Carbon flows in the rhizosphere of ryegrass (*Lolium perenne*)

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Summary – Zusammenfassung

This study addresses the issue of carbon (C) fluxes through below ground pools within the rhizosphere of *Lolium perenne* using the ^{14}C pulse labeling. *Lolium perenne* was grown in plexiglas chambers on topsoil of a Haplic Luvisol under controled laboratory conditions. ^{14}C-CO_2 efflux from soil, as well as ^{14}C content in shoots, roots, soil, dissolved organic C (DOC), and microbial biomass were monitored for 11 days after the pulsing. *Lolium* allocates about 48 % of the total assimilated ^{14}C below the soil surface, and roots were the primary sink for this C. Maximum ^{14}C content in the roots was observed 12 hours after the labeling and it amounts to 42 % of the assimilated C. Only half of the ^{14}C amount was found in the roots at the end of the monitoring period. The remainder was lost through root respiration, root decomposition, and rhizodeposition. Six hours after the ^{14}C pulse labeling soil accounted for 11 %, DOC for 1.1 %, and microbial biomass for 4.9 % of assimilated C. ^{14}C in CO_2 efflux from soil was detected as early as 30 minutes after labeling. The maximum ^{14}C-CO_2 emission rate (0.34 % of assimilated ^{14}C h^{-1}) from the soil occurred between four and twelve hours after labeling. From the 5th day onwards, only insignificant changes in carbon partitioning occurred. The partitioning of assimilated C was completed after 5 days after assimilation. Based on the ^{14}C partitioning pattern, we calculated the amount of assimilated C during 47 days of growth at 256 g C m^{-2}. Of this amount 122 g C m^{-2} were allocated to below ground, shoots retained 64 g C m^{-2}, and 70 g C m^{-2} were lost from the shoots due to respiration. Roots were the main sink for below ground C and they accounted for 74 g C m^{-2}, while 28 g C m^{-2} were respired and 19 g C m^{-2} were found as residual ^{14}C in soil and microorganisms.

Key words: carbon turnover / root exudation / *Lolium perenne* / ryegrass / rhizodeposition / rhizosphere / root respiration / ^{14}C pulse labeling

Kohlenstoffflüsse in der Rhizosphäre von Weidelgras (*Lolium perenne*)

In dieser Arbeit wurden die Kohlenstoff (C)-Flüsse in der Rhizosphäre von *Lolium perenne* mit Hilfe von ^{14}C-Pulsmarkierung unter kontrollierten Laborbedingungen auf einem lehmigen Haplic Luvisol (Parabraunerde) untersucht. Über 11 Tage nach der Markierung wurde die ^{14}C-Dynamik in Blättern, Wurzeln, Boden, gelöstem organischen Kohlenstoff (DOC), CO_2-Efflux aus dem Boden und mikrobieller Bodenbiomasse verfolgt.

Lolium verlagerte 48 % des assimilierten ^{14}C unter die Bodenoberfläche, wobei C primär in die Wurzeln eingebaut wurde. 12 Stunden nach der Markierung erreichte ^{14}C in den Wurzeln ein Maximum von 42 % des assimilierten ^{14}C. Nur die Hälfte dieser Menge wurde nach 11 Tagen in den Wurzeln wiedergefunden. Der Rest wurde durch Wurzelatmung, Wurzelabbau und Rhizodeposition verbraucht. 6 Stunden nach der Markierung wurden im Boden 11 %, im DOC 1,1 % und in der mikrobiellen Biomasse 4,9 % des assimilierten C gefunden. ^{14}C in dem wurzelbürtigen CO_2 wurde schon 30 Minuten nach der Markierung festgestellt, wobei das Maximum des wurzelbürtigen CO_2-Effluxes (0,34 % des assimilierten ^{14}C h^{-1}) zwischen vier und zwölf Stunden nach der Markierung festgestellt wurde. Nach fünf Tagen wurden keine signifikanten Änderungen mehr in der Umverteilung des assimilierten Kohlenstoffs gemessen. Im Laufe von 47 Tagen assimilierte *Lolium* 256 g C m^{-2}, davon wurden 122 g C m^{-2} unter die Bodenoberfläche transportiert, 64 g C m^{-2} wurden in den Blättern wiedergefunden und 70 g C m^{-2} wurden durch die Blattatmung verbraucht. In die Wurzel wurden 74 g C m^{-2} eingebaut, 28 g C m^{-2} wurden in der Rhizosphäre veratmet und 19 g C m^{-2} verblieben im Boden sowie in der mikrobiellen Biomasse.

1 Introduction

Carbon turnover in soils has been a focus of research for many years (*Sauerbeck*, 1968; *Jenkinson*, 1971; *Dalenberg* and *Jager*, 1981). During the last decade, studies have been intensified (reviewed by *Whipps*, 1990, and *Kuzyakov* and *Domanski*, 2000; *Merbach*, 1992; *Cheng* et al., 1993; *Ladd* et al., 1994; *Parshotam* et al., 2000). This interest arises from the fact that soils contain large amounts of C (*Eswaran* et al., 1993) and are considered as a sink or source for CO_2, one of the greenhouse gases (*Dixon*, 1995; *Johnson* et al., 1995; *Mielnick* and *Dugas*, 2000). CO_2 emission from soil consists on three sources: root respiration, microbial decomposition of rhizodeposits (dead roots and all organic C released from the living roots), and utilization of native soil organic matter (SOM) by soil microorganisms. Among these three sources, however, only the last one affects total C balance between the atmosphere and terrestrial C reservoirs. This is because C derived from root respiration and microbial respiration of rhizodeposits represents the C amount lost by plants during their growth. The share of these components to total CO_2 emission from the soil varies depending on a number of factors. The most important ones are the plant species and the stage of plant development (*Meharg* and *Killham*, 1990;

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Whipps, 1990; *Swinnen* et al., 1995; *Grayston* et al., 1996; *Kuzyakov* et al., 1999), the nutritional status of the soil (*Merckx* et al., 1987; *Liljeroth* et al., 1990), the soil type (*Johnen* and *Sauerbeck*, 1977; *Merckx* et al., 1985), the SOM content, and also the temperature during the growth (*Martin*, 1977; *Dormaar* and *Sauerbeck*, 1983; *Whipps*, 1984). Additionally, rhizodeposits strongly affect the activity of soil microorganisms and also influence the decomposition of SOM. Therefore, information on the relationship between rhizodeposits, microbial activity, and decomposition of SOM plays a key role in understanding C cycling within the rhizosphere. Moreover, soils can act as a source of or a sink for CO_2 depending on the root exudation activity and the activity of rhizomicrobial population (*Helal* and *Sauerbeck*, 1986; *Grayston* et al., 1996; *Kuzyakov* et al., 1999).

Most information on the use of root-derived organic C and decomposition of SOM by microorganisms has been obtained by means of pulse labeling or continuous labeling with stable (^{13}C) or radioactive (^{14}C) isotopes (reviewed by *Whipps*, 1990; *Kuzyakov* and *Domanski*, 2000). Studies carried out on cereals (wheat, barley) have shown that they deposit in the soil much more C at the beginning of growth compared to the mature-stages. For example, wheat allocated beneath the ground between 30 % and 40 % of net assimilated C during elongation and only about 20 % at dough ripening (*Swinnen* et al., 1994) or even as small as 5 % (*Gregory* and *Atwell*, 1991). Similar results were also obtained in studies carried out on barley (*Gregory* and *Atwell*, 1991; *Swinnen*, 1994). Moreover, the distribution of beneath ground translocated C between the pools also varies during plant development. The roots of young plants retained about 50 % of transferred C while only about 10 % of C directed to the roots was incorporated into root tissues by mature plants (*Gregory* and *Atwell*, 1991; *Zagal* et al., 1993; *Swinnen*, 1994). The remaining C was lost from the roots through root respiration and rhizodeposition. Similar to cereals, *Brouwer* (1983) and *Kuzyakov* et al. (1999) have reported a decrease of below ground C transport during grass development.

Lost organic carbon serves as a nutrient and energy source for rhizosphere microorganisms, which convert it to CO_2. The rhizosphere respiration given in literature for cereals and grasses varies between 51 % (*Kuzyakov* et al., 1999) and 89 % (*Cheng*, 1996) of total CO_2 efflux from the soil. Root respiration contributes to about 40 % to 50 % of rhizosphere respiration (*Warembourg* and *Billès*, 1979; *Cheng* et al., 1993; *Kuzyakov* et al., 1999). However, *Helal* and *Sauerbeck* (1991) showed that the share of root respiration in beans and maize was much lower and amounted to 24 % and 16 % of rhizosphere respiration, respectively. On the contrary, *Swinnen* (1994) found the root respiration of 30-days-old wheat to be as high as 90 % of rhizosphere respiration.

Most results reported in the literature show a static picture of ^{14}C partitioning. The dynamics of ^{14}C translocation is seldom presented although such data is important to calculate flow rates between different C pools and to estimate the end of translocation of the fixed C. Finally, the dynamic models can be built on the basis of those parameters.

Previous work done in our laboratory (*Kuzyakov* et al., 1999) was focused on the quantification of root respiration of *Lolium perenne* during its development. The aim of this study was not only the quantification of CO_2 coming up from the soil but also investigation of the dynamics of C translocation between all below ground pools and estimation of the C input into the soil by *Lolium perenne*.

2 Materials and methods

2.1 Soil

Soil samples were taken from the Ah horizon (0–10 cm) of a Haplic Luvisol (Parabraunerde) at the experimental farm Karlshof of the University of Hohenheim in March 1999. The wet soil samples were passed through a 5-mm sieve, air-dried, and mixed. Basic sample characteristics are given in Tab. 1.

2.2 Growing conditions, chambers, and labeling procedure

Nine seedlings of *Lolium perenne*, spec. Gremie, were grown in each pot (height 10 cm, 14 cm i.d.) in 1.5 kg of soil with final density 1.06 g cm^{-3}. The following conditions were maintained during plant growth: soil moisture 60 % w/w (± 10 %) of plant available water capacity (AWC), light intensity 400 µE m^{-2} s^{-1}, day length 14 hours, day/night temperature 27/20 °C. No fertilizer was applied.

Plexiglas chambers used for the labeling procedure consisted of two compartments of which the upper one was used only during the labeling. The labeling took place in the middle of the tillering when the plants were 43 days old. ^{14}C was applied as $Na_2{}^{14}CO_2$ and 2.5 M H_2SO_4 was used for $^{14}CO_2$ volatilization.

The day before the labeling, plants were sealed at the base using silicon rubber. Plants were allowed to assimilate the label for 8 hours. Afterwards, removing of the cover was followed by the flushing of the upper compartment to eliminate the remaining unassimilated $^{14}CO_2$. We have used membrane pumps to keep the airflow through the soil at a flow rate 100 cm^3 min^{-1}. The airflow through the soil started immediately after the $^{14}CO_2$ was generated. Plants were harvested in different intervals after the labeling, i.e. 6, 12, 24, 48, 96, 192, and 262 hours after the labeling. Three pots were destructively sampled at each harvesting time.

Table 1: Basic characteristics of the Ah horizon of loamy Haplic Luvisol from the experimental farm Karlshof.
Tabelle 1: Eigenschaften des Ah-Horizontes des Bodens aus der Versuchsstation Karlshof (lehmige Parabraunerde, Haplic Luvisol)

Parameter	Value
pH ($CaCl_2$)	6.8
C_{org} (%)	1.2
N_t (%)	0.13
C/N	9.3
N-NO_3 (mg (100 g soil)$^{-1}$)	0.32
N-NH_4 (mg (100 g soil)$^{-1}$)	0.15
Sand (%)	4.40
Silt (%)	73
Clay (%)	23
FC[1] (%, w/w)	33
AWC[2] (%, w/w)	24
$CaCO_3$ (%)	0

[1]FC and [2]AWC, field capacity (pF = 1.8) and available water capacity (pF 1.8 – pF 4.2)

2.3 Sample analysis

During the experiment, the CO_2 evolving from the bottom chamber compartment was trapped with 20 ml of 0.5 M NaOH solution by continuously pumping with a membrane pump. The trap for $^{14}CO_2$ evolved from the soil was changed every two hours immediately after labeling, but after the 5th day only four times a day. Total CO_2 collected in NaOH solution was measured by titration with 0.2 M HCl against phenolphthalein after addition of 0.5 M $BaCl_2$ solution (*Black*, 1965). ^{14}C-activity in solution was measured with the scintillation cocktail Rothiscint-22x (Roth Company, Germany) on 1-ml aliquot added to 7-ml scintillator after the disappearance of chemiluminescence. The ^{14}C counting efficiency was about 89 % and the ^{14}C-activity measurement error did not exceed 2 %.

At each of the seven harvesting dates, the bottom compartment was opened, each plant was cut at the base, and the soil was pulled out. Roots were separated from the soil ("bulk soil") by handpicking. All picked roots were washed in 400 ml of deionized water to remove the soil ("rhizosphere soil") adhering to the roots. The solution obtained from root washing was centrifuged and filtered through filter paper 589^3 Blue ribbon (Schleicher & Schuell GmbH, Dassel, Germany) and used for further analysis. Plant material and soil samples were dried at 60 °C before analysis. Dry samples of shoots, roots, and soil were pulverized in a ball mill prior to analysis for radioactivity and for total C content.

Total C and N contents in shoots, roots, and soil samples were measured on a C-N-Analyzer (Carlo-Erba, Milano, Italy). After the combustion of 1 g of sample within an oxidizer unit, radioactivity of shoots, roots, and soil samples were measured with the scintillation cocktail Permafluor E+ on a liquid scintillation counter Tri-Carb 2000CA (Canberra Packard Co. Ltd. Frankfurt/M, Germany).

Microbial biomass C and N were determined by a fumigation-extraction method (*Vance* et al., 1987). Non fumigated and fumigated with chloroform soil samples were extracted with 0.5 M K_2SO_4 for 30 min (1:3 soil:extractant ratio) and filtered. The obtained solution was analyzed for C and N on a Dimatoc-100 analyzer (Dimatec Co., Essen, Germany). The results were converted to microbial C using the following relationship:

$$C_{mic} = C_{flush} * 2.34 \quad \text{(Eq. 1)}$$

where: C_{mic} – microbial carbon; C_{flush} – difference between fumigated and non fumigated sample; 2.34 – conversion factor (*Ross*, 1990). The ^{14}C in the obtained solutions was determined by scintillation counting and multiplied by the same factor. We assumed that C amounts from the K_2SO_4 extraction of non fumigated soil samples and from root washing corresponded to the fraction of dissolved organic carbon (DOC).

2.4 Statistics and calculations

All measurements were conducted with three replications for each harvesting time. All tests for differences between means were done using the t-test at the 95 % confidence level.

All ^{14}C pools are calculated as percentages of total assimilated ^{14}C to get the real distribution of newly assimilated C. $^{14}CO_2$ efflux from the soil was calculated as % of total assimilated C h^{-1}. The total assimilated C was calculated according to the equation:

$$^{14}C_{ass} = {}^{14}C_{input} - {}^{14}C_n - {}^{14}C_r, \quad \text{(Eq. 2)}$$

where: $^{14}C_{ass}$ – activity of total assimilated ^{14}C; $^{14}C_{input}$ – total activity introduced as $Na_2{}^{14}CO_2$; $^{14}C_n$ – activity of NaOH solution after flushing the upper chamber; $^{14}C_r$ – ^{14}C not volatilized after H_2SO_4 addition.

The amounts of C presented in different pools (roots, root/microbial respiration, rhizodeposition) were calculated in g C m^{-2} according to the equation:

$$C_i = \frac{C_s \cdot {}^{14}C_i}{{}^{14}C_s}, \quad \text{(Eq. 3)}$$

where: C_i – carbon stock in the pool [g C m^{-2}]; C_s – carbon content in shoots [g C m^{-2}]; $^{14}C_i$ – ^{14}C transferred to the individual pool [% of total assimilated ^{14}C]; $^{14}C_s$ – ^{14}C content in shoots [% of total assimilated ^{14}C]. The above equation was chosen after *Saggar* et al. (1997) and *Kuzyakov* et al. (1999). *Kuzyakov* and *Cheng* (2001) have shown using ^{13}C natural abundance and ^{14}C pulse labeling that this equation can be used for calculation of C pools after a single pulse labeling. The C amount in shoots was chosen as the reference pools since it was the most accurately measured pool of C in the system. The surface area of the pots was used to calculate the amount of C in each pool per one surface unit.

3 Results and discussion

3.1 Dynamics of $^{14}CO_2$ efflux from the soil compartment

Lolium perenne assimilated 99 % of generated $^{14}CO_2$ during the exposure to the label. In our experiment $^{14}CO_2$ in soil was detected already in the first hour after labeling (Fig. 1). The efflux reached a maximum value of 0.34 % (± 0.08 %) of total assimilated ^{14}C h^{-1} during the first day after the labeling (Fig. 1). Soon after, the emission rate declined and fell to below 0.02 % C h^{-1} after the 5th day. ^{14}C respired by roots and rhizosphere microorganisms during eleven days accounted for about 15 % of the total assimilated carbon. More than 80 % of that amount were released during the first five days. The observed rapid changes of $^{14}CO_2$ emission suggested an intensive metabolism of photoassimilates either by roots (respiration and exudation) or by microorganisms (respiration of exudates). *Keith* et al. (1986), *Rattray* et al. (1995), and *Nguyen* et al. (1999) have reported similar results.

The dynamics of $^{14}CO_2$ efflux from the soil is frequently used as an indicator of the completion of C partitioning in the plants (*Keith* et al., 1986; *Meharg* and *Killham*, 1990). As shown by *Swinnen* et al. (1994) $^{14}CO_2$ efflux from soil under field conditions peaked several days later than in laboratory experiments (*Warembourg* and *Billès*, 1979; *Kuzyakov* et al., 1999; *Nguyen* et al., 1999). This difference led some authors to conclude that C partitioning in plants requires about three weeks under field conditions and less

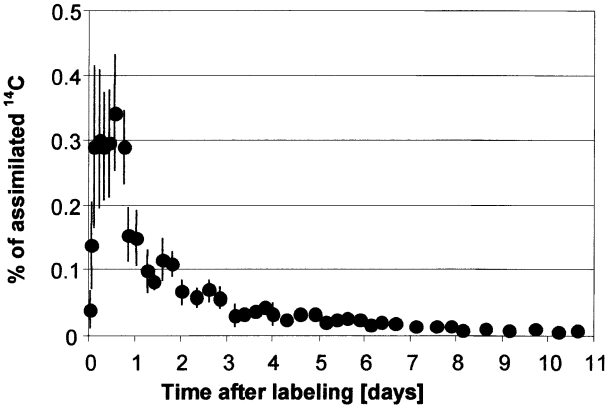

Figure 1: Dynamics of $^{14}CO_2$ efflux from the soil after pulse labeling (means ± SD).
Abbildung 1: Dynamik des $^{14}CO_2$-Effluxes aus dem Boden nach der Pulsmarkierung (Mittelwerte ± SD).

Table 2: Distribution of ^{14}C in the plant-soil system measured at different intervals after the pulse labeling in per cent of total assimilated ^{14}C. Numbers given in parentheses represent standard deviation. Data concerning the rhizosphere respiration are not shown.
Tabelle 2: ^{14}C-Verteilung im System Pflanze-Boden zu verschiedenen Zeiten nach der ^{14}C-Pulsmarkierung (in % des assimilierten ^{14}C). Zahlen in Klammern zeigen die Standardabweichung. Die Daten der Rhizosphärenatmung sind nicht dargestellt.

Time after labeling (hours)	6	12	24	48	96	192	262
Shoots	40 (3.4)	38 (2.5)	30 (1.2)	27 (5.5)	25 (3.5)	27 (7.1)	16 (0.1)
Roots	32 (2.1)	42 (11)	33 (11)	35 (7.2)	29 (4.0)	24 (4.0)	22 (4.3)
Soil	11 (3.5)	5.4 (0.8)	5.9 (1.7)	7.8 (0.4)	6.6 (2.8)	7.6 (1.3)	6.4 (0.8)
Microbial biomass	4.9 (0.51)	4.1 (0.54)	1.9 (0.37)	3.0 (0.50)	1.5 (0.41)	1.8 (0.15)	1.3 (0.31)
DOC total	1.1	0.74	0.66	0.55	0.31	0.28	0.24
● DOC from bulk soil	0.25 (0.12)	0.11 (0.05)	0.14 (0.12)	0.07 (0.02)	0.05 (0.10)	0.07 (0.04)	0.10 (0.10)
● DOC from rhizosphere soil	0.82 (0.16)	0.63 (0.03)	0.52 (0.05)	0.47 (0.09)	0.25 (0.07)	0.21 (0.05)	0.13 (0.06)
^{14}C found below the ground at each sampling*	49.71	52.73	41.18	46.45	37.63	33.96	29.96

* sum of ^{14}C found in roots, soil, microbial biomass, and DOC without root respiration

than one week in the laboratory (*Keith* et al., 1986; *Swinnen* et al., 1994). This difference may be caused by flushing of air through soil columns in the laboratory versus the passive movement of $^{14}CO_2$ by diffusion through the soil layers in the field.

3.2 Allocation of ^{14}C in shoots and roots

The ^{14}C distribution in all investigated pools changed greatly during the experiment (Tab. 2). ^{14}C content during the first hours after the ^{14}C-pulse reached maximum values of 40 % of total assimilated ^{14}C in shoots and 42 % in roots. These peaks were observed 6 hours (shoots) and 12 hours (roots) after the pulse chase. Only about 50 % of these amounts had been subsequently recovered in both shoots and roots on the 11th day (Tab. 2). Shoot respiration and below ground allocation of photosynthates and their use for respiration were responsible for diminishing of assimilated ^{14}C in the shoots (*Swinnen* et al., 1994). *Keutgen* et al. (1995) have shown that wheat exported about 54 % of recently assimilated C from the leaves. *Kuzyakov* et al. (1999) have reported for *Lolium perenne* below ground transport of assimilates in the range of 22 % and 35 % of total assimilation. In this study, 48 % of total assimilated C were transported below ground. This value is higher than those given for ryegrass in other papers (*Meharg* and *Killham*, 1990; *Kuzyakov* et al., 1999).

The losses of ^{14}C from the roots during 11 days after assimilation reached 50 % of the total ^{14}C amount transported to the roots. Immediately after its transport to the roots, the respiration of young photoassimilates was the main reason for diminution of ^{14}C. In turn, releasing of organic compounds and sloughing off the external root tissues were responsible for the decline of ^{14}C in the roots during the next days. Thus, our results confirm the studies of *Warembourg* and *Billès* (1979), *Swinnen* et al. (1994), and *Nguyen* et al. (1999).

3.3 Dynamics of ^{14}C-labeled DOC

Microbial breakdown of the polymeric rhizodeposits and exudation of organic substances with low molecular weight from the roots resulted in the recovery of ^{14}C-labeled DOC in soil. We have determined two fractions of DOC: one by extraction of bulk soil with K_2SO_4 solution and the other root washing with distilled water. If the first pool of labeled DOC consisted only of exudates, then the latter might contain some additives like root hairs, mucilage, or sloughed external root tissue. Because applied filtration through a 0.2 µm filter did not change the ^{14}C activity in the solution, we approach that the contamination of rhizosphere DOC extract with root cell walls was negligible.

In spite of this fact, it was not surprising that DOC obtained from rhizosphere soil contained 2–6 times more of the total assimilated ^{14}C than DOC from bulk soil (Tab. 2). Certain papers have reported more detailed investigations on this topic (*Darrah*, 1991a, b; *Cheng* et al., 1993). The maximum ^{14}C content in the DOC, corresponding to 1.1 % of assimilated ^{14}C, was reached six hours after the labeling and it decreased during the next days (Tab. 2). The observed decline had two distinct phases with different rates of C losses. The first phase with fast changes in ^{14}C content occurred until the 4th day and was followed by the phase with much slower changes. During the first four days roots released ^{14}C mainly as labeled exudates. They have a short half-life time in the rhizosphere due to rapid utilization by microorganisms. This results in a quick disappearance of the labeled exudates. Later, decomposition of labeled root tissue became a major source of labeled DOC found in the rhizosphere. ^{14}C-labeled DOC achieved from the root washing exhibited about an 8-fold decrease during the experimental period while ^{14}C-labeled DOC from bulk soil showed only a 2-fold decrease. The concentration of DOC in the rhizosphere soil was about 3–7 times higher compared to bulk soil (Fig. 2). *Helal* and *Sauerbeck* (1986) investigated the C release into the non-sterile soil during continuous

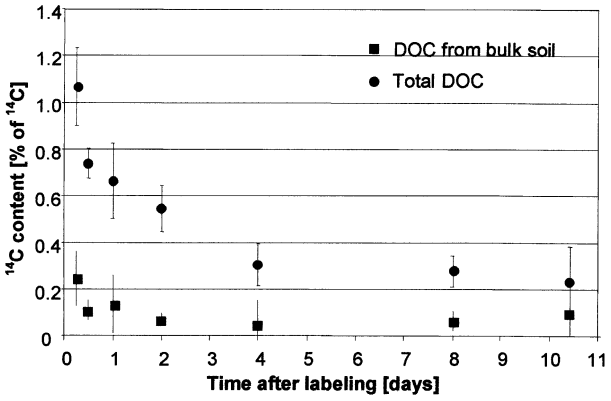

Figure 2: ^{14}C dynamics in total DOC and in DOC recovered in bulk soil. The difference shows the content of ^{14}C in DOC from the rhizosphere soil.
Abbildung 2: ^{14}C-Dynamik im gesamten DOC und im DOC aus dem wurzelfreien Boden. Die Differenz zeigt die ^{14}C-Menge in DOC des Rhizosphärenbodens.

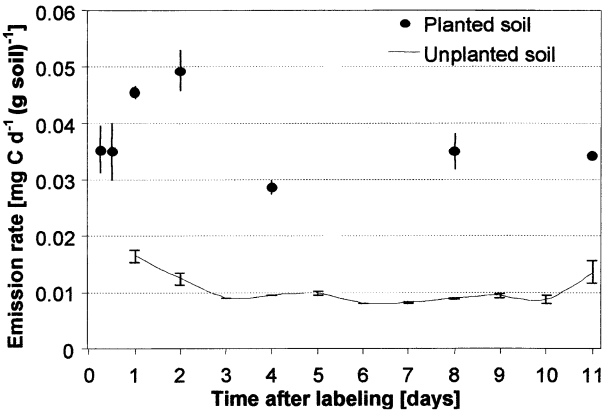

Figure 3: Respiration intensity of unplanted soil (line) and soil with *Lolium perenne* (closed circles) (means ± SD).
Abbildung 3: Intensität des CO_2-Effluxes aus dem unbepflanzten Boden (Linie) und dem Boden mit *Lolium perenne* (geschlossene Kreise) (Mittelwerte ± SD).

feeding of maize with ^{14}C. They divided soil columns into three zones with different proximity to the roots. The central zone was called "root zone" whereas the outer one was ascribed to be a root-free soil. They found that 70 % of labeled organic metabolites released in the soil were found in the central zone and less than 10 % in the outer one, which is similar to the results obtained in our experiment.

3.4 Dynamics of ^{14}C in microbial biomass

The portion of total assimilated ^{14}C recovered in the soil microorganisms significantly decreased with time (Tab. 2). After six hours, when maximum ^{14}C content was attained, microorganisms accounted for 4.9 % of total assimilated ^{14}C with a subsequent decrease to about 1.3 % on the 11th day. In the present experiment the ^{14}C content in soil microorganisms may be influenced by co-extraction of labeled organic substances from fragmented root hairs and fine roots. This possible influence, however, was difficult to assess and is largely unknown. The reduction of the ^{14}C content in MO was greatest during the first four days with about a 3-fold decline in recovered ^{14}C. These fast changes might be attributed to the uptake of rhizodeposits by microorganisms and to successive losses through respiration. Afterwards, the ^{14}C content in microbial biomass varied from 1.3 % to 1.8 %. We assume that incorporation of C from exudates into more stable polymeric constituents of microbial cells was responsible for retention of ^{14}C during the following days. However, the nearly constant ^{14}C content in microbes may also be a result of equilibrium between ^{14}C losses from roots, ^{14}C uptake by microorganisms, and the microbial respiration.

3.5 Effect of *Lolium perenne* on the soil respiration

The presence of *Lolium perenne* increased the CO_2 emission from soil. Measured CO_2 efflux from the soil, except two measurement points, did not change significantly during the experiment. It remained at the levels of 10 µg (without plants) and 35 µg C-CO_2 g^{-1} d^{-1} (with plants) (Fig. 3). Thus, the presence of plants caused a more than 3-fold increase in CO_2 coming from the soil. The reason of the increased CO_2 release from the planted soil at third and forth measurement points was not found.

If the difference between CO_2 efflux from the soil with plants and without plants can be attributed to the contribution of plant roots to the whole CO_2 evolution then roots contribute more than 70 % of the total CO_2 efflux from the soil. *Kuzyakov* et al. (1999), using the same method, have calculated that *Lolium perenne* roots contributed about 50 % to the total soil-derived CO_2. The high C content of soil they used early (4.7 % C_{org}) could change the ratio between the root and microbial contribution to CO_2 emission. As shown in this study, for soils containing less C_{org} the contribution of root respiration is higher.

3.6 Total C yield

The amount of total C recovered in the roots did not change significantly during the experiment (Tab. 3) and amounted to about 1.1–1.2 g C pot^{-1} (about 780 kg C ha^{-1}). Field data for grasslands reviewed by *Redmann* (1992) were about twice this amount. Although the recovered root weight did not change during the allocation period, we observed incorporation of assimilated C into the root tissue. Only turnover of the roots could explain this phenomenon. From about 50 % of total assimilated C allocated below the ground, half was lost due to root respiration and releasing of the organic C from the roots during the 4 days following the pulse chase (see Tab. 2).

An increase of shoot biomass was observed from the 2nd day after the labeling (Tab. 3) which resulted in an increase of the C level from about 0.60–0.70 to 1.0–1.1 g C pot^{-1} (from 430–500 to 710–790 kg C ha^{-1}). This increase in total shoot biomass accompanied the significant changes in the shoot : root (S : R) ratio (Tab. 3). These changes suggested an alternation of the source-sink relationships within the plants. After the growth of the root system necessary for nutrient and water uptake from the soil, green parts were spread to ensure development of the organs responsible for

Table 3: C yield in shoots and roots [g pot^{-1}] and changes of the shoot : root (S : R) ratio of total C during the experiment.
Tabelle 3: C-Menge in Blättern und Wurzeln [g $Gefäß^{-1}$] und die Änderung des Blatt : Wurzel-Verhältnisses während des Experimentes.

Time after labeling (days)	0.25	0.5	1	2	4	8	11
Shoots	0.66^{a}*	0.74^{ab}	0.61^{a}	0.73^{a}	0.88^{bc}	1.1^{cd}	1.0^{d}
Roots	1.1^{a}	1.4^{a}	0.76^{b}	1.1^{a}	1.1^{a}	1.1^{a}	1.2^{a}
S : R ratio	0.62^{a}	0.54^{a}	0.80^{bc}	0.64^{ab}	0.81^{bc}	0.93^{c}	0.85^{c}

* different letters indicate significant differences at $\alpha \leq 0.001$ for shoots and $\alpha \leq 0.05$ for roots and S : R ratios.

the generative reproduction. Similar changes in sinks of assimilated C were reported for wheat and barley (*Swinnen* et al., 1994).

The amount of total assimilated C, C in shoots and roots calculated on the basis of the partitioning pattern on the forth day amounted to 256, 64, and 74 g C m^{-2} during 47 days of growth, respectively. Since 48 % of total assimilation was transferred below the ground, cumulative C input of *Lolium perenne* into the soil corresponded to 122 g C m^{-2} for the whole incubation period. Therefore, the losses of C from roots are equal to 49 g C m^{-2} or to 26 % of total assimilated carbon. Summarized C fluxes, calculated according to Eq. 3., are shown in Tab. 4.

4 Conclusions

- Carbon allocated below ground by *Lolium perenne* accounted for 48 % of total assimilated carbon. This value is higher than that of agricultural cereals. The values of the ^{14}C content in the individual pools reached their maximums 6 or 12 hours after the labeling. This indicates an intensive translocation of ^{14}C-labeled new assimilates from the shoots to the roots and the fast C flows from the roots to the soil and microbial biomass.
- The distribution of ^{14}C between above ground and below ground pools was mostly completed by day five after the labeling as it was indicated by small changes in investigated ^{14}C pools. Therefore the chase period of five days is enough to evaluate the partitioning of C assimilated by plants.
- The total CO_2 emission from planted soil was 35 µg C d^{-1} (g soil)$^{-1}$ and was 3.5 times greater than that of soil without plants. Thus, the most part of the total CO_2 efflux from soil is root-derived CO_2 and it does not affect atmospheric CO_2 concentration.
- Total C input in soil by *Lolium perenne* during 47 days of growth was estimated at about 930 kg C ha^{-1}.
- The calculated net input of the rhizodeposition was equal to 19 g C m^{-2} over 47 days of plant growth. It corresponded to 8 % of total assimilated C or 9 % of net assimilated C. So, the amount of rhizodeposits of *Lolium* can be estimated roughly as percentage of C assimilated during the growth period.

Table 4: C amounts in different pools of the plant-soil system.
Tabelle 4: C-Mengen in verschiedenen Teilen des Systems Pflanze-Boden.

C pool	g C m^{-2}	% of total assimilation	% of net assimilation	% of below ground translocation
total assimilated	256	100	–	–
shoots respiration*	70	27	–	–
shoots	64	25	34	–
transferred below the ground	122	48	66	100
roots	74	29	40	61
losses from roots§	49	19	26	39
– root/microbial respiration	28	11	15	23
– rhizodeposition	19	8	11	16

* calculated as a difference between total assimilation and total C found on the forth day.
§ include root respiration, rhizodeposition, and microbial respiration of rhizodeposits. The root/microbial respiration was calculated by modeling of experimental data (not shown here). Rhizodeposition makes up only for C found in soil and in microorganisms on the forth day.

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