCO<sub>3</sub> was measured on a mass-spectrometer (Europe Scientific).

#### 2.5. Calculations and statistics

Two methods to partition the total CO<sub>2</sub> efflux from soil in the root-derived and soil-derived parts were used: <sup>13</sup>C natural abundance method and <sup>14</sup>C pulse labeling.

To partition the total CO<sub>2</sub> efflux using the <sup>13</sup>C natural

abundance method we applied the following equation (Cheng, 1996):

$$C_3 = C_t \times (\delta_t - \delta_4) / (\delta_3 - \delta_4) \quad (1)$$

where C<sub>t</sub> = C<sub>3</sub> + C<sub>4</sub>, is the total C from below ground CO<sub>2</sub>, C<sub>3</sub> is the amount of C derived from C3 plants, C<sub>4</sub> is the amount of C derived from C4 soil, δ<sub>t</sub> is the δ<sup>13</sup>C value of the C<sub>t</sub>, δ<sub>3</sub> is the δ<sup>13</sup>C value of the C3 plant C, and δ<sub>4</sub> is the δ<sup>13</sup>C value of the C4 soil C. In contrast to Rochette and Flanagan (1998), we assume that there was no isotopic discrimination during trapping, because trapping was carried out by a forced-air circulation with pumps. Based on Cheng (1996), there was no significant isotopic fractionation by microbial decomposition of exudates and SOM.

To calculate the root-derived CO<sub>2</sub> efflux (C<sub>CO<sub>2</sub></sub><sup>root</sup>) from soil using <sup>14</sup>C pulse labeling the following equation was used:

$$C_{CO_2}^{root} = C_{shoots} \times {}^{14}C_{CO_2} / {}^{14}C_{shoots} / \text{time} \quad (2)$$

where C<sub>CO<sub>2</sub></sub><sup>root</sup> is the C amount in the root-derived CO<sub>2</sub> [mg C kg<sup>-1</sup> h<sup>-1</sup>], C<sub>shoots</sub> is the C amount in the shoots [g C kg<sup>-1</sup>], <sup>14</sup>C<sub>CO<sub>2</sub></sub> is the percentage of <sup>14</sup>C in the investigated flow [% of assimilated <sup>14</sup>C], <sup>14</sup>C<sub>shoots</sub> is the <sup>14</sup>C content in the shoots, 7 days after labeling [% of assimilated <sup>14</sup>C], time is the time between the sowing and the <sup>14</sup>C pulse labeling (31 days).

For calculations using the <sup>14</sup>C method the amount of C in shoots was chosen as the reference. This selection was made because shoot mass and the <sup>14</sup>C incorporation in shoots can be measured more accurately compared to all other compartments of the system (roots, CO<sub>2</sub>, soil etc.). Saggart et al. (1997) and Kuzyakov et al. (1999, 2001) used similar methods to estimate total below-ground C translocation. This calculation method allows only a rough estimation of the amount of C passed through each compartment because the parameters of Eq. (2) are not constant during plant development. This calculation method can be used only after whole <sup>14</sup>C distribution in the plant and the achievement of equilibrium after <sup>14</sup>C pulse labelling.

The soil-derived CO<sub>2</sub> efflux was calculated as the difference between the total CO<sub>2</sub> efflux and the root-derived CO<sub>2</sub> obtained by the <sup>13</sup>C natural abundance method.

The experiment consists of the following treatments: (1) soil without plants (analyzed for total CO<sub>2</sub> and δ<sup>13</sup>C-CO<sub>2</sub>), (2) planted soil unlabeled with <sup>14</sup>C under prolonged darkness period (analyzed for total CO<sub>2</sub> and δ<sup>13</sup>C-CO<sub>2</sub>), (3) soil with plants labeled with <sup>14</sup>C under normal darkness period (analyzed for total CO<sub>2</sub> and <sup>14</sup>C-CO<sub>2</sub>), (4) soil with plants labeled with <sup>14</sup>C under prolonged darkness period (analyzed for total CO<sub>2</sub> and <sup>14</sup>C-CO<sub>2</sub>). The treatments 1, 2, and 4 were conducted with four replicates. The treatment three was conducted with eight replicates. The data are presented as means of four replicates ± standard deviation (SD). *t*-test (α ≤ 5%) was used to indicate the significance of differences between treatments. The linear trends of dynamics of CO<sub>2</sub> efflux for the whole investigation period as well as for the some part of it (see Figs.) were calculated by means of the least squares fit.

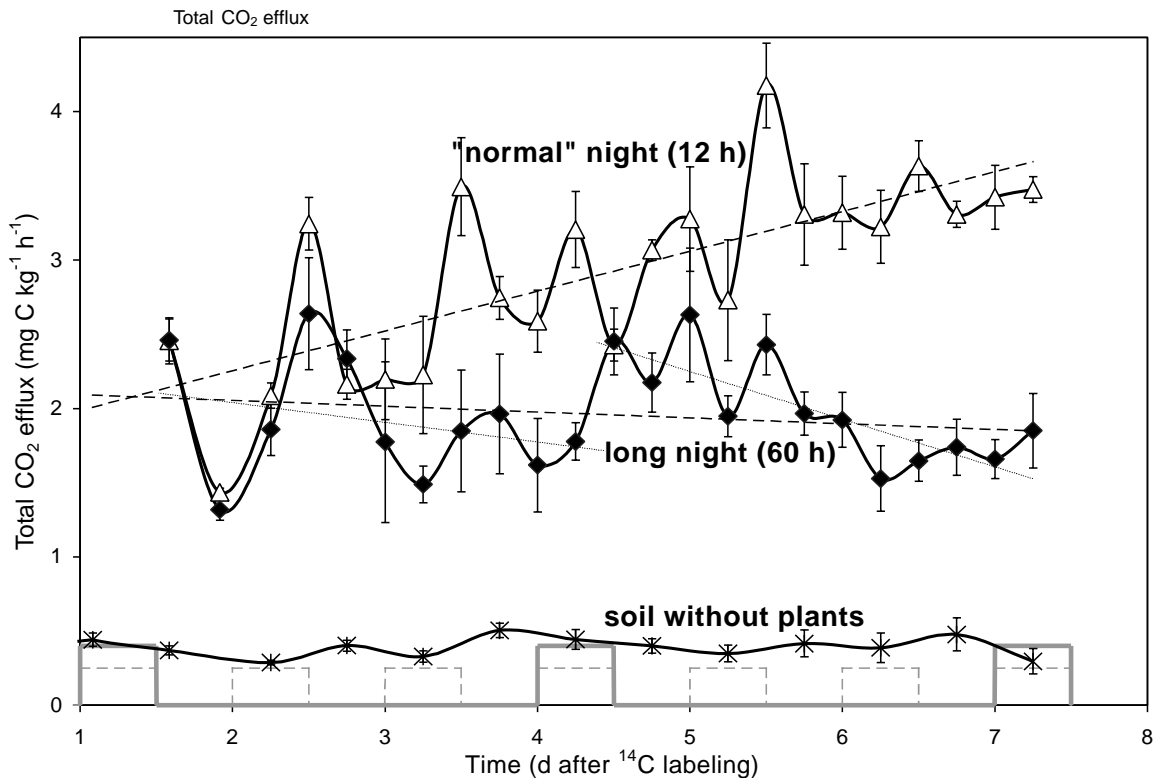


Fig. 1. Total CO<sub>2</sub> efflux from the soil ( $\pm$ SD,  $n = 4$ ) with wheat under different light regimes ( $\Delta$  normal night, 12 h,  $\blacklozenge$  long night, 60 h) and from a bare soil (\*). The dashed lines are linear fits for the whole observation period, the dotted lines are linear fits for each prolonged dark treatment. The light phases are shown as raised gray columns at the bottom.

### 3. Results

#### 3.1. Total below-ground CO<sub>2</sub> efflux

The total below-ground CO<sub>2</sub> efflux from planted soil was influenced by the manipulation of light and dark periods (Fig. 1). At the beginning of the monitoring period, when the light/dark condition was the same (12/12 h day/night) for both planted treatments, the amounts and the diurnal dynamics of below-ground CO<sub>2</sub> efflux were also similar for planted treatments. One day without light led to a decrease of below-ground CO<sub>2</sub> efflux compared to the soil–plant-system with normal (12/12 h) day/night period. This difference increased during day 2 of the prolonged darkness treatment. After day 4 when the light was resumed for 12 h for the prolonged darkness treatment, the difference in total below-ground CO<sub>2</sub> effluxes between the two treatments decreased, but not enough to achieve the same amount of CO<sub>2</sub> efflux from the treatment with a normal day/night period. The difference between treatments increased during the second darkness treatment period when the light was off for another 60 h.

The total CO<sub>2</sub> efflux from the soil–plant system with a normal day/night period increased from 2.0 to 3.5 mg C kg<sup>-1</sup> h<sup>-1</sup> during the 7-day observation period. During the same period, the CO<sub>2</sub> efflux from the soil–plant system with a prolonged night period decreased from 2.0 to 1.7 mg C

kg<sup>-1</sup> h<sup>-1</sup>. So, at the end of day 7 of the treatment with long nights had only half of the total CO<sub>2</sub> efflux compared to the treatment with a normal day/night period. The decrease of CO<sub>2</sub> efflux from the soil–plant-system with the prolonged night period is very clear if the first and the second darkness treatment periods are considered separately (Fig. 1, two sloping straight lines on the CO<sub>2</sub> efflux from soil–plant-system with prolonged night period). This decrease is more than two-fold greater during the second half compared to the first half of the observation period. These results indicated that total below-ground CO<sub>2</sub> efflux was closely coupled with above-ground photosynthesis.

Compared to planted treatments, the CO<sub>2</sub> efflux from unplanted soil is only about 400  $\mu$ g C kg<sup>-1</sup> h<sup>-1</sup> and was stable during the whole observation period (Fig. 1). The CO<sub>2</sub> efflux from unplanted soil amounted to only 12 and 20% of the total CO<sub>2</sub> efflux from the planted soil with normal and prolonged night periods, respectively.

The total CO<sub>2</sub> efflux intensity from each of the two planted treatments had clear diurnal dynamics (Fig. 1). The CO<sub>2</sub> efflux intensity from the treatment with a normal day/night period clearly increased at the end of each light phase. The CO<sub>2</sub> efflux from the treatment with prolonged darkness showed a similar diurnal dynamics as the treatment under the regular day/night condition during the first darkness treatment period, indicating the existence of an endogenous control mechanism. Amplitudes of diurnal changes

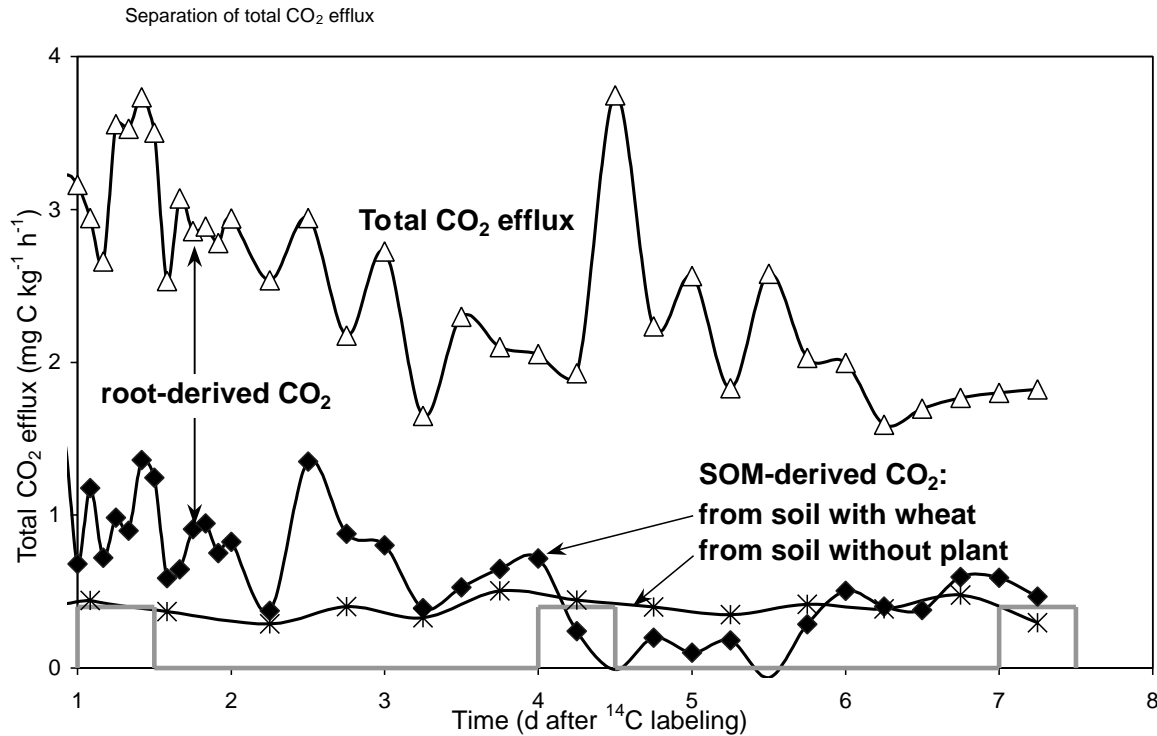


Fig. 2. Separation of total CO<sub>2</sub> efflux from soil with wheat with prolonged dark period ( $\Delta$ ) into SOM-derived ( $\blacklozenge$ ) and root-derived CO<sub>2</sub> and comparison of soil derived CO<sub>2</sub> efflux from bare ( $*$ ) and planted soil. The light phases are shown as raised gray columns. ( $n = 4$ ).

in total below-ground CO<sub>2</sub> efflux from the prolonged darkness treatment were smaller than from the treatment with normal day/night periods, but they were clearly distinct initially, and declined with the duration of darkness. The CO<sub>2</sub> efflux from the soil without plants was nearly constant throughout the observation period without diurnal changes.

### 3.2. Partitioning of CO<sub>2</sub> efflux from soil and priming effects induced by roots

The use of the <sup>13</sup>C natural abundance method (Cheng, 1996; Rochette and Flanagan, 1998) permitted the separation of the soil-derived and root-derived CO<sub>2</sub> from the soil planted with wheat. For this aim the C<sub>3</sub> plant wheat was grown on a soil that was developed under C<sub>4</sub> vegetation (tall grass prairie in Kansas). To use the <sup>13</sup>C natural abundance method for calculating the contribution of rhizosphere respiration to total CO<sub>2</sub> efflux from the soil, it is necessary to know the C isotope values for plant and soil organic matter. The  $\delta^{13}\text{C}$  value of the SOM was  $-14.85 \pm 0.19$ . The  $\delta^{13}\text{C}$  value of wheat shoots and roots was  $-27.42 \pm 0.15$  (SD) and  $-25.01 \pm 0.64$ , respectively.

Soil-derived CO<sub>2</sub> effluxes varied between 5 and 50% of total CO<sub>2</sub> effluxes from the rooted soil (Fig. 2). On average the soil-derived CO<sub>2</sub> efflux from the planted soil amounted to 25% of the total CO<sub>2</sub> efflux. Therefore, 75% of total CO<sub>2</sub> efflux from the planted soil was root-derived. This root-derived CO<sub>2</sub> efflux included root respiration and microbial respiration from decomposing exudates and sloughed root

cells. The root-derived CO<sub>2</sub> efflux from the prolonged night treatment increased during the observation period, although the plants received light only twice for 12 h during a total period of 144 h. Two light-on events day 1 and day 4 led to strong increases of total CO<sub>2</sub> efflux from soil. These increases lasted about 12 h. Then the CO<sub>2</sub> efflux intensity decreased to the previous rate. The increase of CO<sub>2</sub> efflux after the second light period was greater than after the first period. The decrease after the second light period was also greater than after the first one (Fig. 3, two sloping dashed lines).

The absence of light for 60 h resulted in substantial decreases of root-derived CO<sub>2</sub> (Fig. 3). During the second long-night phase this decrease was about 100% faster than during the first long-night phase. After the first 60 h of darkness period a 12 h light period led to a doubling of the root-derived CO<sub>2</sub> efflux for the next 12 h (Fig. 3).

During the first half of the observation period the soil-derived CO<sub>2</sub> from the planted soil was higher than from the unplanted soil (Fig. 2). At the beginning of the second half the soil-derived CO<sub>2</sub> from the planted soil was less than from the unplanted soil. After that, they were similar. The difference between the soil-derived CO<sub>2</sub> from the planted and unplanted soil was a measure of the additional humus mineralization caused by root growth, or priming effect due to rhizosphere activities, especially by root exudates. Easily decomposable exudates led to the increased microbial growth and activity in the rhizosphere and subsequently increasing nutrient acquisition from soil organic matter.

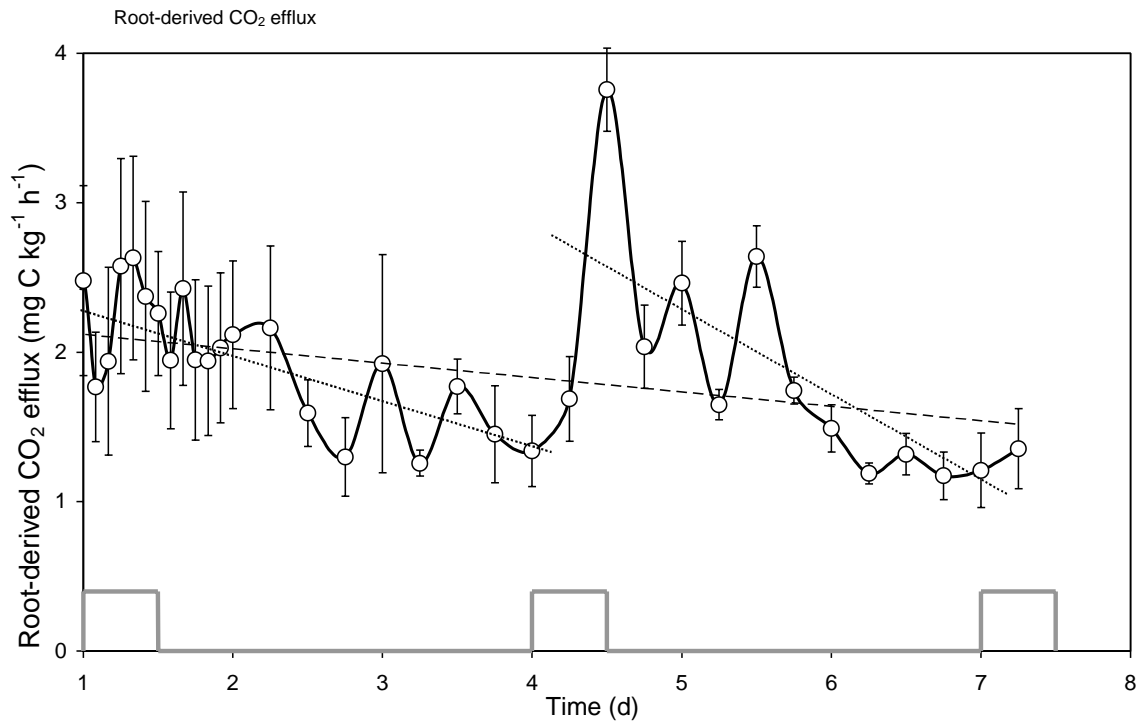


Fig. 3. Root-derived CO<sub>2</sub> efflux ( $\pm$ SD,  $n = 4$ ) from soil with wheat during two prolonged day/night light phases (○). The dashed line is linear fit of the whole observation period; the dotted lines are linear fit for each prolonged dark treatment. The light phases are shown as raised gray columns.

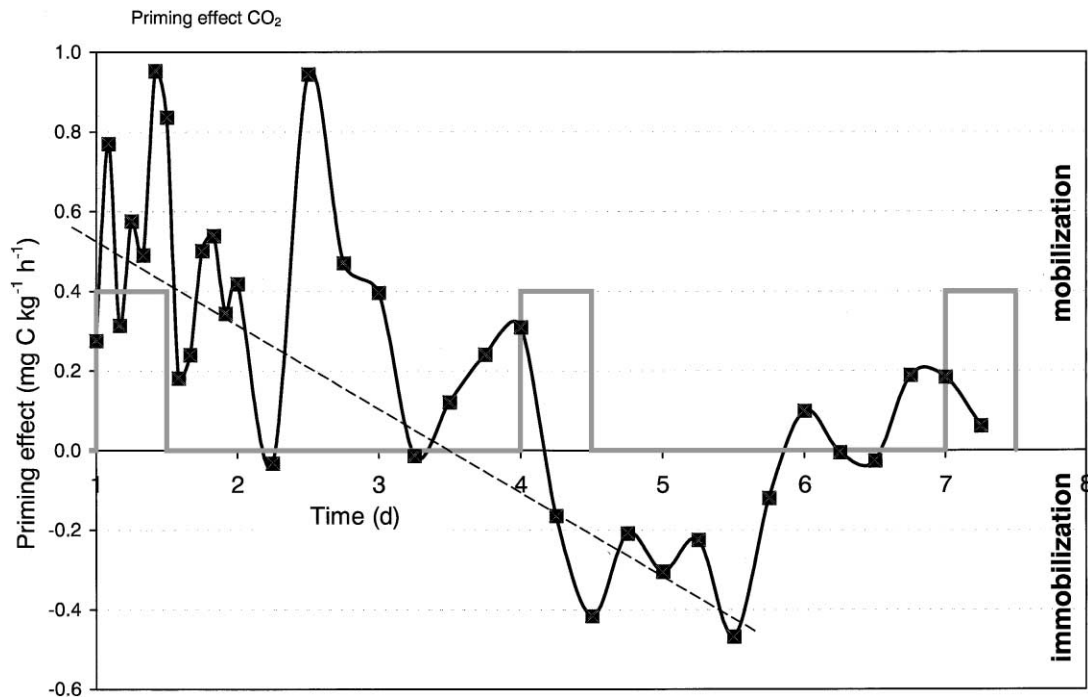


Fig. 4. Priming effect (■) changes in the decomposition of SOM during two prolonged day/night light phases. The dashed lines are linear fit of the first 6 days. The light phases are shown as raised gray columns. ( $n = 4$ ).

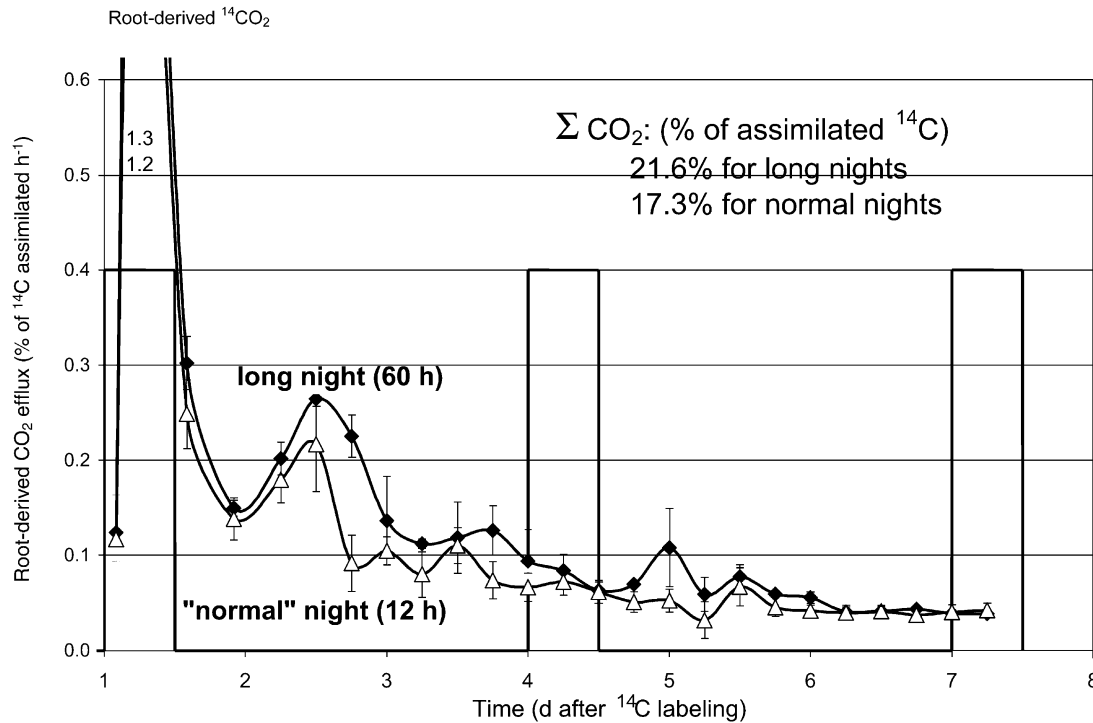


Fig. 5. Root-derived  $\text{CO}_2$  efflux from recently assimilated C measured with  $^{14}\text{C}$  pulse labeling of the shoots under different light regimes ( $\Delta$  normal night with 12 h,  $\blacklozenge$  long night with 60 h). The light phases for prolonged dark period are shown as raised gray columns. ( $n = 4$ ).

During the first 3 days the priming effect was positive and amounted to approximately  $42 \mu\text{g C kg}^{-1} \text{h}^{-1}$  (Fig. 4), or  $33 \text{ kg C ha}^{-1} \text{d}^{-1}$  (calculated for 30 cm soil layer and  $1.1 \text{ g cm}^{-3}$  soil density). Without light, the priming effect decreased and was negative on day 4. The light-on event during day 3 lead to the switch from retardation to acceleration of additional humus decomposition.

### 3.3. $^{14}\text{CO}_2$ efflux from the soil

To observe the respiration dynamics of the recently assimilated C, the plant shoots were labeled with  $^{14}\text{CO}_2$  one day before the beginning of the prolonged night phase. The root-derived  $^{14}\text{CO}_2$  efflux from soil reached the maximum 6 h after pulse labeling (Fig. 5). The first noticeable minimum of  $^{14}\text{CO}_2$  efflux was measured at 24 h after the labeling. At the end of day 2 after labeling (ca 36 h) a second peak was observed regardless of the lighting conditions. This result indicated that there were diurnal changes of rhizosphere respiration of recently assimilated C in both normal light conditions as well as in the absence of light.

The second maximum and also the following  $^{14}\text{CO}_2$  efflux from the soil with plants without light was higher compared to the lighted plants. The  $^{14}\text{CO}_2$  efflux intensity from the plants without light was higher until day 6 after the labeling compared to the plants receiving light. The total amount of root-derived C respired in the rhizosphere was  $17.3 \pm 2.25$  and  $20.6 \pm 0.61\%$  of total assimilated C for plants with and without light, respectively (Fig. 5). The differences between

treatments were significant at  $\alpha \leq 0.05$  during the whole observation period. The difference between both treatments was maximal during day 2 and day 5 after the labeling.

On day 6 after labeling the  $^{14}\text{CO}_2$  efflux intensity from the soil of both variants were similar at ca. 0.04% of assimilated  $\text{C h}^{-1}$ .

## 4. Discussion

### 4.1. Total $\text{CO}_2$ efflux from planted soil and $\text{CO}_2$ partitioning

Our results showed that root-derived  $\text{CO}_2$  was the dominant component in the total  $\text{CO}_2$  efflux from planted soil. This might vary depending on root development and the C content of the soil used. The soil-derived  $\text{CO}_2$  was, on average, 25% and was never more than 50% of the total  $\text{CO}_2$  efflux from soil (Figs. 1 and 2). We used a soil with a high C content; so the contribution of root-derived  $\text{CO}_2$  would be even higher from soils with lower total C contents than from the soil we used in this experiment.

The field conditions in common agricultural practice are different from that used in our laboratory study. In our experiment, only 1 kg of soil was used for five wheat plants in each container. Under common field conditions the volume of top soil per wheat plant would be about five times higher than the amount used in our experiment ( $\sim 1 \text{ kg soil container}^{-1}$ ; calculated for 0.3 m plough layer,  $1.1 \text{ g cm}^{-3}$  soil density, and  $320 \text{ plants m}^{-2}$ ). Therefore, the

calculated share of root-derived CO<sub>2</sub> efflux under the field conditions will amount to about 35–40% of the total CO<sub>2</sub> efflux from soil. Rochette and Flanagan (1998) reported similar values of the contribution of rhizosphere respiration to total soil respiration for maize (C4 plant) on an organic soil (C3 soil). This indicates that plant-derived CO<sub>2</sub> should be separated from soil-derived CO<sub>2</sub> in any study on soil C sequestration, otherwise, soil C loss would be overestimated.

Root-derived CO<sub>2</sub> was very sensitive to changes in photosynthesis. Root-derived CO<sub>2</sub> decreased significantly after 1 or 2 days without photosynthesis (Fig. 1). The absence of photosynthesis for 5–6 day led to the decrease of root-derived CO<sub>2</sub> to approximately 50% compared to plants with a normal day/night period. Stronger decrease of CO<sub>2</sub> efflux from soil during the second prolonged dark period indicated that assimilates were consumed to a larger extent than during the first prolonged dark period of 60 h without light (Fig. 1). The light-on event (12 h) after the first prolonged dark period led to the doubling of the root-derived CO<sub>2</sub> (Fig. 2), even though it was not high enough to reach the same value as for the plants with a normal day/night period.

Our results confirmed the proposition of Craine et al. (1999) that photosynthesis strongly controls total soil CO<sub>2</sub> efflux. Indirect approaches (i.e., shading and removal of aboveground biomass) were employed by Craine et al. (1999). These indirect approaches inherently involved possible confounding factors such as alterations of soil-derived CO<sub>2</sub>, temperature, and plant physiological responses to cutting. Those confounding factors were avoided in our study by using isotope tracers to monitor separately soil-derived CO<sub>2</sub> and root-derived CO<sub>2</sub> without destruction. Our results clearly indicated that rhizosphere respiration is tightly coupled with plant photosynthetic activity. This tight coupling can be inferred from the results of our previous pulse-labeling studies (Cheng et al., 1993, 1994; Kuzyakov et al., 1999, 2001) which showed that photosynthates were transported to roots and metabolized by roots and rhizosphere microorganisms within a few h after initial assimilation. Any factors that affects photosynthesis, or substrate supply to roots and rhizosphere microorganisms, is an important determinant of root-derived CO<sub>2</sub> efflux, and thereby, total CO<sub>2</sub> efflux from soils, such as irradiation, water stress, nutritional status, and herbivory activities. This strongly encourages the inclusion of photosynthesis as crucial controlling factor for total soil CO<sub>2</sub> efflux in global studies of C cycling in addition to temperature and other abiotic factors.

#### 4.2. Diurnal changes in CO<sub>2</sub> efflux

In our experiment, plants were grown at a uniform day and night temperature (22°C). Microbial decomposition of the native soil organic matter (which is strongly influenced by temperature) should be the same during both day and

night phases, if it was not affected by rhizosphere activities. CO<sub>2</sub> efflux from unplanted soil was constant and independent of the day/night changes (comp. CO<sub>2</sub> efflux from unplanted soil, Figs. 1 and 2). However, there was a clear diurnal change in the CO<sub>2</sub> efflux from soil planted with wheat. In the second half of each light period and shortly after the switch off of the light, the CO<sub>2</sub> efflux from planted soil increased to about 20–50% above the ‘night’ low values (Figs. 1–3, and 5). Most likely it is connected with a possible increase of exudation of organic substances from roots and increase of root respiration a few h after the photosynthesis begin. Root-derived CO<sub>2</sub> should be the main component contributing to these diurnal changes. Naturally, fast assimilation of C by photosynthesis and the following fast transport of this C into the roots lead to the rapid appearance of recently assimilated C in the root-derived CO<sub>2</sub>. Therefore, the intensity of root-derived CO<sub>2</sub> follows the diurnal dynamics of photosynthesis. However, root-derived CO<sub>2</sub> also showed a 24-h diurnal cycle during the prolonged dark period, indicating that the diurnal cycle was also regulated by plant endogenous mechanisms.

Some investigations have shown the diurnal changes of CO<sub>2</sub> efflux pattern under the field conditions (Baldocchi et al., 1986; Kim and Verma, 1992; Oberbauer et al., 1996). In most cases the increase of CO<sub>2</sub> efflux from soil in the afternoon was explained by increased soil temperatures. There is no doubt that a rise of soil temperature leads to an increase of CO<sub>2</sub> efflux, but our results demonstrate that the diurnal pattern of root-derived CO<sub>2</sub> efflux was coupled with the plant photosynthetic cycle, and was independent from soil temperature. This indicates that the diurnal soil CO<sub>2</sub> efflux is controlled by photosynthesis cycle together with temperature changes, thereby invalidating the approach of estimating day-time soil CO<sub>2</sub> efflux based on night-time rates after adjustment of temperature differences only, without any consideration of photosynthetic cycles. This result also provides an explanation for the high degree of unaccounted variation in some correlation analyses (Baldocchi et al., 1986; Kim and Verma, 1992) between temperature and total soil CO<sub>2</sub> efflux due to the exclusion of photosynthesis-related variables. This also implies that one measurement per day is insufficient for accurate estimation of total CO<sub>2</sub> efflux from soil under field conditions.

#### 4.3. Use of assimilates by wheat for rhizodeposition

Two apparently contrasting results connected with the use of assimilates by wheat were found: (1) The total root-derived CO<sub>2</sub> efflux from planted soils with the normal day/night setting was higher than that from plants with prolonged night (Fig. 1). It can be explained by the lack of assimilates for exudation and root respiration by plants in the absence of light. (2) In contrast, the <sup>14</sup>CO<sub>2</sub> efflux is higher by the plants with prolonged night compared to that from plants with the normal day/night changes (Fig. 5). Both results were significant throughout the



whole observation period. These apparently contrasting results can be explained by the fact that the plants use more assimilates for growth of cell tissue when they can assimilate new C. In the absence of light no new assimilates are utilized for tissue growth. However, the maintenance energy requirement remains nearly the same. Therefore, the plants have to use the recently assimilated C to cover the energy losses for maintenance respiration as well for exudation.

#### 4.4. Root-derived CO<sub>2</sub>—comparison of two methods

Two methods for estimating root-derived CO<sub>2</sub> were used in this study: the <sup>13</sup>C natural abundance method and artificial pulse labeling with <sup>14</sup>C. The average amount ( $\pm$ SD) of root-derived CO<sub>2</sub> during the observation period was  $1.80 \pm 0.27$  (four replications) and  $1.67 \pm 0.37$  mg C kg<sup>-1</sup> h<sup>-1</sup> (all 12 replications from variants with <sup>14</sup>C labeling) ( $\pm$ SD) for <sup>13</sup>C natural abundance and <sup>14</sup>C pulse labeling, respectively, which did not differ significantly from each other.

According to the principle that the <sup>13</sup>C natural abundance method allows an exact calculation of the share of the root-derived CO<sub>2</sub> of the total CO<sub>2</sub> efflux from soil. Soil–plant pairs impose limitations to the <sup>13</sup>C natural abundance method: C3 plants growing in a C4 soil, or vice versa, are unusual. Hence, the field application of this method is restricted to places where soils developed under C3 vegetation allow the growth of C4 plants and vice versa. Also high-resolution and high-sensitivity mass-spectrometry is necessary for <sup>13</sup>C analyses because a maximal range of only ~14‰ is available for all variations of the <sup>13</sup>C/<sup>12</sup>C ratio in CO<sub>2</sub> (it is calculated as a difference between the  $\delta^{13}\text{C}$  value of cell tissue of C3 and C4 plants). At the same time, the variability of  $\delta^{13}\text{C}$  value in soil or plant is at least about  $\pm 1$ –2‰ (Cheng, 1996) or more (Farquhar et al., 1989). The two requirements mentioned above, limits a wider application of this method. However, this method can easily be used under field conditions (Rochette and Flanagan, 1998), because special equipment for plant labeling is not necessary.

The second method used in our study to estimate root-derived CO<sub>2</sub>: artificial pulse labeling of shoots with <sup>14</sup>C also has many limitations. Eq. (2) given above for calculation of percent distribution of assimilated C assumes that: (1) the partitioning pattern of assimilated C does not change significantly during growth and that (2) distribution of labeled assimilates is almost linear. The <sup>14</sup>C distribution at one stage of development cannot be applied to another because partitioning patterns undergo change during plant growth. The most important limitation of pulse labeling is that the results of C allocation observed for a specific growth stage cannot be directly applied for the whole growing season. However, a series of labeling pulses applied at regular intervals during plant growth have been found to provide a reasonable estimate of the cumulative below-ground C input (Keith et al., 1986; Gregory and Atwell, 1991; Jensen,

1993; Swinnen et al., 1994; Warembourg and Estelrich, 2000; Kuzyakov et al., 1999, 2001). The calculation of the root-derived CO<sub>2</sub> from the total CO<sub>2</sub> efflux according <sup>14</sup>C distribution after pulse labeling could not be used under conditions of rapidly changing the <sup>14</sup>C incorporation in the shoots (i.e. defoliation, strong reduced photosynthesis, etc.). As shown by Hodge and Millard (1998) <sup>14</sup>C pulse chase methodology is an important physiological tool, although it should not be used in isolation.

In our experiment both methods gave similar results. This means that the distribution of assimilates by wheat did not change considerably at least until the end of the fifth week after germination. Additionally, the increase of dry mass of wheat was nearly linear. It means that both methods can be used to estimate the amount of root-derived CO<sub>2</sub> by young wheat plants. It may be possible to use the Eq. (2) for the vegetative stage of different grasses as it was done in experiments of Saggari et al. (1997) and Kuzyakov et al. (1999, 2001). The allocation pattern of assimilated C changes considerably during the transition from the vegetative stage to the reproductive stage. Therefore, artificial <sup>14</sup>C pulse labeling cannot be applied during this transition to estimate root-derived CO<sub>2</sub> and total rhizodeposition.

#### 4.5. Rhizosphere priming effect

Priming effects (PE) are short-term changes (in most cases increases) in decomposition rates of soil organic matter induced by input of organic and mineral substances (i.e. exudates, plant remainders, fertilizers) in soil. PE have been measured in many studies after application of organic or mineral fertilizers to the soil (reviewed by Jenkinson et al., 1985; Kuzyakov et al., 2000). However, there is conflicting evidence in the literature on the effects of plant roots on soil organic matter (SOM) decomposition. Roots have been found to have both stimulatory and inhibitory effects on SOM decomposition. Laboratory experiments under controlled conditions have shown that when <sup>14</sup>C-labeled plant materials were decomposed in soil planted with maize, ryegrass, wheat or barley, <sup>14</sup>CO<sub>2</sub> release from the soil was reduced compared to bare soil controls (Reid and Goss, 1982, 1983; Sparling et al., 1982). The authors of these reports proposed that this inhibitory effect of living roots on SOM decomposition was due to competition between the roots and the rhizosphere microflora for substrates. In contrast, a stimulatory effect of living roots on SOM decomposition has been reported in other laboratory experiments (Helal and Sauerbeck, 1986, 1989; Cheng and Coleman, 1990; Kuzyakov et al., 2001). The breakdown of soil aggregates by growing roots and the stimulation of the rhizosphere microflora by exudation of easy available organic compounds were proposed as mechanisms, which resulted in the increased decomposition of SOM. Furthermore, other research has shown that SOM decomposition is dependent upon the length of exposure to living roots. In a 2 year study, the presence of plants suppressed

the decomposition of newly-incorporated  $^{14}\text{C}$ -labeled plant material during the first 200 days but stimulated the mineralization of  $^{14}\text{C}$  in the soil during the later stage (200 days until 2 years) when compared to bare soil (Sallih and Bottner, 1988).

Methodological differences among those studies have been assumed to be an important reason for the controversy. Our study, with the simultaneous use of both methods, shows that both methods produce similar results. Methodological differences were probably not as critical as it was assumed. Independent of the calculation method, the priming effect was positive during the first 3 days. The absence of light caused the reduction of exudation and subsequent decrease of the priming effect (Fig. 4, sloping dashed line). Since easily-decomposable substrates decreased due to the absence of photosynthesis, the ability of microorganisms to decompose additional SOM also decreased. After photosynthesis resumed on day 4 the microbial community switched from decomposing SOM to easily decomposable root exudates. This led to the reduction of SOM decomposition for the following 2 days. During this time the microbial activity increased, available N was used, and microorganisms returned to the accelerated SOM decomposition during day 6 and day 7. Qualitative changes in exudation by prolonged night-time may also be important in modifying microbial processing of root C flow, and also the balance of mineralization and immobilization processes. Although in the prolonged darkness treatment the light was absent for 5 days during 1 week, an average positive priming effect of about  $22 \mu\text{g C kg}^{-1} \text{h}^{-1}$  was recorded during the whole observation period. This value corresponds to about  $17 \text{ kg C ha}^{-1} \text{d}^{-1}$  of extra mineralization of SOM (calculated for a 30 cm soil layer and  $1.1 \text{ g cm}^{-3}$  soil density).

We cannot measure the dynamics of PE using  $^{14}\text{C}$ -pulse labeling. However, the total amount of root derived  $\text{CO}_2$  measured with  $^{14}\text{C}$  during the investigation was the same as the value obtained from the  $^{13}\text{C}$  method. Therefore, the amount of additional decomposition of SOM (positive PE) for the whole period was similar irrespective of the tracer method used.

The tight coupling of priming effect with photosynthesis suggests that root exudates are the main agent responsible for the rhizosphere priming effect. Root turnover (Sallih and Bottner, 1988) and breaking down of soil aggregates (Helal and Sauerbeck, 1986, 1987) by roots have been proposed to be other possible agents for a rhizosphere priming effect. These two processes are probably do not change quickly enough to play a significant role, because of the short duration of our experiment.

These results show that the cultivation of wheat leads to the increasing decomposition intensity of soil organic matter. However, the results of many long-term field experiments show the opposite picture: The plant cultivation lead to the accumulation of SOM compared to the bare soil (Körschens and Müller, 1994). This contradiction can be explained by annual cycles of the accumulation-

decomposition intensity of SOM. During the cultivation of plants in the spring and early summer, the exudation intensity of growing plants is very high, and it leads to the increased microbial growth and activity in the rhizosphere. The acceleration of the SOM decomposition intensity follows them. In summer and autumn the active plant growth is finished and the exudation is negligible or is absent. The main input of organic substances consists from root and shoot remainders which decomposition is much slower than that of exudates. Therefore the microbial activity and the decomposition intensity of SOM are reduced. The humification of the plant residues prevails.

## Acknowledgements

We thank Dr John Blair for helping us obtain the valuable Tallgrass prairie soils used in this experiment. This research was supported by a grant from the United States Department of Agriculture and by the German Research Foundation (DFG) in the form of a research fellowship for Y. Kuzyakov. We thank Professor J.S. Waid for his linguistic improvement of the manuscript.

## References

- Baldocchi, D.D., Verma, S.B., Matt, D.R., Anderson, D.E., 1986. Eddy-correlation measurements of carbon dioxide efflux from the floor of a deciduous forest. *Journal of Applied Ecology* 23, 967–975.
- Bidulph, O., 1969. Mechanisms of translocation of plant metabolites. In: Eastin, J.D., Haskins, F.A., Sullivan, C.Y., Van Bavel, C.H.M. (Eds.). *Physiological Aspects of Crop Yield*. American Society of Agronomy, Madison, WI, pp. 143–164.
- Bottner, P., Sallih, Z., Billes, G., 1988. Root activity and carbon metabolism in soils. *Biology and Fertility of Soils* 7, 71–78.
- Bottner, P., Cortez, J., Sallih, Z., 1991. Effect of living roots on carbon and nitrogen of the soil microbial biomass. In: Atkinson, D. (Ed.). *British Ecological Society Special Publication 10*. Blackwell Scientific Publications, Oxford, pp. 201–210.
- Cheng, W., Coleman, D.C., 1990. Effect of living roots on soil organic matter decomposition. *Soil Biology & Biochemistry* 22, 781–787.
- Cheng, W., Coleman, D.C., Carroll, C.R., Hoffman, C.A., 1993. In situ measurement of root respiration and soluble C concentrations in the rhizosphere. *Soil Biology & Biochemistry* 25, 1189–1196.
- Cheng, W., Coleman, D.C., Carroll, C.R., Hoffman, C.A., 1994. Investigating short-term carbon flows in the rhizospheres of different plant species, using isotopic trapping. *Agronomy Journal* 86, 782–788.
- Cheng, W., 1996. Measurement of rhizosphere respiration and organic matter decomposition using natural  $^{13}\text{C}$ . *Plant and Soil* 183, 263–268.
- Craine, J.M., Wedin, D.A., Chapin, F.S., 1999. Predominance of ecophysiological controls on soil  $\text{CO}_2$  flux in a Minnesota grassland. *Plant and Soil* 207, 77–86.
- Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. *Annual Reviews of Plant Physiology and Plant Molecular Biology* 40, 503–537.
- Gregory, P.J., Atwell, B.J., 1991. The fate of carbon in pulse labelled crops of barley and wheat. *Plant and Soil* 136, 205–213.
- Helal, H.M., Sauerbeck, D., 1986. Effect of plant roots on carbon metabolism of soil microbial biomass. *Zeitschrift für Pflanzenernährung und Bodenkunde* 149, 181–188.
- Helal, H.M., Sauerbeck, D.R., 1987. Direct and indirect influences of plant

- roots on organic matter and phosphorus turnover in soil. *INTECOL Bulletin* 15, 49–58.
- Helal, H.M., Sauerbeck, D., 1989. Carbon turnover in the rhizosphere. *Zeitschrift für Pflanzenernährung und Bodenkunde* 152, 211–216.
- Hoagland, D.R., Arnon, D.I., 1950. The water-culture method for growing plants without soil. California Agricultural Experimental Station, Circular, 347.
- Hodge, A., Paterson, E., Thornton, B., Millard, P., Killham, K., 1997. Effects of photon flux density on carbon partitioning and rhizosphere carbon flow of *Lolium perenne*. *Journal of Experimental Botany* 48, 1797–1805.
- Hodge, A., Millard, P., 1998. Effect of elevated CO<sub>2</sub> on carbon partitioning and exudate release from *Plantago lanceolata* seedlings. *Physiologia Plantarum* 103, 280–286.
- Jenkinson, D.S., Fox, R.H., Rayner, J.H., 1985. Interactions between fertilizer nitrogen and soil nitrogen *m*- the so-called ‘priming’ effect. *Journal of Soil Science* 36, 425–444.
- Jensen, B., 1993. Rhizodeposition by <sup>14</sup>C<sub>2</sub>-pulse-labelled spring barley grown in small field plots on sandy loam. *Soil Biology & Biochemistry* 25, 1553–1559.
- Johnen, B.G., Sauerbeck, D., 1977. A tracer technique for measuring growth, mass and microbial breakdown of plant roots during vegetation. In: Lohm, U., Persson, T. (Eds.). *Soil Organisms as Components of Ecosystems*, Vol. 25. Ecological Bulletins, Stockholm, pp. 366–373.
- Keith, H., Oades, J.M., Martin, J.K., 1986. Input of carbon to soil from wheat plants. *Soil Biology & Biochemistry* 18, 445–449.
- Kim, J., Verma, S.B., 1992. Soil surface CO<sub>2</sub> flux in a Minnesota peatland. *Biogeochemistry* 18, 37–51.
- Körschens, M., Müller, A., 1994. Nachhaltige Bodennutzung, gemessen am Ertrag sowie an C- und N-Bilanzen. *Archiv für Acker-, Pflanzenbau und Bodenkunde* 38, 373–381.
- Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. *Review. Zeitschrift für Pflanzenernährung und Bodenkunde* 163, 421–431.
- Kuzyakov, Y., Kretschmar, A., Stahr, K., 1999. Contribution of *Lolium perenne* rhizodeposition to carbon turnover of pasture soil. *Plant and Soil* 213, 127–136.
- Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. *Soil Biology & Biochemistry* 32, 1485–1498.
- Kuzyakov, Y., Ehrensberger, H., Stahr, K., 2001. Carbon partitioning and below-ground translocation by *Lolium perenne*. *Soil Biology & Biochemistry* 33, 61–74.
- Mary, B., Fresneau, C., Morel, J.L., Mariotti, A., 1993. C and N cycling during decomposition of root mucilage, roots and glucose in soil. *Soil Biology & Biochemistry* 25, 1005–1014.
- Martin, J.K., Merckx, R., 1992. The partitioning of photosynthetically fixed carbon within the rhizosphere of mature wheat. *Soil Biology & Biochemistry* 24, 1147–1156.
- Meharg, A.A., 1994. A critical review of labelling techniques used to quantify rhizosphere carbon-flow. *Plant and Soil* 166, 55–62.
- Meharg, A.A., Killham, K., 1990. Carbon distribution within the plant and rhizosphere in laboratory and field grown *Lolium perenne* at different stages of development. *Soil Biology & Biochemistry* 22, 471–477.
- Nguyen, C., Todorovic, C., Robin, C., Christophe, A., Guckert, A., 1999. Continuous monitoring of rhizosphere respiration after labelling of plant shoots with <sup>14</sup>C<sub>2</sub>. *Plant and Soil* 212, 191–201.
- Oberbauer, S.F., Gillespie, C.T., Cheng, W., Sala, A., Gebauer, R., Tenhunen, J.D., 1996. Diurnal and seasonal patterns of ecosystem CO<sub>2</sub> efflux from upland tundra in the foothills of the Brooks Range, Alaska, USA Arctic and Alpine Research 28, 328–338.
- Qian, J.H., Doran, J.W., Walters, D.T., 1997. Maize plant contributions to root zone available carbon and microbial transformations of nitrogen. *Soil Biology & Biochemistry* 29, 1451–1462.
- Rochette, P., Flanagan, L.B., 1998. Quantifying rhizosphere respiration in a corn crop under field conditions. *Soil Science Society of America Journal* 61, 466–474.
- Raich, J.W., Schlesinger, W.H., 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus* 44, 81–99.
- Reid, J.B., Goss, M.J., 1982. Suppression of decomposition of <sup>14</sup>C carbon isotope-labelled plant roots in the presence of living roots of maize and perennial ryegrass. *Journal of Soil Science* 33, 387–395.
- Reid, J.B., Goss, M.J., 1983. Growing crops and transformations of <sup>14</sup>C-labelled soil organic matter. *Soil Biology & Biochemistry* 15, 687–691.
- Saggar, S., Hedley, C., Mackay, A.D., 1997. Partitioning and translocation of photosynthetically fixed <sup>14</sup>C in grazed hill pastures. *Biology and Fertility of Soils* 25, 152–158.
- Sallih, Z., Bottner, P., 1988. Effect of wheat (*Triticum aestivum*) roots on mineralization rates of soil organic matter. *Biology and Fertility of Soils* 7, 67–70.
- Sparling, G.P., Cheshire, M.V., Mundie, C.M., 1982. Effect of barley plants on the decomposition of <sup>14</sup>C-labelled soil organic matter. *Journal of Soil Science* 33, 89–100.
- Swinnen, J., Van Veen, J.A., Merckx, R., 1994. <sup>14</sup>C pulse-labelling of field-grown spring wheat: an evaluation of its use in rhizosphere carbon budget estimations. *Soil Biology & Biochemistry* 26, 161–170.
- Swinnen, J., Van Veen, J.A., Merckx, R., 1995. Root decay and turnover of rhizodeposits estimated by <sup>14</sup>C pulse-labelling in field-grown winter wheat and spring barley. *Soil Biology & Biochemistry* 27, 211–217.
- Warembourg, F.R., Paul, E.A., 1977. Seasonal transfers of assimilated <sup>14</sup>C in grassland: plant production and turnover, soil and plant respiration. *Soil Biology & Biochemistry* 9, 295–301.
- Warembourg, F.R., Billes, G., 1979. Estimating carbon transfers in the plant rhizosphere. In: Harley, J.L., Scott Russell, R. (Eds.). *The Soil–Root Interface*. Academic Press, London, pp. 183–196.
- Warembourg, F.R., Estelrich, D.H., 2000. Towards a better understanding of carbon flow in the rhizosphere: a time-dependent approach using carbon-14. *Biology and Fertility of Soils* 30, 528–534.
- Whipps, J.M., 1987. Carbon loss from the roots of tomato and pea seedlings grown in soil. *Plant and Soil* 103, 95–100.