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Carbon partitioning and below-ground translocation by *Lolium perenne*

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Abstract

Carbon (C) balance, rhizodeposition and root respiration during development of *Lolium perenne* were studied on a loamy Gleyic Cambisol by $^{14}CO_2$ pulse labeling of shoots in a two-compartment chamber under controlled laboratory conditions.

The losses from shoot respiration were about 36% of the total assimilated C. The highest respiration intensity was measured in the first night after the labeling, and diminishes exponentially over time.

Total $^{14}CO_2$ efflux from the soil (root respiration, microbial respiration of exudates and dead roots) in the first eight days after the ^{14}C pulse labeling increased with plant development from 2.7 to 11% of the total ^{14}C assimilated by plants. A model approach used for the partitioning of rhizosphere respiration showed that measured root respiration was between 1.4 and 3.5% of assimilated ^{14}C, while microbial respiration of easily available rhizodeposits and dead root residues was between 0.9 and 6.8% of assimilated C. Both respiration processes increased during plant development. However, only the increase in root respiration was significant. The average contribution of root respiration to total $^{14}CO_2$ efflux from the soil was approximately 46%.

Total CO_2 efflux from the soil was separated into plant-derived and soil-derived CO_2 using ^{14}C labeling. Additional decomposition of soil organic matter (positive priming effects) in rhizosphere was calculated by subtracting the CO_2 efflux from bare soil from soil-derived CO_2 efflux from soil with plants. Priming effects due to plant rhizodeposition reach 60 kg of C ha^{-1} d^{-1}. ^{14}C incorporated in soil micro-organisms (extraction–fumigation) amounts to 0.8–3.2% of assimilated C. The total below-ground transfer of organic C by *Lolium perenne* was about 2800 kg of C ha^{-1}. The C input into the soil consists of about 50% of easily available organic substances.

Keywords: Carbon turnover; Exudation; *Lolium perenne*; Priming effect; Rhizodeposition; Rhizosphere; Root respiration; ^{14}C pulse labeling; CO_2

1. Introduction

Quantification of carbon (C) input of perennial crops into soils is essential in the study of soil organic matter dynamics. The total amount of below-ground C translocation by plants is far more difficult to quantify as compared to the above-ground yield. Isolation of roots by the traditional soil washing procedure underestimates the total C input to soil; with a considerable portion of the fine roots and root hairs being lost during the root washing. Some parts of rhizodeposits especially exudates, lysates, sloughed-off cells etc., are not accounted for.

Rhizodeposition of root exudates (easily available C source) greatly influences C turnover in soils, and can lead to C accumulation or C consumption due to its influence on the microbial activity in the rhizosphere.

Labeling of plants with ^{14}C has been widely used to quantify rhizodeposition as it allows for a distinction between root-derived organic C and native soil organic C (Whipps, 1990). Two types of ^{14}C labeling are commonly used (i.e. continuous labeling and pulse labeling). Pulse labeling with ^{14}C, compared to continuous labeling, has the advantage of being easier to handle (Whipps, 1990), giving more information on photosynthate distribution at specific development stages of the plants (Meharg and Killham, 1990), and it can be used for the kinetic investigation of $^{14}CO_2$ evolution from soil (Warembourg and Billes, 1979; Kuzyakov et al., 1999). In addition, pulse labeling coupled with monitoring of $^{14}CO_2$ evolution dynamics from soil can be used to distinguish between different stages of rhizosphere respiration (Kuzyakov et al., 1999). A series of pulse labeling at regular intervals during the growth period have been found to provide a reasonable estimate of the cumulative below-ground C input (Keith et al., 1986; Gregory and Atwell, 1991; Swinnen et al., 1995a,b; Jensen, 1993).

Results on the contribution of cereals (wheat, barley) to C accumulation and turnover of soil organic matter have shown that agricultural plants seldom transfer more than

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Table 1
Basic characteristics of the A_h horizon of the fine loamy Gleyic Cambisol from an Allgäu pasture (south Germany) used in the experiment (Kleber, 1997) (FC[1], field capacity (pF = 1.8); AWC[2], available water capacity (pF 1.8–4.2))

pH($CaCl_2$)	Corg %	Nt %	C/N	Clay (<2 μm)	Silt (2–63 μm)	Sand (63–2000 μm)	FC %	AWC %	$CaCO_3$ %
5.2	4.7	0.46	10	28.4	47.1	24.5	50	23	0

33% of assimilated C into the soil (Zagal et al., 1993; Swinnen et al., 1995a,b). About one-third to half of this C can be respired by roots and micro-organisms in the first days after $^{14}CO_2$ assimilation (Gregory and Atwell, 1991; Cheng et al., 1993; Swinnen, 1994; Swinnen et al., 1995a).

There is little information about the contribution of pasture plants to C accumulation in soil and turnover of soil organic matter. Pasture plants can differ from annual agricultural crops such as wheat or barley, with which most of the investigations about rhizodeposition have been conducted. This is because agricultural grain plants were selected and cultivated in order to maximize the harvest index and floor yield. This led to differences in partitioning of assimilates from photosynthesis. In addition, the total below-ground C transfer by pasture plants can be higher than that of annual agricultural crops because of prolonged vegetative development. Annual agricultural crops are known to finish the main root growth at emergence of grains and by maturity the roots stop to grow. Pasture plants are cut before or shortly after the emergence of grains. The roots stay in the soil and their growth and development continues.

The plants lose an essential portion of assimilated C by shoot respiration during the first days after assimilation. This amount must be taken into consideration by calculating recovery rates of assimilated ^{14}C in experiments.

The aims of this research were:

1. to determine the distribution and below-ground C transfer of assimilated ^{14}C at different development stages of a typical representative of pasture plants (*Lolium perenne* L.);
2. to estimate the ^{14}C losses during respiration of shoots as part of the C balance of the plant;
3. to separate $^{14}CO_2$ efflux from soil into root respiration and microbial respiration of exudates;
4. to estimate the acceleration of turnover of soil organic matter due to rhizodeposition of plants;
5. to estimate the incorporation of ^{14}C exudates in the rhizosphere micro-organisms.

2. Materials and methods

We studied the C rhizodeposition and root respiration of *Lolium perenne* L. on a fine loamy Gleyic Cambisol by means of $^{14}CO_2$ pulse labeling, using a two-compartment chamber (Warembourg and Kummerow, 1991; Martin and Merckx, 1992) under controlled laboratory conditions.

2.1. Soil

The soil was taken from the top 10 cm (A_h horizon) of a long-term pasture in Allgäu found on a 3–4% slope (South Germany, Kleber, 1997). The soil samples taken in February 1998, were air dried, homogenized and passed through 5- and 4-mm sieves to separate large roots and stones. Basic characteristics of the soil are presented in Table 1. Each pot for plant growing was filled with 3.5 kg of air-dried soil to a specific gravity of 1.2 g cm^{-3}.

2.2. Chamber and labeling

The labeling apparatus previously described by Kuzyakov et al. (1999) consists of a two compartment Plexiglas chamber. The lower compartment (diameter 138 mm and height 200 mm) was for the soil and plant roots, and the upper compartment (diameter 138 mm and height 300 mm) for the shoots and $^{14}CO_2$ generation. The lower compartment has a collector for drained water. Both compartments are separated from each other by a Plexiglas lid with drill holes (diameter 8 mm) for plants. A day before labeling, each hole with a plant was sealed with silicon paste NG 3170 from Thauer and Co., Dresden (Gregory and Atwell, 1991; Swinnen et al., 1995a). The seal was tested for air leaks.

The labeling took place at five times during growth of *Lolium* (at 52, 56, 76, 89 and 103 days after sowing; compare Table 2). Plants in different chambers were used for each labeling. Each container was labeled separately. 580 kBq of ^{14}C as $Na_2{}^{14}CO_3$ solution was put in a 2-ml Eppendorf micro test tube in the upper compartment of the chamber and the chamber was then closed. 1 ml of 5 M H_2SO_4 was added to the $Na_2{}^{14}CO_3$ solution in the

Table 2
Plant height, days after sowing, and development stages of *Lolium perenne* at which ^{14}C labeling was conducted

Plant height (cm)	20	22	28	30	32
Days after sowing	52	56	76	89	103
Development stage	Tillering begin		Tillering middle		Tillering end

microtest tube through a teflon tube. This allowed the complete evolution of $^{14}CO_2$ into the chamber atmosphere. Assimilation took place within 7 h after the pulsing of $^{14}CO_2$. After the labeling period of 7 h, trapping of CO_2 from the upper compartment was started to remove the remaining unassimilated $^{14}CO_2$. The air of the upper chamber was pumped through 20 ml of 0.5 M NaOH solution to remove $^{14}CO_2$. Then, the top of the chamber was removed. By the third labeling, the upper part of the chamber was not removed after 7 h and was kept closed for 8 days to estimate the total shoot respiration and system losses. The trap for $^{14}CO_2$ evolved from the shoot respiration by third labeling was changed two times a day, according to the day/night changes.

2.3. Growing conditions

Nine pre-vernalized (seeds were exposed for 11 days to a temperature of +2°C) seedlings of *Lolium perenne* L, sp. Gremie, were grown in each pot at 3.5 cm distance. The plants were grown at 27 ± 1°C day, and 22 ± 1°C night temperature with a day-length of 14 h and light intensity of approximately 400 μmol m^{-2} s^{-1}. The soil water content of each chamber was adjusted daily with deionized water to half of the daily consumption over 60% of the available field capacity. The soil water content never dropped below 50% of the available field capacity.

Two N treatments were investigated. The plants of the variants with additional N were fertilized five times during the experiment with 564 mg KNO_3 as water solution. This amount corresponds to about 50 kg of N ha^{-1} per treatment and to 250 kg of N ha^{-1} per total growing period. Always, the addition of mineral N took place one day before labeling to get the impact of fertilizing on C partitioning. The plants of the variants without additional N fertilizing did not receive KNO_3.

To compare total unlabeled CO_2 evolution with and without *Lolium perenne*, the soil without plants were also incubated in the same pots, under the same conditions and with the same two levels of N fertilization.

2.4. Microbial biomass C and N

Microbial biomass C and N was determined by a fumigation–extraction method (Vance et al., 1987; Ross, 1990). Fumigated and non-fumigated soil samples (25 g) were extracted with 200 ml of 0.5 M K_2SO_4 for 30 min and filtered. An aliquot of K_2SO_4 solution was analyzed for organic C and total N with Dimatoc-100. The ^{14}C activity of another aliquot was determined by scintillation counting. The additional oxidisable C, N and ^{14}C counts obtained from the fumigated soils were taken to represent the microbial-C and N flush and converted to microbial biomass C and N using the relationship (Ross, 1990):

Microbial C = C flush *2.34

The same equation was used for the calculation of ^{14}C and N in microbial biomass. The analyses for microbial biomass C and N were made in duplicate from the top soil layer (0–5 cm) of each container.

2.5. Sample analysis

During the experiment, the CO_2 evolved from the lower compartment was trapped in 20 ml of 0.5 m NaOH solution by continuous pumping (100 cm^3 min^{-1}) with a membrane pump. The trap for $^{14}CO_2$ evolved from the soil was changed every hour immediately after labeling, but after 5 days only twice daily. Eight days after labeling the lower chamber was opened, each plant was cut at the base and each root-soil column pulled out. The soil was subsampled in four 5-cm layers, homogenized, and analyzed separately. Each soil sample was divided into four parts. (1) About 100 g soil were frozen and stored for future determination of microbial biomass and mineral N. (2) About 700 g were used for the root washing by hand, on a 0.5-mm sieve. (3) 25 g of the soil were dried at 105°C for the dry mass analysis and (4) 75 g at 60°C for ^{14}C and chemical analyses. Shoots and roots were also dried at 60°C. Dry samples of shoots, roots and soil were mixed and pulverized in a ball mill (Retsch), prior to analysis, for radioactivity and for total C and N determination.

Radioactivity of shoots, roots and soil samples was measured after combustion of 1 g of sample within an oxidizer unit (Canberra Packard Co. Ltd, Model 307) with the scintillation cocktail Permafluor E^+ (Canberra Packard Co. Ltd) by a Liquid Scintillation Counter Tri-Carb 2000CA (Canberra Packard Co. Ltd).

^{14}C-CO_2 collected in an NaOH solution was measured with the scintillation cocktail Rothiscint-22x (Roth Company) on 1-ml aliquots of NaOH after the decay of chemiluminescence. The ^{14}C counting efficiency was about 89 ± 1% and the ^{14}C-activity measurement error did not exceed 2%. The absolute ^{14}C-activity was standardized by the addition of the NaOH solution as a quencher to the scintillation cocktail and using a two-channel ratio method of extended standard (tSIE).

$^{14}CO_2$ effluxes from the soil were measured with two replicates. Variability between the replicates did not exceed 15%. ^{14}C measurements of shoot, root and soil were conducted with four replicates.

Total content of CO_2 collected in the NaOH solution was measured by titration with 0.2 M HCl against phenolphthalein, after addition of 2.0 M $BaCl_2$ solution (Black, 1965). Total C and N content in the shoot and root was measured on a C-N-Analyser (Carlo-Erba). The total C content in shoots was about 40 ± 3% of dry mass and was accepted as a constant. The total C content in washed roots varies considerably because of the different contents of mineral soil particles. The C content in the roots was considered by all calculations according to C analysis.

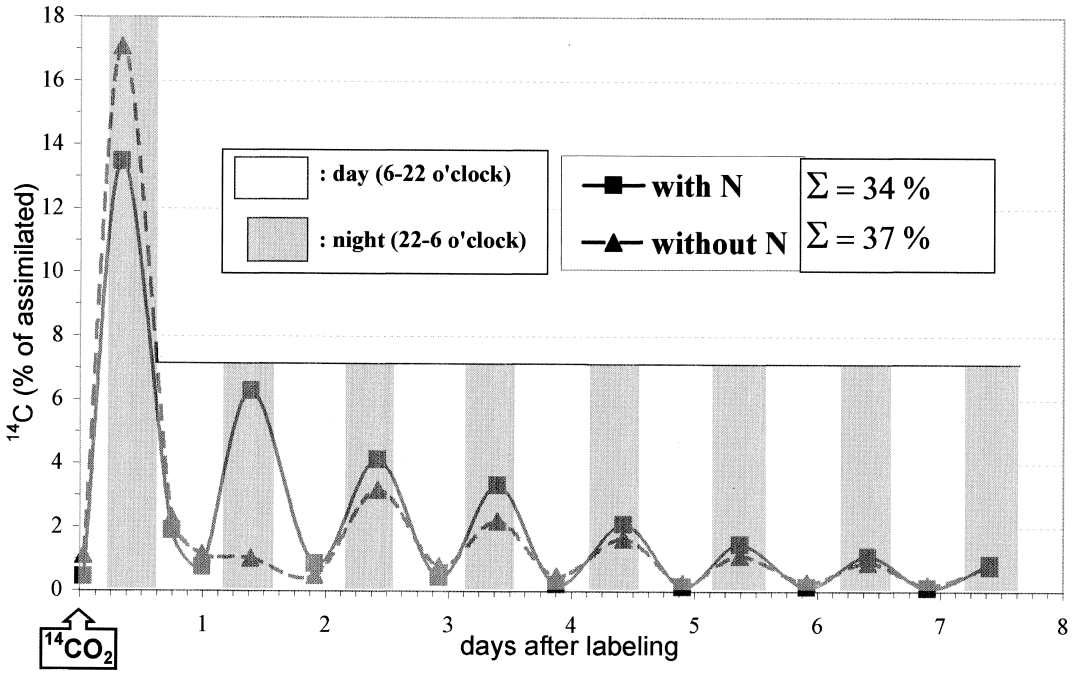

Fig. 1. Dynamics of shoot respiration of *Lolium perenne* for eight days after $^{14}CO_2$ pulse labeling of shoots. Third labeling period. Day and night phases are shown.

2.6. Calculations and statistics

The ^{14}C data for each replicate were expressed as percentages of net assimilated ^{14}C. We define the net assimilated ^{14}C using the expression:

$$^{14}C \text{ assimilated} = {}^{14}C \text{ input} - {}^{14}Cna - {}^{14}Crem - {}^{14}Ccw \quad (1)$$

where:

^{14}Cna, ^{14}C not assimilated after 7 h;
$^{14}Crem$, ^{14}C remainder in 2-ml Eppendorf micro test tube;
^{14}Ccw, ^{14}C in condensed water on the walls of the top chamber.

The C amount (g m^{-2}) for different flows has been calculated by using the following equation:

$$C^{flow}_{amount} = C_{shoots} \times {}^{14}C_{flow}/{}^{14}C_{shoots} \quad (2)$$

where:

C^{flow}_{amount}, C amount in the investigated flow (g m^{-2});
C_{shoots}, C amount in the shoots (g m^{-2});
$^{14}C_{flow}$, percent of ^{14}C in the investigated flow (% of assimilated ^{14}C);
$^{14}C_{shoots}$, ^{14}C content in the shoots eight days after labeling (% of assimilated ^{14}C).

For all calculations, the amount of C in the shoots was chosen as the reference. This selection was made because the mass of the shoots and the ^{14}C incorporation in the shoots can be measured more accurately, as compared to all other compartments of the system (roots, CO_2, soil etc.). The mass of the shoots for a square meter has been calculated using the surface area of the experimental pot. Parameters C^{flow}_{amount}, $^{14}C_{flow}$, $^{14}C_{shoots}$ were taken from the experimental results and were different for each development stage investigated. Using Eq. (2) we calculated the flows of: (1) total below-ground C translocation; (2) rhizosphere derived CO_2; and (3) the amount of root exudates and root respiration.

Saggar et al. (1997); Kuzyakov et al. (1999) have used the same method for the estimation of total below-ground C translocation. This method of calculation allows only a rough estimation of the C amount passed through each flow, as the parameters of Eq. (2) are not constant during plant development. This calculation method can be used only after total ^{14}C distribution in the plant and the achievement of the stage near equilibrium. Caution is advised regarding the results of C flow amounts, because of the interpolation between the labeling periods.

In order to separate the processes of root respiration, microbial respiration of root exudates and microbial respiration of dead roots and root parts, a simple model for $^{14}CO_2$ efflux from soil after $^{14}CO_2$ pulse labeling has been used. The model and the parameters used are described in detail in earlier works (Kuzyakov et al., 1999). The model describes separately the below-ground flows of labeled C and can divide total $^{14}CO_2$ efflux from soil into the three fluxes mentioned above. We estimated root respiration and exudation by fitting modeled $^{14}CO_2$ efflux to measured $^{14}CO_2$ efflux. The fitting occurs by the change of two parameters

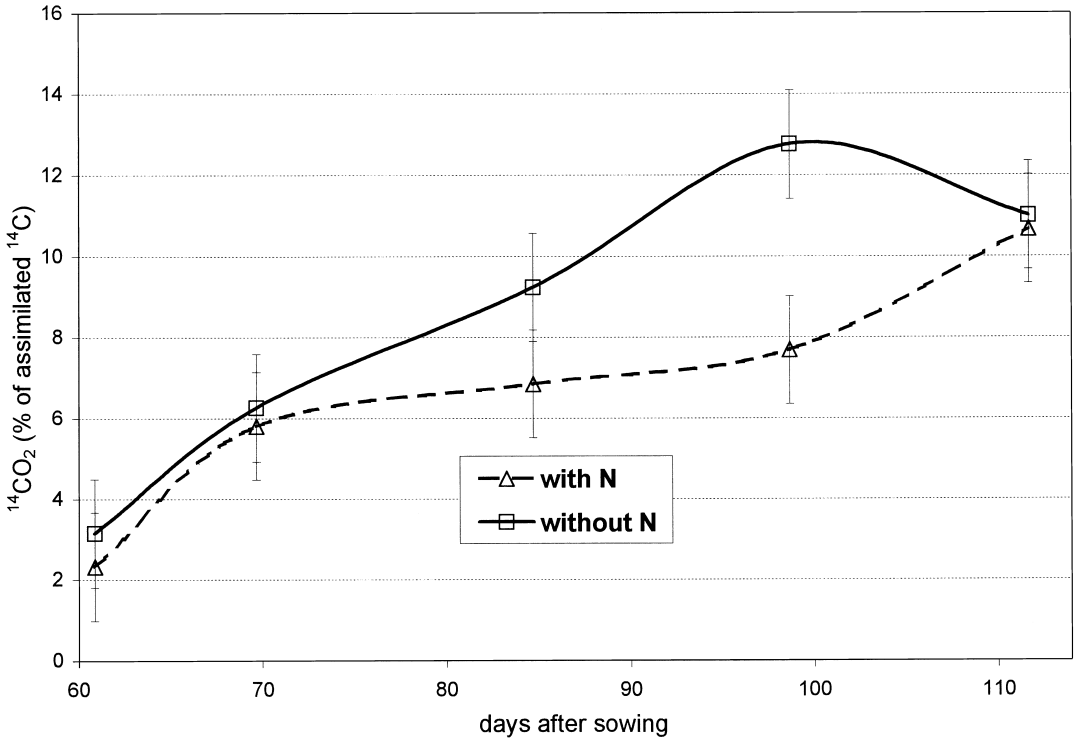

Fig. 2. Cumulative $^{14}CO_2$ efflux from the soil in the first eight days after ^{14}C pulse labeling, as a percentage of the total assimilated C. Means ± LSD ($p = 0.05$).

responsible for the rates of root respiration and exudation. We have fitted the model with the measured $^{14}CO_2$ efflux dynamics at each development stage of *Lolium perenne*, investigated separately by minimizing the least squares procedure using the Marquardt method.

The experiments were made with two replicates with respect to N fertilizing. The soil from each container was divided in four 5-cm-layers. Each of them was analyzed separately. Analysis of variance and LSD test at 5% error probability assessed the significance of differences between variants.

The ^{14}C data as percentages of net assimilated C were subjected to analysis of variance to determine the statistical significance of the effect of N fertilizing and the stage of plant growth.

3. Results

Lolium assimilated most of the $^{14}CO_2$ in the atmosphere in the upper chamber during the first 7 h, with only 0.1–1.4% of $^{14}CO_2$ input remaining. With photosynthesis rates of about 5 μmol of $CO_2\ m^{-2}\ s^{-1}$ (Anten, 1995), all of the CO_2 in the upper chamber must be assimilated during the first hour. The natural variability between plants can lead to the different amounts of ^{14}C assimilated in the different chambers. To increase the uptake of $^{14}CO_2$ in each chamber, we used longer labeling periods than those calculated according to the photosynthesis rates. Along with the separate input of $^{14}CO_2$ in each replication, this led to the decrease of the variability in the assimilated ^{14}C amount between the plants.

3.1. Shoot respiration

The total losses of assimilated C by shoot respiration over eight days were 33.9 and 37.3% by unfertilized plants and plants fertilized with 150 kg of N ha^{-1}, respectively. The differences between fertilized variants were not significant ($\alpha \leq 0.05$). The respiration losses were maximal on the first night after labeling, and diminished exponentially with time (Fig. 1). On the eighth day, the loss intensity was about 1% of assimilated C per day. Therefore, the loss intensity for the following days can be predicted to be about 0.5–1% d^{-1}.

As expected, there were significant differences between daytime and night-time respiration. The entire night-time respiration over a period of eight days after labeling was about 30.4% of assimilated C, compared with the daytime respiration of only 5.2%.

We measured the shoot respiration only once during the development of *Lolium* — during the third labeling period — because the closed upper chamber led to higher air humidity and air temperature compared with the open air. These conditions could affect the below-ground distribution of assimilates (see below).

3.2. Total $^{14}CO_2$ efflux from the soil and separating root respiration and microbial respiration of rhizodeposits

The total $^{14}CO_2$ efflux from the soil in the first eight days after ^{14}C pulse labeling increased during plant development

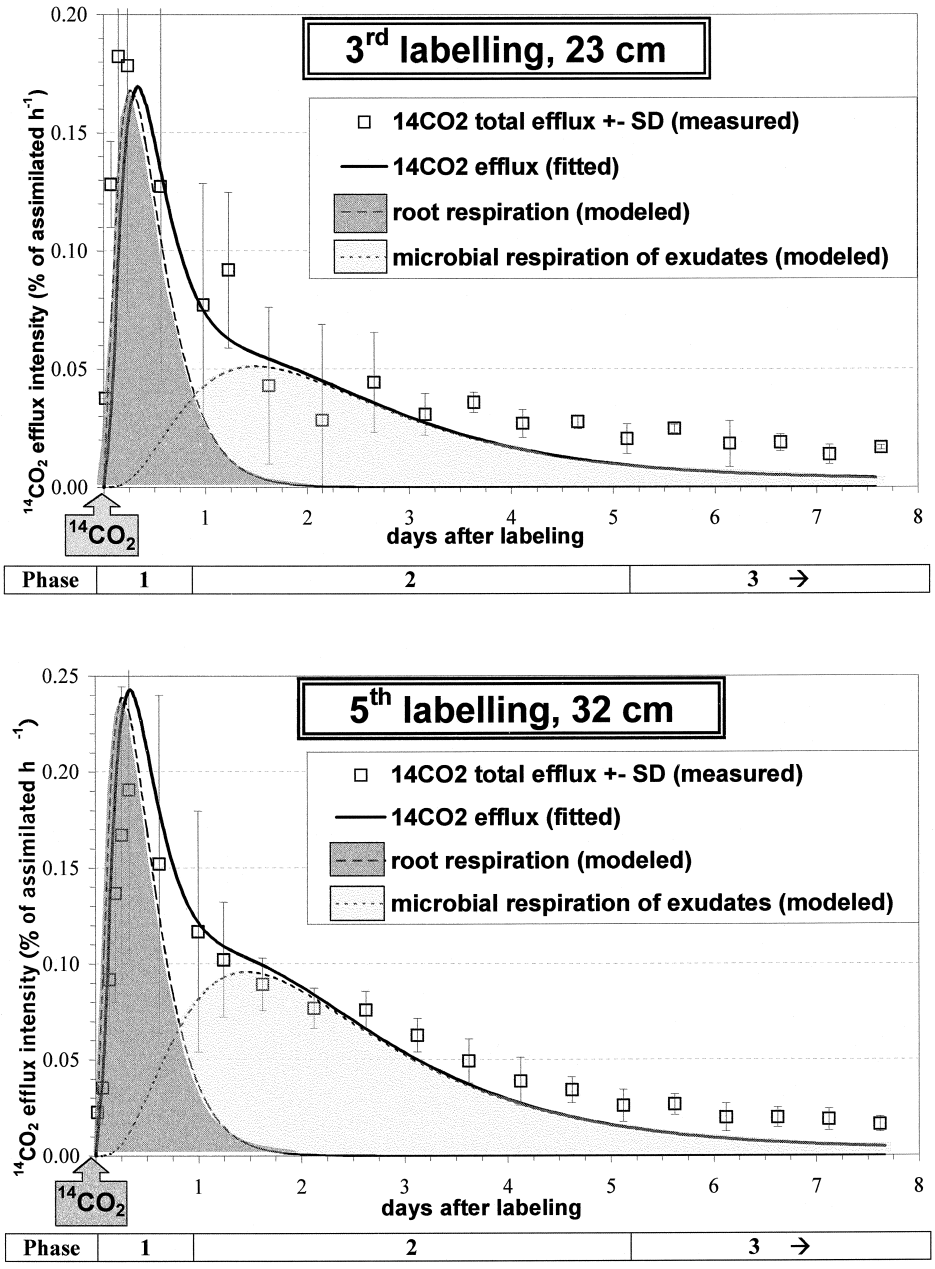

Fig. 3. Measured (means ± SD) and fitted $^{14}CO_2$ efflux from the soil and differentiation between root respiration and microbial respiration of root exudates and dead roots according to the model described earlier (Kuzyakov et al., 1999). $^{14}CO_2$ efflux at third labeling (at the top) and at fifth labeling (at the bottom) is shown (Each point of $^{14}CO_2$ efflux is placed in the middle between two neighboring points of NaOH exchange.).

from 2.7 to 11% of assimilated ^{14}C (Fig. 2). The differences between N treatments were significant ($\alpha \leq 0.05$) only at the fourth labeling. However, the untreated N plants have a tendency for more intensive rhizosphere respiration during the whole growth period (Fig. 2). The maximal $^{14}CO_2$ efflux in the soil was measured at the fourth labeling, and reached 13% of assimilated C.

The intensity of $^{14}CO_2$ efflux from the soil after pulse labeling is presented in Fig. 3. Data for only two stages of development are shown here: the third and fifth labeling stages at 23 and 32 cm in height, respectively. The maximum intensity of $^{14}CO_2$ efflux was reached eight hours after labeling. According to the intensity, three phases of $^{14}CO_2$ efflux could be distinguished: during the first day— the highest intensity, between the second and fifth day medium intensity, and after the fifth day the lowest intensity.

The total $^{14}CO_2$ efflux from the soil comprises of: (1) root respiration, (2) microbial respiration of root exudates, and (3) microbial respiration of dead roots and root parts (Whipps, 1990; Helal and Sauerbeck, 1991). The separation of CO_2 efflux from different flows used in this research is based on the assumption that these three processes are

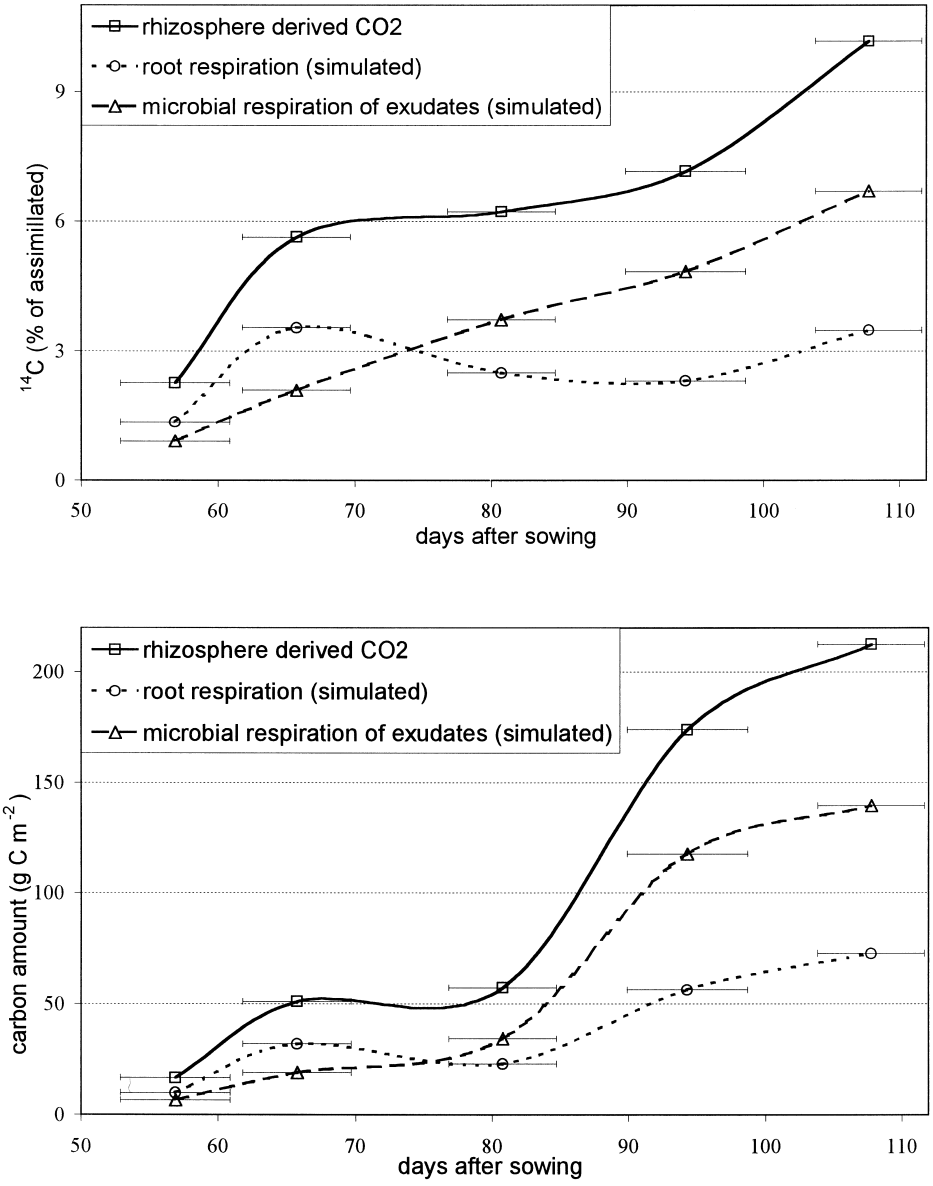

Fig. 4. Measured total rhizosphere respiration and calculated root respiration and microbial respiration of root exudates and dead roots during the development of *Lolium perenne*. In % of assimilated C (at the top) and in grams of C m^{-2} (at the bottom).

predominant in total CO_2 efflux at different times following pulse labeling. The most rapid process is the CO_2 efflux from root respiration, which predominates in the first day after labeling (Fig. 3, phase 1). The CO_2 evolution by microbial respiration of root exudates occurs later than root respiration because it consists of a chain of successive processes: exudations from the root, intake by micro-organisms and respiration of micro-organisms. The microbial respiration of root exudates predominates between the second and fifth days after labeling (Fig. 3, phase 2). The microbial respiration of dead roots is very slow (on the time scale used) and, therefore, it has only a negligible contribution to the total $^{14}CO_2$ efflux from the soil in the first days after pulse labeling. It predominates the total CO_2 efflux after the fifth day (Fig. 3, phase 3).

The use of the model of below-ground C translocation described earlier (Kuzyakov et al., 1999) allows the simulation of root respiration and rhizomicrobial respiration (Fig. 3). The fitted curve and the subdivision in (1) root respiration and (2) microbial respiration of root exudates and dead roots substantiate these assumptions. In order to calculate the total amount of C passed through root respiration and root exudation separately, an integration of each process was performed. The calculated root respiration was in the range 1.5–3.5%, and the microbial respiration of easily available rhizodeposits and dead root residues was in the range of 0.9–6.8% of assimilated ^{14}C. These relationships are close to previously reported ones (Kuzyakov et al., 1999). Root exudation increases strongly during plant development. The root respiration increases significantly

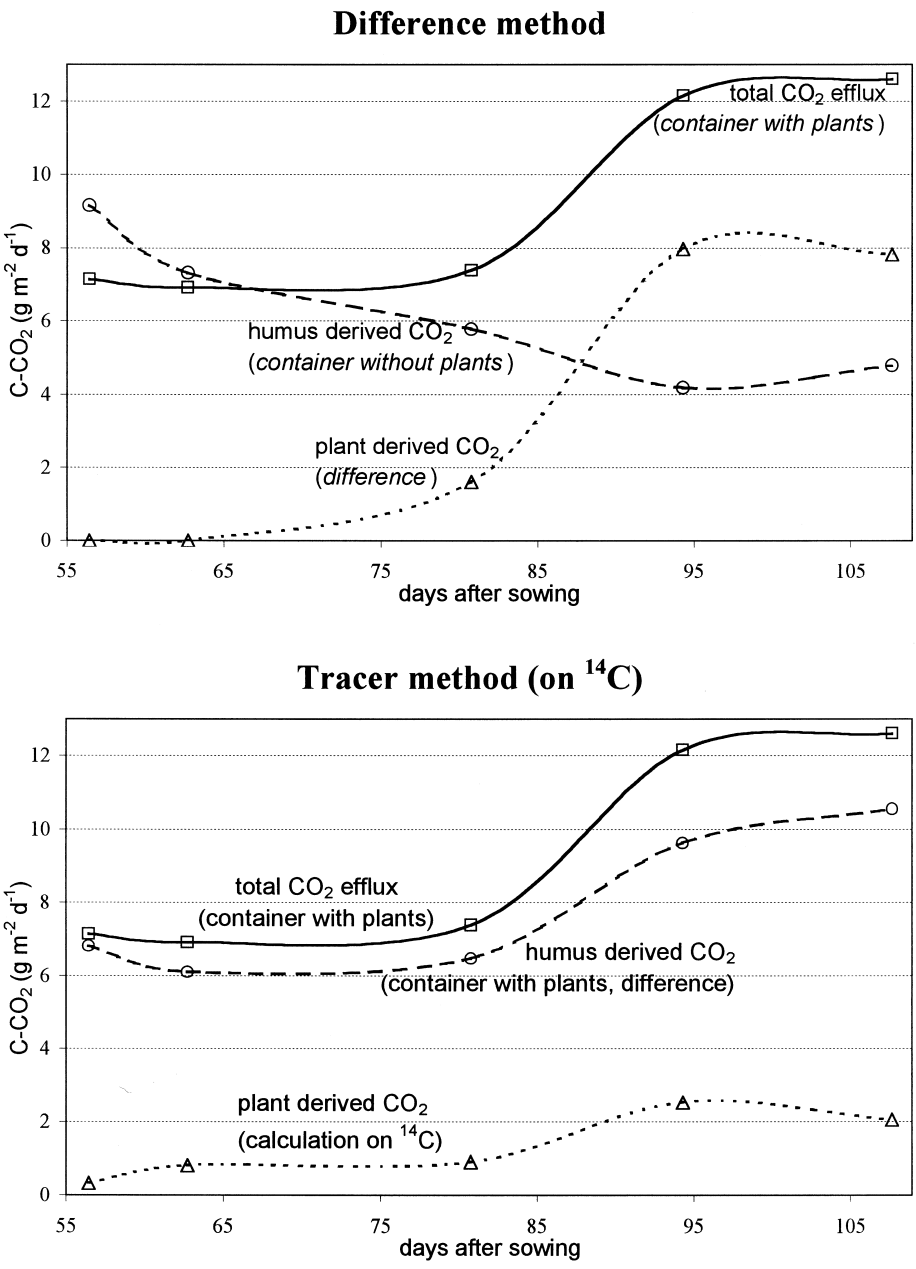

Fig. 5. Total CO_2 efflux from the soil and separation between rhizosphere-derived and soil-derived CO_2 by difference method (at the top) and ^{14}C method (at the bottom).

only between the first and the other labeling periods (Fig. 4, top). The separation between root respiration and microbial respiration used, shows that the contribution of root respiration to the total $^{14}CO_2$ efflux from the rhizosphere fluctuated between 32 and 63%, with an average of 46%.

Using Eq. (2), we calculated the amount of organic C translocated, as root exudates in the soil. It increases during the development of *Lolium* from 6.7 to 140 g of C m^{-2} (Fig. 4, bottom). Despite the fact that the percentage of assimilated C used for root respiration increases only slightly during plant development, the total amount of C used for root respiration increased by about seven times. The increase of total C evolved by root respiration, as well as exuded by roots, is in accordance with plant growth and with the increased amount of total assimilated C.

3.3. Contribution of L. perenne to the total CO_2 evolution from the soil

We used two methods to calculate the contribution of roots of *L. perenne* to the total CO_2 efflux.

1. Difference method

   In the first method, we compared the total unlabeled CO_2

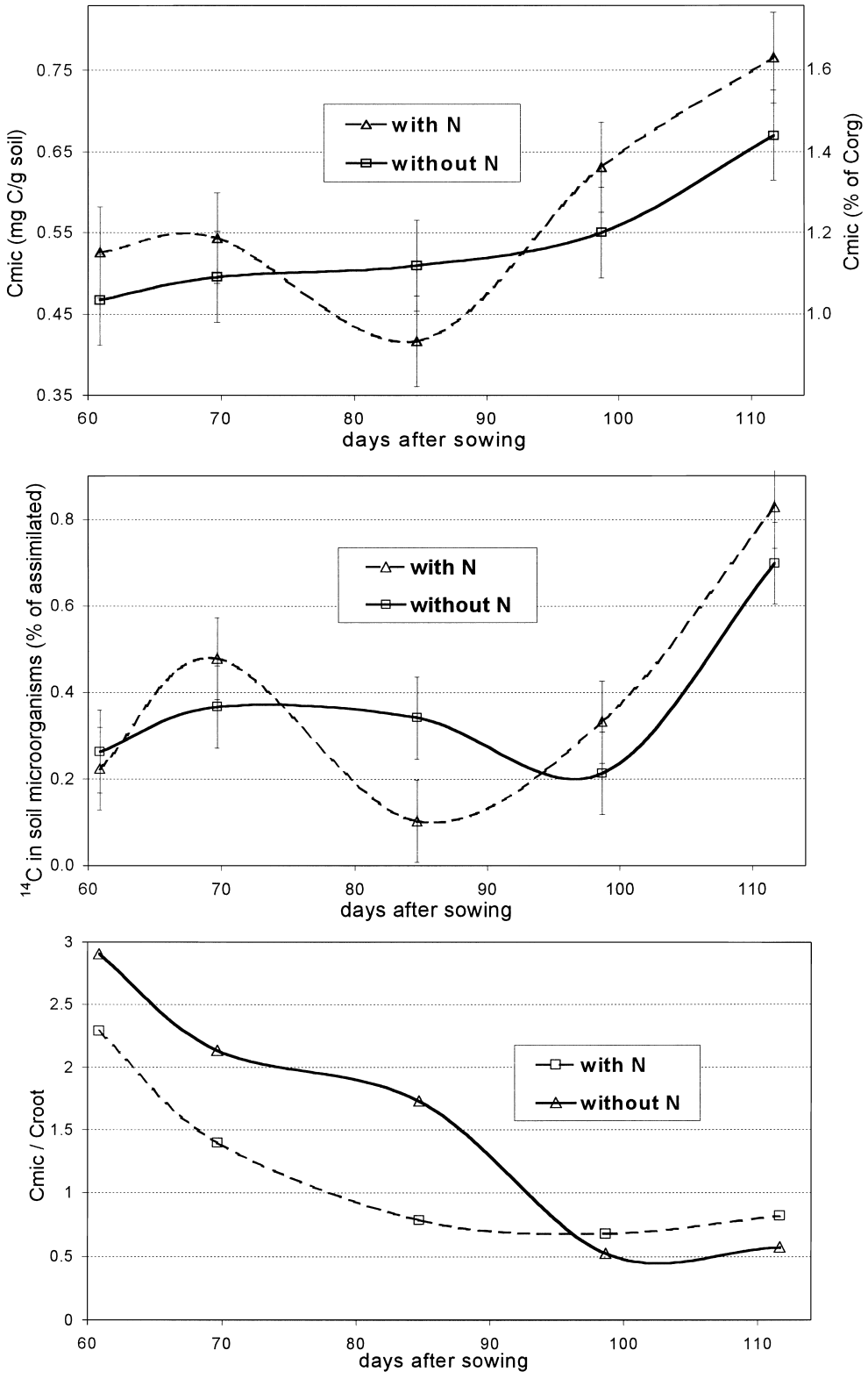

Fig. 6. Micro-organism dynamics in the soil (extraction–fumigation) (at the top), incorporation of ^{14}C in micro-organisms (in the middle), and the relation between the C amount in micro-organisms and the C amount in roots (at the bottom).

evolution from the soil with plants and from the bare soil incubated under the same conditions: that is, the same amount of soil in the pot, same soil moisture and fertilization, same air temperature and airflow. We assumed that the difference between CO_2 efflux from the soil with *L. perenne* and from the bare soil is equal to the contribution of plant roots to the total CO_2 efflux. In this difference approach, the interactions between the decomposition of soil organic matter and root growth/exudations are not taken into account.

The CO_2 efflux from the soil with *L. perenne* increased during the plant development from 7.1 to 12.6 g of C m^{-2} d^{-1} (Fig. 5, top). The CO_2 efflux from the bare soil decreased during the same time from 9.2 to 4.8 g of C m^{-2} d^{-1}. According to the difference method, we estimated that the CO_2 originating from the rhizosphere of *L. perenne* increased from 0 to 8 g of C m^{-2} d^{-1} (Fig. 5, top).

2. ^{14}C labeling method

In the second method, the amount of rhizosphere respiration of *Lolium* was calculated using Eq (2). For the calculation of rhizosphere respiration, we used $^{14}CO_2$ efflux from the soil (% assimilated) instead of the $^{14}C_{flow}$. In contrast to the difference method, the ^{14}C labeling method takes into consideration the interactions between root growth/exudations and the decomposition of soil organic matter.

According to the ^{14}C labeling method, the root-derived CO_2 increased from 0 to 2.5 g of C m^{-2} d^{-1} (Fig. 5, bottom). The difference between the total CO_2 efflux from the soil and the rhizosphere CO_2 represents the CO_2 originating from the soil organic matter. According to this method, the CO_2 efflux originating from the humus reflects the interactions between the decomposition of soil organic matter and root growth and exudations. It shows that the CO_2 originating from the organic matter of soil with *Lolium* increased during plant development from 6.8 to 10.6 g of C m^{-2} d^{-1}.

3.4. ^{14}C incorporation in soil micro-organisms

The amount of soil micro-organisms increases significantly during plant development, from 1.1 to 1.5% of soil organic matter (Fig 6, top). The C amount in micro-organisms in our soil is half of the C_{mic} content reviewed by Anderson and Domsch (1989) for most European soils (2–3% of soil organic matter). This relatively low amount of C_{mic} corresponds with a very high content of organic matter in the soil investigated (4.7% Corg). Martin and Merckx (1992) reported nearly the same content of micro-organisms using similar equipment for an investigation of C translocation in the soil and its partitioning for wheat. With the exception of the third labeling period, the variants treated with N have a tendency to contain more micro-organisms than those without additional N. Although the micro-organism content increases by 1.5 times during plant development, the maximum content of micro-organisms per root mass was at the beginning, then decreased exponentially during plant development (Fig. 6, middle).

Rhizosphere micro-organisms utilize root exudates very fast. For this reason, eight days after labeling, only 0.1–0.8% of assimilated C was located in micro-organisms in the upper soil horizon (0–5 cm). The ^{14}C incorporation in micro-organisms increased significantly only at the last labeling date (Fig. 6, bottom). It is possible that the increase of root mass in the soil, and therefore the increased total amount of root exudates and sloughed-off root cells per unit of micro-organisms, is responsible. During the first four labeling periods, ^{14}C incorporation was about 0.3% of assimilated C and nearly constant. The differences in ^{14}C incorporation in micro-organisms between N-treated and untreated variants were significant only in the third labeling period, when the upper chamber part was closed during the experiment.

3.5. Total carbon transfer of L. perenne into the soil

The total ^{14}C content in the bottom chamber compartment (soil and roots of the plant cutting, eight days after labeling) was in the range of 2.6–6.7% of assimilated C. About one-third of the below-ground translocated C was found in the roots washed with a 0.5 mm sieve. The washing of sieves smaller than 0.5 mm led to a dramatic increase of mineral particles in the washed roots. We believe that the finest roots, containing most of the ^{14}C-radioactivity eight days after pulse labeling, were lost during the washing process with a 0.5 mm sieve. The $^{14}CO_2$ evolution from the soil eight days after labeling was negligible ($<0.35\%$ d^{-1}) because all labeled root exudates were decomposed and root respiration from labeled assimilates ended. Therefore, most residual ^{14}C found below ground was located in the roots, soil micro-organisms and incorporated into soil organic matter.

The below-ground C translocation of *L. perenne* was described above, as a part of total assimilated C. This corresponds to the partitioning of the labeled C after the pulse labeling, although the amount of total assimilated C remains unknown. However, in most studies related to C balance and C turnover in soils, the mass units are important. For an estimation of the total amount of C that *L. perenne* transferred below the soil surface at different stages of development, we used Eq. 2 to calculate the amount of C flows as described above. In this equation, we used the ^{14}C content in the soil eight days after labeling instead of the amount of $^{14}C_{flow}$ (%). This allowed a rough estimation of the C amount transferred below ground. According to this method, *L. perenne* transfer of C into the soil was about 19 g m^{-2} of C when growth began (stage 1), and about 140–160 g of C m^{-2} at the fifth growth stage. It corresponded to 48 g m^{-2} organic matter and 350–400 g m^{-2} dry organic mater, respectively. After the addition of exudates respired by

rhizosphere micro-organisms over eight days following labeling (Fig. 4), the total transfer of organic C into the soil was equal to 280 g of C m^{-2} (2800 kg of C ha^{-2}) at the end of development.

The root mass obtained by means of conventional root washing procedures is equal to 1.7 g per container at the beginning of the experiments and 9.0 g per container at the end of plant development. This is equivalent to about 110 and 580 g of roots m^{-2}, respectively (44 and 230 g of C m^{-2}). The last result roughly corresponds with the root amounts monitored in a long term Allgäu pasture, from where the soil for our experiments was taken: 550–750 g of roots m^{-2}, depending on N fertilization and the season (Kretzschmar, 1999).

4. Discussion

4.1. Shoot respiration

The estimation of shoot respiration (photorespiration + dark respiration) is useful for evaluating the total budget of assimilated C. *L. perenne* lost about 36% of total assimilated C by shoot respiration over eight days following assimilation (Fig. 1). This C loss would be higher if the shoot respiration was measured over a longer time. The other results showed that between 20 and 50% of fixed C was lost by shoot respiration immediately after assimilation, in plants with a C3 pathway (Warembourg and Morral, 1978). Therefore, shoot respiration is an essential part of the C balance of plants, and its estimation gives additional information about the C partitioning between above- and below-ground plant parts. In the experiments with ^{14}C or ^{13}C labeling of plants for the estimation of C input into the soil, most authors presented the below-ground distribution of labeled C in two forms:

1. percentage of total assimilated ^{14}C (such as in this and previous research by the authors), sometimes in percentage of input; and
2. percentage of labeled ^{14}C in the plant and soil pools found after the experiment, with the assumption that recovered ^{14}C is equal to the net assimilated C.

It is very important to distinguish between these forms because the results are quite different. As shown above, shoot respiration can be estimated at more than 40% of total assimilated C. Therefore, the relative below-ground translocated C is overestimated in the publications with the presentation of data as a percentage of recovered ^{14}C or ^{13}C. Nevertheless, the presentation form of distribution of labeled C does not affect the total C input into the soil (in kg ha^{-1} or other mass units). Both, the total recovered ^{14}C and the portion of assimilated ^{14}C translocated below ground, decrease at the same rate by using the presentation in percent of total assimilated C compared to the presentation as recovery rates.

4.2. Total $^{14}CO_2$ efflux from the soil

The beginning of $^{14}CO_2$ efflux from the soil was recorded within the first hour after labeling of the shoots of *L. perenne* (Fig. 3). Cheng et al. (1993) found the beginning of $^{14}CO_2$ evolution from the soil with winter wheat and rye to be within 30 min after pulse labeling. Along with this, other results (Gregory and Atwell, 1991; Kuzyakov et al., 1999) showed that the assimilation of CO_2 and the downward transport of C in plants are very rapid processes. Biddulph (1969) estimated the rate of flow of assimilates towards the roots to be about 100 cm h^{-1}. These high transport rates prove that the downward transport of assimilates is an active process that is much faster than the diffusion by a concentration gradient (Warembourg and Morral, 1978).

The increase of $^{14}CO_2$ efflux from the soil during plant growth (Fig. 2) contradicts the results about C translocation dynamics by agricultural cereals (Keith et al., 1986; Gregory and Atwell, 1991; Swinnen, 1994), as well as with some experiments with *L. perenne* (Meharg and Killham, 1990; Kuzyakov et al., 1999). In these references, the rhizosphere respiration diminished during plant development. Contrary to previous experiments with non-vernalized *L. perenne* (Kuzyakov et al., 1999), we used prevernalized seedlings in this study. Thus, the vernalization of seedlings can be responsible for the change in the below-ground C translocation pattern during plant development.

The partitioning of CO_2 efflux from the soil in actual root respiration and respiration of micro-organisms utilizing the exudates and root residues, is very important for the C balance of the soil. Exudates and root remains are energy rich; they enlarge the underground C stock and are metabolized by soil microflora. These C sources, which are easily available for micro-organisms, contribute to the C turnover of the soil, especially to higher microbial activity in the rhizosphere when compared with root-free soil. In contrast, CO_2 originating from root respiration cannot be used at the next trophic level and does not affect the turnover of organic matter since it is energy poor. Nevertheless, CO_2 originating from root respiration contributes to the total CO_2 evolution from the soil and is frequently measured in field experiments, where soil is studied as a source of the increasing CO_2 concentration in the atmosphere. Therefore, the subtraction of root respiration from the total CO_2 efflux is a necessary prerequisite for estimating the contribution of soils to the increase of the CO_2 concentration in the atmosphere.

Many approaches for separating root respiration and microbial respiration of exudates have been previously used. However, most of them give an inaccurate value of C partitioning. Sterilization of the soil, including roots used in some earlier experiments, is an inadequate method for separating root respiration and microbial respiration of rhizodeposits because of the stimulation of root exudation and root development by soil micro-organisms. Meharg and Killham (1995) showed that some micro-organisms could

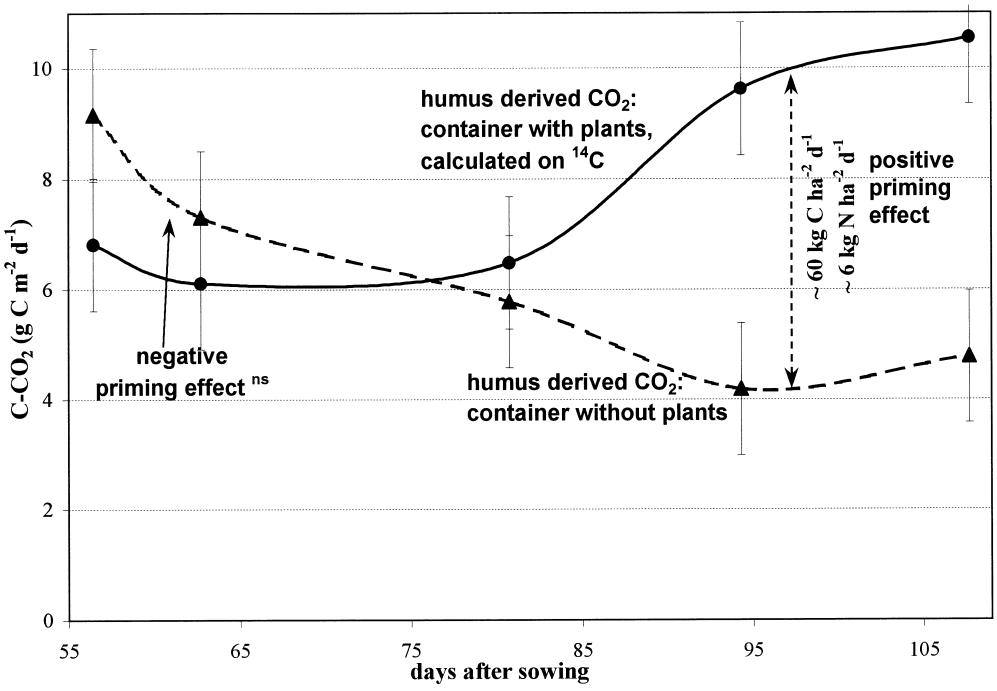

Fig. 7. Humus derived CO_2 efflux from the soil with *Lolium perenne* and from the bare soil and positive priming effect induced by plants.

increase the root exudation of *L. perenne* 34 times in comparison with sterile soil. Merbach and Ruppel (1992) found a 13-fold increase of root exudations in wheat. However, most results indicate that micro-organisms stimulate rhizodeposition by 1.5–3.0 times.

Two other different approaches, one based on an isotope dilution method (Cheng et al., 1993) and another on the addition of ^{14}C labeled artificial rhizodeposits to the soil (Swinnen, 1994), were used to separate root respiration and microbial respiration of rhizodeposits and to exclude inadequate soil sterilization. However, these experiments showed contrary results. For example, Cheng et al. (1993) found that root respiration of three-week-old wheat plants was about 41% of the total respiration of rhizosphere. Swinnen (1994), on the other hand, estimated the contribution of root respiration of 30-day-old wheat and barley plants to the total rhizosphere respiration to be 89–95%. However, both methods were not optimal. The isotope dilution method of Cheng et al. (1993) allows the calculation of the share of root respiration and rhizomicrobial respiration only during 4–5 h after labeling. However, this relationship changes over time, because rhizomicrobial respiration lasts longer than root respiration (Kuzyakov et al., 1999). In the ^{14}C labeled artificial rhizodeposits method (Swinnen, 1994), the treatment of both variants (^{14}C labeling of plants and artificial ^{14}C rhizodeposits) was not equal. The input of unlabeled artificial rhizodeposits in the variant with ^{14}C labeling of plants can improve the suggested method.

The separation of CO_2 efflux derived from root respiration and rhizomicrobial respiration used in this research is based on $^{14}CO_2$ efflux dynamics. Three phases of the root-derived $^{14}CO_2$ efflux from the soil can be distinguished (Fig. 3):

1. Root respiration dominates the total root-derived $^{14}CO_2$ efflux one day after labeling.
2. After the first day and until the fifth day, the rhizomicrobial respiration of exudates amounts to the major part of the root-derived $^{14}CO_2$ efflux.
3. After the fifth day, the decomposition of sloughed-off cells and dead roots is responsible for the root-derived $^{14}CO_2$ efflux from the soil.

This modeling approach allows the separation of stages with different $^{14}CO_2$ efflux rates without soil sterilization or other treatments, but can be used for pulse labeling only. According to the modeling approach used, root respiration contributes about 46% of the total $^{14}CO_2$ from the rhizosphere. These results are close to those found by Cheng et al. (1993) using the isotope dilution method for three-week-old wheat plants growing on an arable sandy soil.

4.3. Priming effect due to root growth of L. perenne

Both the difference and ^{14}C labeling methods were used in this research for estimating the contribution of *L. perenne* roots and of the microbial decomposition of humus substances to the total CO_2 efflux from the soil. Unlike the ^{14}C labeling method, the difference method does not consider the interactions between root exudations and decomposition of soil organic matter. Therefore, the difference between the results obtained with both methods

corresponds to the interactions between root exudations and the decomposition of soil organic matter. These interactions — the indirect changes of the decomposition intensity of soil organic matter due to root growth and exudation — can be referred to as a priming effect.

A comparison of results from the two methods used for estimating the contribution of *L. perenne* roots and of the microbial decomposition of humus substances to the total CO_2 efflux shows remarkable differences (Fig. 5). The CO_2 efflux originating from soil organic matter from containers with *L. perenne* increased during plant development, and at the end of the experiment had twice as much CO_2 efflux as soil without plants (Fig. 7). This acceleration in decomposition of soil organic matter due to root growth and exudation can be referred to as a positive priming effect (Kuzyakov et al., in press). The root exudates of plants are one of the most important causes of real positive priming effects. In this experiment, the positive priming effect led to the additional humus decomposition of about 6 g of C m^{-2} d^{-1} at the end of plant development. The C/N ratio of organic matter of the soil investigated was 10 (Table 1). If we accepted that the C/N ratio of all fractions of soil organic matter is nearly the same, then the additional humus decomposition corresponded to the N mineralization of about 6 kg of N ha^{-1} d^{-1}. This very high level of extra humus decomposition is only possible in a C-rich soil under good aeration conditions. Correspondingly, in soils with lower humus content the level of priming effects should be lower. This additional N mineralization, which is a result of rhizodeposition, sufficiently covers the N demand of growing plants.

Positive priming effects during plant growth were found in previous experiments using ^{14}C labeling, for distinguishing between humus-derived CO_2 and root-derived CO_2 (Helal and Sauerbeck, 1986, 1989; Bottner et al., 1988, 1991; Mary et al., 1993; Swinnen et al., 1995b). However, negative priming effects were found in experiments using natural ^{13}C fractionation by C3 and C4 plants for separation between CO_2 originating from humus and rhizosphere-derived CO_2 (Cheng, 1996). It is important to compare both methods in the same experiment to find the reasons for the different results obtained from the two approaches.

The priming effect increases during plant development. This is in accordance with the increased amount of micro-organisms in the soil (Fig. 6, top), which follows the total root mass (data not presented). However, the amount of micro-organisms increases less than the amount of roots (Fig. 6, bottom). The reason is that the exudation intensity of younger roots is higher when compared with older ones (Swinnen 1994; Groleaurenaud et al., 1998). Therefore, young roots exude a larger amount of the easily available C per unit of root mass (Fig. 6, bottom). This is responsible for the higher C_{mic} content related to root mass in younger roots when compared with older roots. Therefore, the turnover of C in the rhizosphere of young roots is more intensive. The doubling of the root-borne ^{14}C in micro-organisms at the end of plant development (Fig. 6, middle) can be explained by the slower C turnover in the rhizosphere with older roots compared with the younger roots. This slower C turnover leads to higher remains of ^{14}C in micro-organisms. For example, Ladd et al. (1995) pointed out that faster turnover rates would result in a lower accumulation of ^{14}C biomass.

We only investigated the ^{14}C incorporation in soil micro-organisms in the upper soil horizon (0–5 cm). If the ^{14}C incorporation in the roots of the three deeper horizons (5–20 cm at 5 cm) were of the same order, then about 0.8–3.2% of assimilated C would be located in soil micro-organisms eight days after labeling. Similar results for the incorporation of about 1.6% of ^{14}C (% of recovery rates) into the soil microbial biomass were found by Helal and Sauerbeck (1986) for the rhizosphere of maize. Van Ginkel et al. (2000) found about 2.4% of recovered ^{14}C in the microbial biomass using continuous labeling of *L. perenne*.

4.4. Total C input by L. perenne into the soil

The relative below-ground C translocation by *L. perenne* into the soil, found in this research, is smaller than in previous reports. For example, data for ^{14}C incorporation in the roots of *L. perenne* reported by Meharg and Killham (1990, 1991) (4–29%) and by Zagal (1994) (32–42%) are higher. However, these results are presented as a percentage of net assimilated C and would be 30–40% smaller if expressed in percent of total assimilated C. Additionally, the use of older plants in our experiment can also result in the relatively smaller below-ground C translocation noted by Meharg and Killham (1990, 1991) and Zagal (1994).

We found that C input by *Lolium* into the soil corresponded to about 280 g of C m^{-2} at the end of development ($\approx$ 2800 kg of C ha^{-1}). Half of this consisted of root tissue and carbon incorporated into micro-organisms and soil organic matter. The other half comprised of easily available organic substances: exudates, secretions, fine roots and sloughed-off cells that were mineralized to CO_2 over eight days. Whipps (1990) reported that the C input from wheat into the soil during the vegetation period was 1200–2900 kg of C ha^{-1}. In relation to grasses, Saggar et al. (1997) found that C translocation by different plants varied between 2450 and 4430 kg of C ha^{-1}. The calculated C input of 2800 kg of C ha^{-1} in this experiment, refers to 1.85% of the C stock in the total pasture soil profile from which the experimental soil was taken. If we assume that the humification coefficient for the root-derived C is 10%, and that the C level in the soil investigated is approximately constant, then the soil organic matter has a mean residence time of about 1850 years.

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