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ORIGINAL PAPER

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Carbon partitioning in plant and soil, carbon dioxide fluxes and enzyme activities as affected by cutting ryegrass

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Abstract The effect of a single cut (simulated grazing) and regrowth of *Lolium perenne* on CO_2 efflux from soil (loamy Haplic Luvisol), on below-ground C translocation and on the distribution of plant C among different soil particle size fractions was investigated under controlled conditions with and without N fertilization by pulse labelling with ^{14}C 7 times (four before and three after the cutting). The amount of ^{14}C respired from the rhizosphere of *Lolium* decreased by a factor of about 3 during 1 month of growth. At the same time the amount of ^{14}C stored in soil increased. Cut and non-fertilized plants respired less C in the rhizosphere compared to the uncut plants and cut fertilized plants. About 80% of the root-derived CO_2 efflux originated from the C assimilated after defoliation, and 20% originated from the C assimilated before cutting. N fertilization decreased the below-ground C losses (root respiration and exudation) during regrowth. The shoot is the main sink of assimilated C before and after the defoliation. N fertilization led to higher C incorporation into the shoot parts growing after defoliation compared to unfertilized plants. A lower incorporation of ^{14}C was observed in the roots of N fertilized plants. The relative growth rates (expressed as ^{14}C specific activity) of roots and stubble were minimal and that of shoot parts growing after defoliation was maximal. Twelve percent of ^{14}C was found in the newly grown leaves after regrowth; nevertheless, 4.7% and 2.4% of ^{14}C in the new shoot parts were translocated from the root and shoot reserves of unfertilized and fertilized plants, respectively. Most of the C retranslocated into the new *Lolium* leaves originates from the stubble and not from the roots. Between 0.5% and 1.7% of ^{14}C recovered in shoots and below-ground C pools was found in the soil microbial biomass. Cutting and fertilization did not change ^{14}C incorporation into the microbial biomass and did not affect xylanase, invertase, and protease activities. Tracing the assimilated C in particle size fractions revealed maximal incorporation for the sand and clay fraction.

Keywords Carbon allocation · Cutting and regrowth · Defoliation · *Lolium perenne* · Rhizodeposition

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Introduction

Quantifying the C input of grasses into soils is essential in the study of soil organic matter dynamics and CO_2 efflux from pastures. Although the relative C translocation into soil is higher for pasture plants than agricultural cereals, the absolute C input is similar (1,500 kg C ha^{-1} $year^{-1}$) when the same growth period is considered (Jensen 1993; Swinnen et al. 1995; Kuzyakov and Domanski 2000; Kuzyakov 2001). This is because agricultural cereals have a higher yield than grassland.

The partitioning pattern of below-ground translocated C is similar in cereals and grasses (Kuzyakov and Domanski 2000). One-half of the translocated C is found in the roots and about one-third is evolved as CO_2-C from the soil by root respiration and microbial utilization of root-borne organic substances. The remaining below-ground translocated C is incorporated into the soil microorganisms and soil organic matter.

Not only the total C input into the soil but also the distribution of assimilated C in time and space can be interesting. The portion of assimilated C allocated below-ground by cereals decreases during plant growth (Keith et al. 1986; Swinnen 1994; Gregory and Atwell 1991), whereas in pasture plants, both decreases and increases have been reported (Warembourg et al. 1990; Zagal 1994; Kuzyakov et al. 1999; 2001). The changes in the translocation pattern of *Lolium* are related to the vernalization of seedlings (Kuzyakov et al. 2001) or to annual and perennial variations of species (Warembourg et al. 1990).

Another important difference between grasses and crops is the periodic mowing of grasses. Defoliation

of grasses leads to a more complex redistribution of assimilated C. Shortly after defoliation, the total assimilation activity drops considerably due to the very small leaf area. Shortly after clipping, the number of tillers per plant is reduced (Wilsey 1996; Mackie-Dawson 1999). Defoliation increases sink strength in the newly grown leaves and reduces the below-ground C allocation (Detling et al. 1979; Holland and Detling 1990; Wilsey 1996; Mackie-Dawson 1999). In addition, perennial grasses store some of the C assimilated before defoliation in roots and stubble, and then utilize these C reserves for regrowth after cutting (Davidson and Milthorpe 1966; Gifford and Marshall 1973; Bokhary 1977; Boot 1989; Johansson 1993). The regrowth rate largely depends on the mobilization of these organic reserves. An important part of the C will be relocated into the growing shoots, and another part remains in roots and will be intensively used for root growth, respiration, and exudation. As shown by Ta et al. (1990) for alfalfa, the reutilization of C reserves after defoliation is especially important for N_2 fixation by nodulated roots.

Despite such differences in the C allocation between grasses and cereal crops, and the mowing commonly conducted in grasslands, only few studies have dealt with the C redistribution in grasses and in soil after defoliation (Bushby et al. 1992; Johansson 1993). We were unable to find any studies concerning the incorporation of root-derived C into the soil microbial biomass in response to defoliation and its effect of enzyme activities in the rhizosphere of grasses.

The aims of this study were: (1) to determine the pattern of root-derived CO_2 efflux and below-ground C translocation of a typical pasture plant, *Lolium perenne* L., before and after defoliation, (2) to estimate the amount of C retranslocated from roots and stubble into the newly growing shoots during regrowth, (3) to trace the assimilated C in soil, and (4) to compare the enzyme activities in soil after regrowth under two different N regimes. Tracing the assimilated C should help to understand below-ground allocation into the following pools: roots, root and microbial respiration, microbial biomass, different particle size fractions of soil. The microbial use of plant-derived substrates was tested by estimating enzyme activities.

Materials and methods

Soil

The soil, a silt loamy Haplic Luvisol, was taken in May 1999 from the top 10 cm (Ap horizon) of the Karlshof long-term field experimental station of the University of Hohenheim (5 km south of Stuttgart). It was sieved (2 mm), air dried and mixed. The electrostatic method was used to remove small roots and plant remains (Kuzyakov et al. 2001). The soil contains no $CaCO_3$ and has the following characteristics: pH 6.0, total C 1.2%, total N 0.13%, clay 20%, silt 8%. The bottom part of each experimental pot was filled with 2.4 kg air-dried soil.

Chamber and labelling

The labelling apparatus previously described in detail by Kuzyakov et al. (1999) consists of a two-compartment plexiglas chamber. The lower compartment (diameter 138 mm and height 200 mm) is for the soil and plant roots and the upper compartment (diameter 138 mm and height 300 mm) is for the shoots and $^{14}CO_2$ evolution. Both compartments are separated by a plexiglas lid with drilled holes (diameter 8 mm) for plants. In contrast to our previous studies and to most other research, the holes with plants were not sealed before and during the labelling. This allows undisturbed plant growth throughout the experiment because silicon or paraffin, usually used for sealing, impedes growth if the growth period is longer than 1 week after sealing.

The labelling took place 7 times (at 25, 30, 35, 40, 45, 50, and 55 days after sowing) during growth of *Lolium*; 4 times before cutting and 3 times after cutting. Each container was labelled separately: 48 kBq ^{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. One millilitre of 3.5 M lactic acid (Nguyen et al. 1999) was added to the $Na_2{}^{14}CO_3$ solution in the micro test-tube through Teflon tubing. This allowed the complete evolution of $^{14}CO_2$ into the chamber atmosphere. Assimilation took place within 1 h after the pulsing of $^{14}CO_2$. The trapping of CO_2 from the upper compartment was started after a 1-h labelling period in order to remove the remaining unassimilated $^{14}CO_2$. This was carried out by pumping the air of the upper chamber through 20 ml of 0.5 M NaOH. Then the top of the chamber was removed and the trapping of CO_2 from the bottom chamber began. The trapping of CO_2 from the soil was carried out by pumping the air of the bottom chamber through two washing flasks with 20 ml of 0.5 M NaOH solution. The second washing flask was connected to the membrane pump, whose outlet was open to the atmosphere. The amount of CO_2 and the ^{14}C activity in both washing flasks were measured separately. Between 96% and 98% of the CO_2 evolved from soil was trapped by two flasks connected in series. The airflow was set at 125 ml min^{-1}.

Growth conditions

Non-vernalized seeds of *Lolium perenne* L. cv. Gremie were sown on filter paper in Petri dishes and germinated at 22°C. On the sixth day, when the seedlings were 3 cm tall, nine germinated seeds were transferred into each container with soil. The plants were grown at 3.5-cm intervals at 27±1°C day and 22±1°C night temperatures with a day length of 14 h, and a light intensity of approximately 400 µmol m^{-2} s^{-1} PAR at plant height. The soil water content of each chamber was adjusted daily to maintain a moisture value of 60% of the available field capacity.

Three treatments were carried out. In the first treatment (uncut labelled) the plants were labelled 7 times and were not cut, whereas in the second (cut labelled) the plants were labelled 4 times before cutting and 3 times after it. In the third treatment (cut unlabelled) the plants were labelled 4 times before cutting and were not labelled thereafter. The plants were cut after 42 days, at exactly 4 cm above the soil surface (Mackie-Dawson 1999; Paterson and Sim 1999). Plants were unfertilized or fertilized with 150 kg N ha^{-1} given as KNO_3 and applied in three equal amounts after 15, 28 and 43 days.

To compare total unlabelled CO_2 evolution with and without *Lolium perenne*, the soil without plants was also incubated in the same type of pot, under the same conditions, and with the same two levels of N fertilization.

Enzyme activities

Xylanase, invertase and protease activities in soil were determined after the experiment as reported by Kandeler et al. (1999). For the determination of xylanase activity, 0.3-g moist soil samples were incubated with 5.0 ml of 1.7% (w/v) xylan solution from oat spelt

suspended in 2 M acetate at pH 5.5 and 5.0 ml of 2 M acetate buffer for 24 h at 50°C. Reducing sugars released during the incubation period were then measured colorimetrically (Schinner and von Mersi 1990; Schinner et al. 1996). To measure invertase activity, 0.3-g moist fractions were incubated with 5.0 ml of 50 mM sucrose solution and 5.0 ml of 2 M acetate buffer (pH 5.5) for 3 h at 50°C. Reducing sugars were determined as described for xylanase activity. Results of invertase activity and xylanase activity were expressed as mg glucose equivalents (GE) g^{-1} 3 h^{-1} and mg GE g^{-1} 24 h^{-1}, respectively. Protease activity was determined as reported by Ladd and Butler (1972) with the following modifications: 0.2 g soil was incubated for 2 h in 5 ml buffered casein solution (pH 8.1) and 5 ml TRIS buffer (0.05 M, pH 8.1) at 50°C. The aromatic amino acids released were extracted with 0.92 M trichloroacetic acid and measured colorimetrically by the Folin-Ciocalteu reagent. The results were expressed as µg tyrosine equivalents (Tyr) g^{-1} 2 h^{-1}.

Physical fractionation of the soils

The incorporation of plant-assimilated ^{14}C in different particle size fractions was investigated as reported by Kandeler et al. (2000) and Stemmer et al. (1998). Field-moist soil (35 g) was dispersed in 100 ml cooled distilled water by a probe-type ultrasonic disaggregator (50 Js^{-1} for 120 s). Coarse and medium sand (250–2,000 µm) and fine sand (63–250 µm) were separated by manual wet sieving with about 400 ml cooled distilled water. Silt-sized particles (2–63 µm) were separated from the clay fraction (<2 µm) by centrifugation at approximately 150 *g* for 2.0 min at 15°C. The pellets were resuspended in water and centrifuged 3 times under the above-mentioned conditions to purify the silt fraction. The combined supernatants were centrifuged at 3,900 *g* for 30 min at 15°C to yield clay-sized particles (0.1–2 µm, according to an equispherical diameter and a particle density of 2.65 g cm^{-3}). Compared with the particle size method using sodium pyrophosphate as a dispersion agent (Kandeler et al. 1996), the particle size distribution obtained after the fractionation procedure yielded similar amounts of the sand fractions, but a higher amount of the 2- to 63-µm particles. The applied sonication energy thus did not disrupt microaggregates completely. Therefore, the fractionation procedure using the application of low energy sonication (0.2 kJ g^{-1} soil) prevented the release of stable organic matter that is physically protected within microaggregates in natural sites (Stemmer et al. 1999).

Chemical analysis and calculations

During the experiment, the CO_2 evolved from the soil was trapped in 20 ml of 0.5 M NaOH solution by continuous pumping (125 cm^3 min^{-1}) with a membrane pump. The trap for $^{14}CO_2$ was changed twice daily.

Five days after the last labelling (day 60 after germination) the lower chamber was destructively sampled. The total above-ground biomass was similar for uncut and cut plants, therefore the comparison of the these is acceptable. Shoots were divided into two parts: (1) the shoot part higher than 4 cm above the soil, termed "new leaves", and (2) shoot parts less than 4 cm above the soil, termed "stubble". The shoot parts cut 4 cm above the soil on day 42 are termed "cut leaves". After the end of the experiment each root-soil column was pulled out. The roots were carefully washed twice in 200 ml deionized water with the addition of Micropur containing Ag^+ to suppress the microbial decomposition of organic substances before analysis (Gransee and Wittenmayer 2000). Because the whole soil column was penetrated with *Lolium* roots the whole soil is considered as rhizosphere.

Shoots, roots and soil were dried at 60°C and then mixed and pulverized in a ball mill (Retsch) prior to radioactivity analysis and determination of the total C and N content.

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

The labelled ^{14}C-CO_2 collected in NaOH solution and ^{14}C in washing water were measured with the scintillation cocktail Eco-Plus (Roth) 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 adding NaOH solution as a quencher to the scintillation cocktail and using a two-channel ratio method of extended standard (tSIE).

The amount of CO_2 trapped by the NaOH solution was measured by titration with 0.25 M HCl in the presence of phenolphthalein after addition of 0.5 M $BaCl_2$ solution (Black 1965). Total C and N contents in shoot and root were measured using a C-N analyser (Carlo-Erba).

Microbial biomass C was determined by a fumigation-extraction method (Vance et al. 1987; Ross 1990) with the determination of the C content of 0.5 M K_2SO_4 extracts by Dimatoc-100. The ^{14}C activity in the aliquot was determined by scintillation counting. The C and ^{14}C counts obtained from the fumigated soils were taken to represent the microbial-C and ^{14}C flush and converted to microbial biomass C using the relationship (Ross 1990): microbial C=C flush×2.34. The ^{14}C activity in the K_2SO_4 extract from soil without fumigation and the ^{14}C activity found in water remaining after root washing were accepted as ^{14}C in dissolved organic C (DOC). To consider the natural ^{40}K radioactivity in K_2SO_4 extracts, the 0.5 M K_2SO_4 extract from unlabelled soil was used as a background standard by scintillation counting.

The $^{14}CO_2$-C data for each replicate were expressed as percentages of assimilated ^{14}C. The net assimilated ^{14}C (^{14}C assimilated) was calculated from:

$$^{14}C\text{assimilated} = {}^{14}C\text{input} - {}^{14}C\text{na} - {}^{14}C\text{rem} \quad (1)$$

where: ^{14}C input is ^{14}C input activity for each container (48 kBq); ^{14}Cna is ^{14}C not assimilated after 1 h; ^{14}Crem is the ^{14}C remaining in a 2-ml Eppendorf micro test-tube.

The ^{14}C data for shoots, roots and soil were calculated on the basis of total ^{14}C recovered from plants (shoots and roots), soil respiration and soil for each container.

The experiment was carried out with three replicates with respect to cutting, ^{14}C labelling and N fertilization. However, all plants under the same fertilization regime were treated identically before cutting and were therefore accepted as replicates. Thus there were nine replicates for each N treatment before cutting. The ^{14}C data as percentages were subjected to Tukey's multiple comparison test (honestly significant difference, HSD) at P=0.05 to determine the significance of the effect of cutting and N fertilizer on CO_2 efflux and above-ground and below-ground C allocation.

Results and discussion

Effect of growth and defoliation on $^{14}CO_2$ efflux from the soil

After 25 days, the plants were labelled in the $^{14}CO_2$ atmosphere by seven ^{14}C pulses. As shown by Warembourg and Estelrich (2000), a series of pulse labelling yields more information about the allocation of assimilate than continuous labelling. Shortly after each labelling, $^{14}CO_2$ appeared in the bottom compartment as shown by the $^{14}CO_2$ efflux peaks following each 5-day label (Fig. 1). The $^{14}CO_2$ efflux after each pulse labelling was similar to that already reported (Warembourg and Esterlich 2000; Nguyen et al. 1999; Kuzyakov et al. 1999, 2001) and its intensity presented as a percentage of ^{14}C assimilated by each labelling allows an easy comparison of peaks after

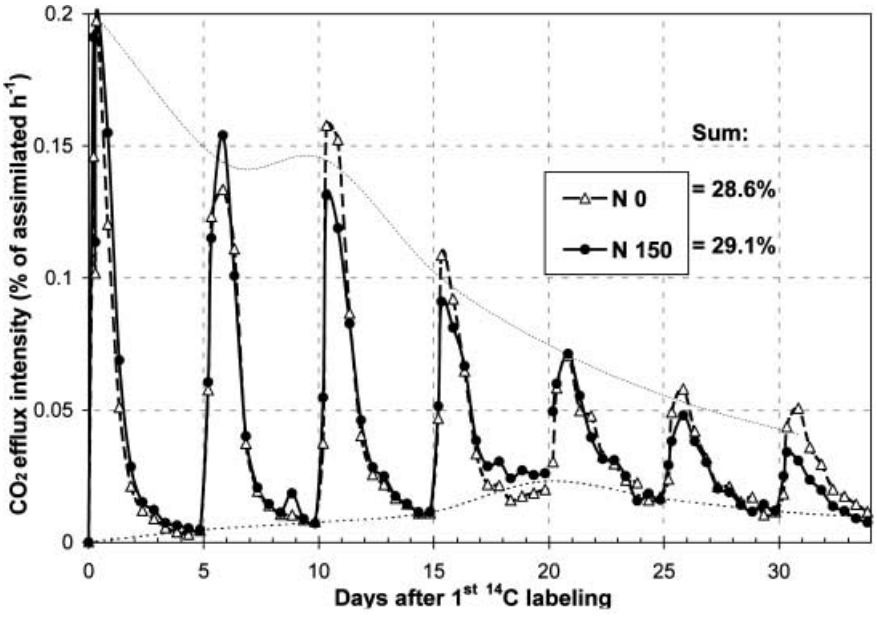

Fig. 1 Root-derived CO_2 efflux intensity ($^{14}CO_2$) from soil planted with *Lolium perenne* at two levels of N fertilization and labelled 7 times during growth. The plants of these treatments were not cut. The *upper dotted line* represents the decrease in the maximal $^{14}CO_2$ efflux intensity and the *bottom dotted line* represents the residue of assimilated C remaining in the soil. *N 0* Unfertilized, *N 150* 150 kg N ha^{-1} given as KNO_3

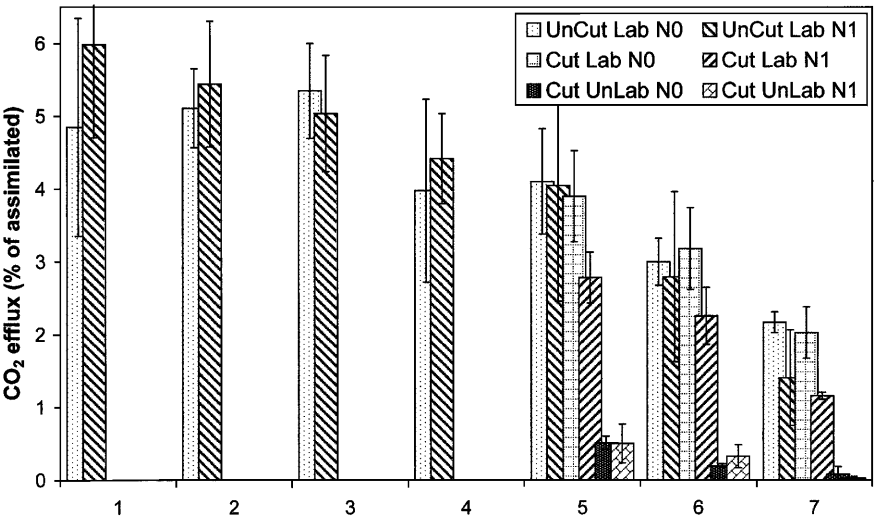

Fig. 2 Cumulative root-derived CO_2 efflux ($^{14}CO_2$) (±SD) from soil planted with *L. perenne* for chase periods after each labelling. Two levels of N fertilization [unfertilized (*N0*) and 150 kg N ha^{-1} (*N1*)] and three treatments in respect to cutting (*Cut*) and labelling (*Lab*) after cutting (UnCut Lab, Cut Lab, and Cut UnLab) are shown

each labelling. A strong decrease is observed in the portion of assimilated C released as rhizosphere respiration (Fig. 1, the line connecting the maxima of the $^{14}CO_2$ efflux intensity).

After the first labelling, the total $^{14}CO_2$ efflux amounted to 5% and 6% of assimilated C and after the seventh labelling only to 2% and 1.5% of assimilated C for unfertilized and for N fertilized plants, respectively (Fig. 2). Therefore, the ^{14}C used for root respiration and exudation decreased by a factor of about 3 over 35 days. A decrease in the C portion used for rhizosphere respiration during development in grasses has been reported in most other studies (Warembourg et al. 1990; Meharg and Killham 1990; Kuzyakov et al. 1999). Increases (Zagal 1994; Kuzyakov et al. 2001) or nearly constant values (Johansson 1993) have also been observed during plant growth. All studies, however, show an increase in the absolute amount of CO_2 respired in the rhizosphere during plant growth.

Domanski et al. (2001) demonstrated that 5 days after assimilation the C allocated below-ground is converted into root tissue, microbial biomass or is incorporated in soil organic matter. In the present study, the amount of C with slower turnover in the soil increased for the first 20 days and then decreased slightly, as shown by the line connecting the minima of the $^{14}CO_2$ efflux intensity on Fig. 1. Thus, 45 days after *Lolium* growth, the mineralization of easily decomposable substances exceeded the organic C input by roots in soil.

N fertilization had no significant effect on the root-derived $^{14}CO_2$ efflux from uncut plants (Fig. 2), probably because sufficient inorganic N is present in this soil due to the high mineralization potential and good aeration of the investigated Luvisol. A decrease in below-ground respiratory losses has been previously reported in fertile soil (Warembourg et al. 1990) or N fertilized soil (Swinnen et al. 1995). On the other hand, Bushby et al.

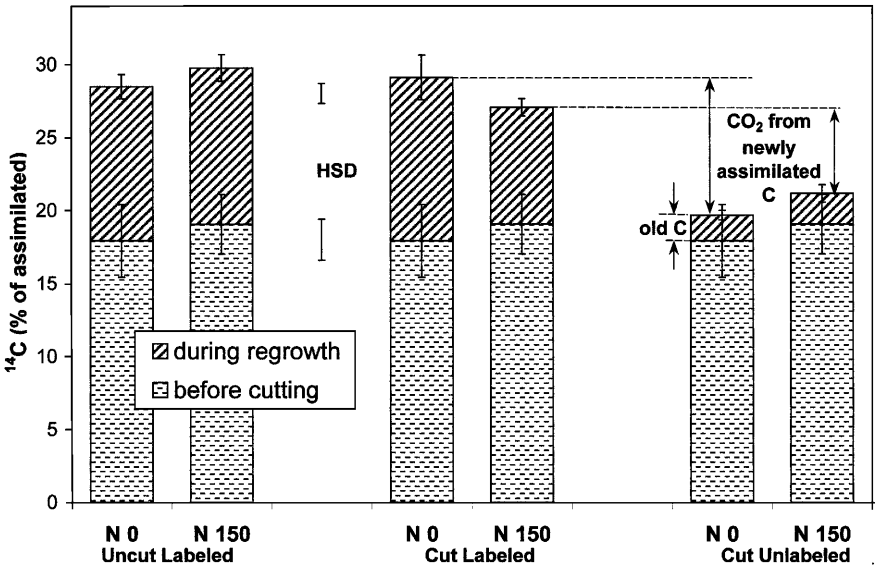

Fig. 3 Total root-derived CO_2 efflux ($^{14}CO_2$) (±SD) from soil respired before and after cutting at two levels of N fertilization of *L. perenne*. Tukey's multiple comparison test [honestly significant difference (HSD), P=0.05] between the treatments was calculated for CO_2 efflux before and after cutting

(1992) reported an increased efflux of rhizosphere-derived ^{14}C-CO_2 after mineral N addition to *Panicum* grass; the same behaviour was shown by Liljeroth et al. (1990), who conducted a continuous ^{14}C labelling experiment with two wheat varieties.

Cutting the plants on day 42 changed the $^{14}CO_2$ efflux pattern (Fig. 3). The maximal amount of root-derived CO_2 was evolved from soil with uncut plants and cut unfertilized plants. The rhizosphere respiration of cut non-fertilized plants during regrowth was about 3.2% higher (α=0.005) than that of fertilized plants (Fig. 3). Reduced rhizosphere respiration after cutting has been reported for green panic grass in the absence of N fertilization (Bushby et al. 1992). Paterson and Sim (1999), on the other hand, found higher root exudation after repeated defoliation of *L. perenne* grown in nutrient solution. Higher exudation of fertilized plants grown on non-sterilized soil instead of nutrient solution would lead to microbial decomposition of exudates and thereby increase CO_2 efflux. Despite the significant effect of defoliation on the CO_2 efflux from soil under controlled conditions in our study, this small difference would probably not be significant on a field scale due to the high spatial and temporal variability of CO_2 fluxes.

The least $^{14}CO_2$ was respired from the rhizosphere of the cut plants which were not labelled after defoliation. The total $^{14}CO_2$ respired here was derived from labelled C assimilated before defoliation. This respired C stems from the C reserves stored by *Lolium* in roots and stubble before defoliation and from the mineralization of sloughed off root cells, root hairs and microbial biomass turnover. The different values from plants labelled and not labelled after cutting reflects the amount of newly assimilated C (assimilated after cutting) in the rhizosphere respiration (Fig. 3, CO_2 from newly assimilated C). This newly assimilated C amounts to 9.4% and 5.9% for unfertilized and fertilized plants, respectively, and corresponds to about 80% of the total root-derived $^{14}CO_2$ evolved after defoliation.

There was no significant effect (only a tendency) of N fertilization on the $^{14}CO_2$ efflux of uncut plants and plants before defoliation. However, fertilization does have a significant (α=0.05) effect on efflux after defoliation (Fig. 2). After cutting, the values of N fertilized plants were lower than those of unfertilized plants. The latter lost about one-third more assimilates via root respiration and exudation during regrowth compared with the fertilized plants (Fig. 3, compare the upper parts of the two bars in the middle). We conclude that N fertilization lowered the unproductive C losses, especially during the regrowth phase when C assimilation is strongly reduced (Visser et al. 1997) and when the energy demand for water and nutrient transport remains high and must be partially covered from C reserves. Thus, the nutrient uptake from the soil declines rapidly after defoliation until a positive daily C balance for the whole plant has been re-established (Clement et al. 1978; Mackie-Dawson 1999).

Labelled and total C in different parts of *L. perenne* and soil

The grown shoots are the main sink of assimilated C before and after defoliation. On day 42, most ^{14}C was found in the shoot parts cut at 4 cm above the soil surface (Fig. 4). Similar results on the preferred incorporation of assimilated C into growing shoots of *Festuca pratensis* were obtained by Johansson (1993).

The fertilized plants tended to translocate more C into the cut shoot parts (19.1%) than did the unfertilized plants (17.4%), although the difference was not significant ($HSD_{0.05}$=3.22). The amount of ^{14}C recovered in newly grown shoot parts from cut plants is much lower than that in the shoot parts of the uncut plants >4 cm above the soil surface. Nonetheless, the total ^{14}C in cut and newly grown parts is about twice as high as in the upper part of uncut shoots.

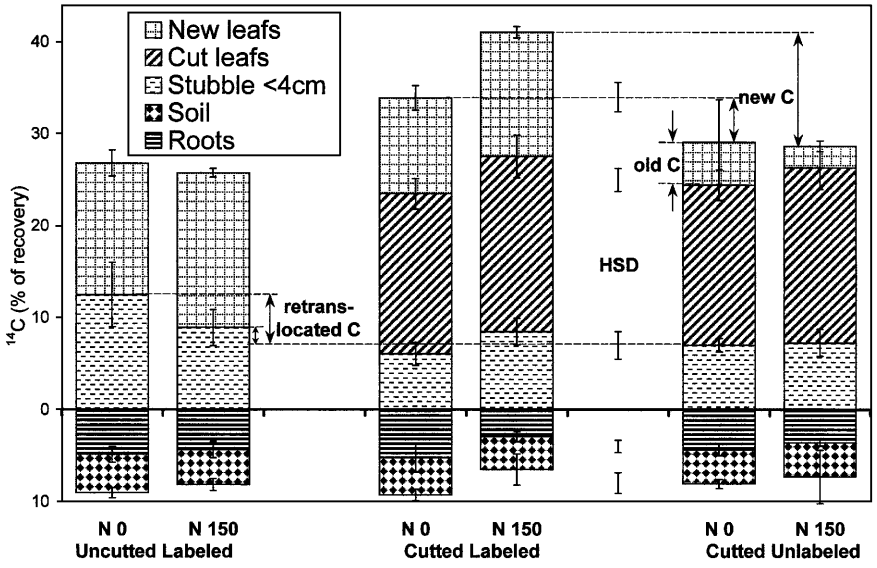

Fig. 4 Distribution of assimilated ^{14}C (±SD) in different above-ground parts, roots and soil at two levels of N fertilization in different defoliation and labelling treatments. Tukey's multiple comparison test (HSD, P=0.05) between the treatments was calculated for the different plant parts. For other abbreviations, see Figs. 1 and 3

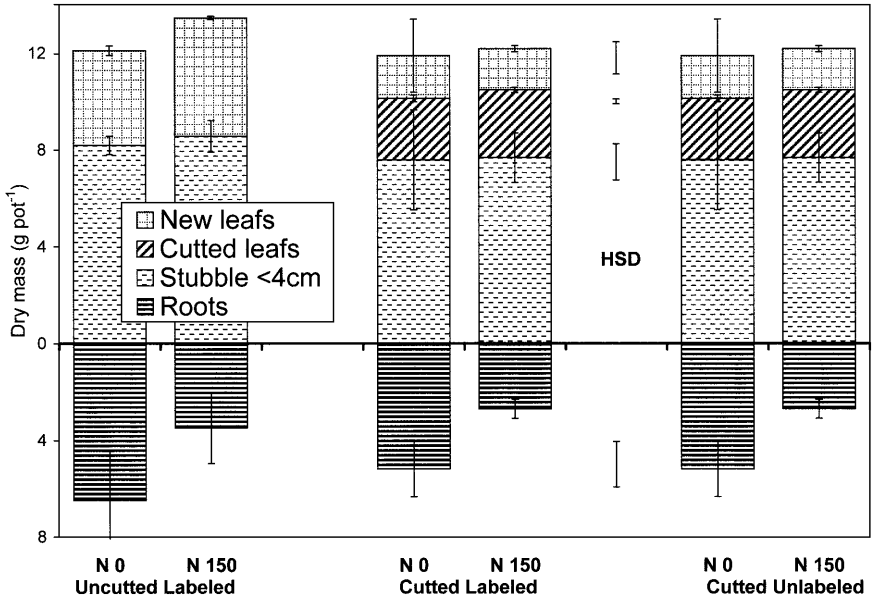

Fig. 5 Distribution of total plant dry mass (±SD) in different above-ground and below-ground parts of *L. perenne* at two levels of N fertilization in different defoliation and labelling treatments. Tukey's multiple comparison test (HSD, P=0.05) between the treatments was calculated for each plant part. For other abbreviations, see Figs. 1 and 3

The *Lolium* roots contained only 3.0–5.3% ^{14}C at the end of the experiment. Similar amounts were recovered in the soil. Johansson (1993) recovered the same amount of ^{14}C in the soil, although the ^{14}C allocation in *F. pratensis* roots was about 2 times higher. In contrast to our experiment, Johansson (1993) used continuous labelling, which was responsible for C incorporation during the whole growing period. The small amount of ^{14}C recovered in roots could be connected with the high intensity of root turnover. N fertilization decreases the ^{14}C content in roots and soil residue in all defoliation treatments, although this result was only significant for the roots of the cut unlabelled treatment.

Our experiment yielded only a small, non-significant decrease in ^{14}C (Fig. 4) and total C (Fig. 5) in the roots after cutting. Bushby et al. (1992) reported a significant ^{14}C decrease in both crowns and roots after defoliation. The roots of defoliated blue grama respired >60% of the labile organic substances in 4 weeks (Chung and Trlica 1980). Olson and Wallander (1999) reported that roots of unclipped *Euphorbia esula* were stronger sinks for C than roots of clipped *E. esula*. According to this literature data, defoliation decreases both the total root mass and the incorporation of assimilated C in below-ground plant parts (Holland and Detling 1990).

N affects the ^{14}C redistribution in the shoot parts grown after defoliation (P<0.01): it leads to a significantly higher transfer into the grown shoot parts compared with unfertilized plants. At the same time, a lower incorporation of ^{14}C into the roots of N fertilized plants was observed (P<0.05). Therefore, N fertilization leads to stronger above-ground C allocation during regrowth at the expense of below-ground C translocation.

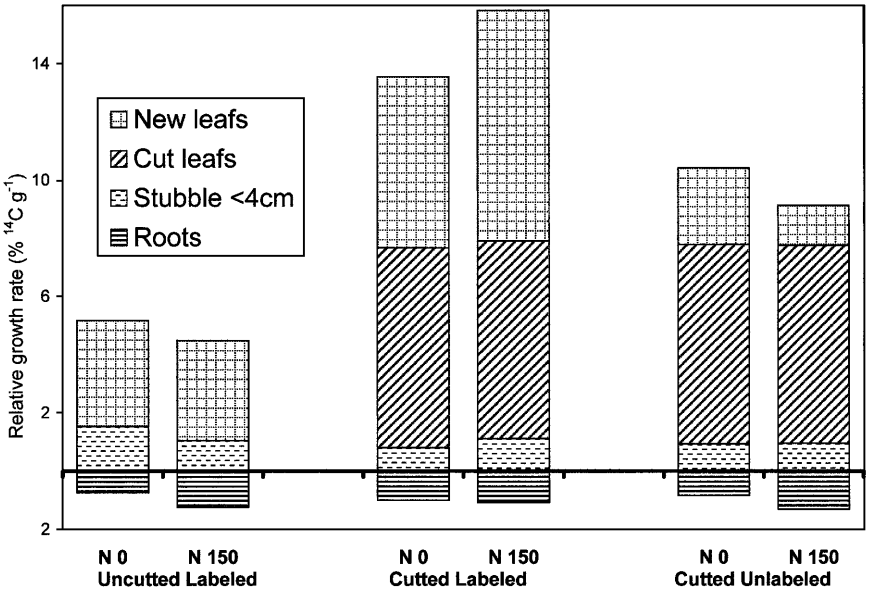

Fig. 6 Relative growth rate of different above-ground and below-ground parts of *L. perenne* at two levels of N fertilization in different defoliation and labelling treatments. For other abbreviations, see Figs. 1 and 3

A similar effect of N fertilization on the C partitioning in the above- and below-ground plant parts was found based on dry mass (Fig. 5): it strongly decreased the root mass (ca. 1.9 times) compared with unfertilized plants and at the same time increased total shoot mass. The ratio between the total above-ground plant parts and root mass is about 2.1 and 4.2 for unfertilized and fertilized plants, respectively. A similar fertilization-induced shift of C translocation from roots into the shoots was measured after defoliation of *Panicum maximum* (Bushby et al. 1992). The amount of $^{14}CO_2$ respired by roots and rhizosphere microorganisms (see above) shows smaller differences between the N treatments than was found for the total root mass. Thus, the respiration intensity of N fertilized roots is higher than that of unfertilized plants.

The stubble shorter than 4 cm makes up more than the half of the above-ground biomass (Fig. 5). Nonetheless, this stubble has the lowest ^{14}C of the three above-ground plant parts (Fig. 4) because most of the lower shoot parts (stubble) grew before the first ^{14}C labelling on day 25.

To estimate the relative growth rate (RGR) of different plant parts, the amount of ^{14}C was divided by the dry mass and specific activity was calculated (Fig. 6). The ^{14}C specific activity of roots and the stubble shorter than 4 cm is similar and represents minimal RGR compared with the other plant parts. The stubble of the uncut plants has more ^{14}C per gram dry mass than that of the cut plants. The specific activity of the upper shoot parts of uncut plants is only half that of cut parts or newly grown parts. This shows the amount of unproductive C losses of the shoots of the uncut plants due to shoot respiration. Thus, the mowing commonly practised in grasslands not only harvests more hay, but at the same time avoids the unproductive respiratory C losses of uncut shoots.

The higher specific activity of the newly grown shoots of fertilized versus unfertilized plants is interesting (Fig. 6, upper parts of the two middle bars). It shows that N fertilization increases the growth rate of the shoots after defoliation. At the same time, the amount of ^{14}C translocated after cutting from the stubble and roots of fertilized plants into the newly grown shoots is only half that of unfertilized plants (Fig. 6, upper parts of the two right-hand bars). This means that the fertilized plants use less C reserves stored in roots and stubble for the newly grown shoots than do unfertilized plants. These allocation patterns are also confirmed by the higher specific activity of roots of fertilized plants (Fig. 6, bottom parts of the two right-hand bars).

C retranslocation during regrowth

An important distinction between agricultural cereals and perennial pasture grasses is the reutilization and retranslocation of C reserves stored in roots and stubble for the growth of new shoots as well as for exudation, root and shoot respiration after cutting. The comparison between the second and third treatment of our experiment allows an estimation of the utilization of “old C” (C assimilated before defoliation) during regrowth as well as of the “new C” assimilated after defoliation. Figure 3 shows that about 1.7% of the assimilated C for the unfertilized plants and 2.1% for the fertilized plants which is respired in the rhizosphere after cutting originates from “old C”; 4.7% and 2.4% of ^{14}C recovered in the new shoot parts after the experiment were translocated from the root and shoot reserves (Fig. 4). Thus, about 45% and 17% (here 100%=total mass of new leaves) of the ^{14}C in newly grown shoot parts originate from the C reserves of unfertilized and fertilized plants, respectively. These results correspond with the data of Morvan-Bertrand et al. (1999), who found 1% of “old C” in the new leaf tissue of *L. perenne* 28 days after cutting. Johansson (1993), however, reported a higher proportion of “old C” in the newly grown shoots of *F. pratensis*. These discrepancies depend not only on the plant spe-

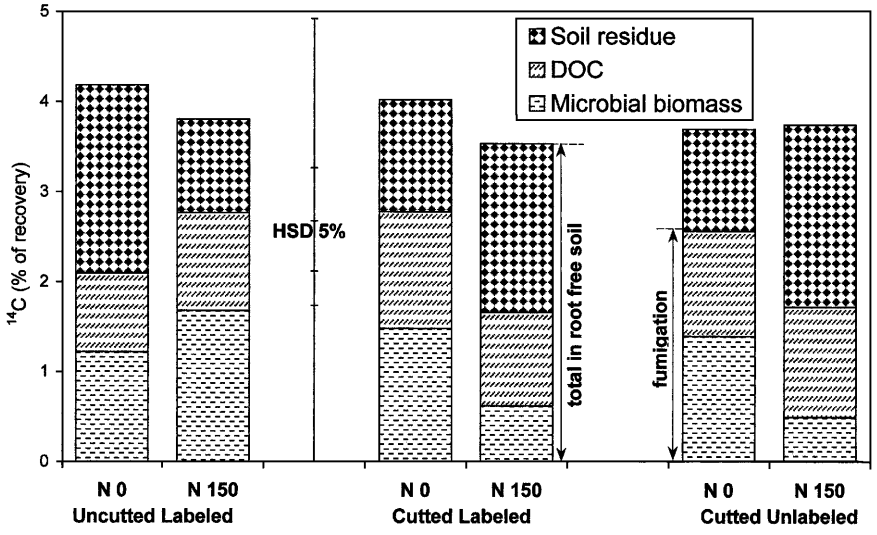

Fig. 7 Distribution of plant-assimilated C in soil residue, microbial biomass and dissolved organic C (*DOC*) at two levels of N fertilization in different defoliation and labelling treatments. Tukey's multiple comparison test (HSD, P=0.05) between the treatments was calculated for each compartment. For other abbreviations, see Figs. 1 and 3

cies, but also on the growing period after defoliation. Dynamics' studies show that the relationship between the "old" and "new" C as well as N in the growing shoots strongly depends on the time after defoliation (Avice et al. 1996; Briske et al. 1996; Morvan-Bertrand et al. 1999; Visser et al. 1997). The use of stored C peaks shortly after clipping and diminishes with time. Two days after defoliation of *L. perenne* the new shoot tissue contains between 60% (Schnyder and de Visser 1999) and 91% (Morvan-Bertrand et al. 1999) of the C derived from reserves assimilated prior to defoliation.

Comparing the ^{14}C in stubble and roots of uncut versus cut plants unlabelled after cutting allows the estimation of sources of C retranslocated during regrowth (Fig. 4). Most of the "old C" found in new leaves was retranslocated from stubble and not from roots. For unfertilized and fertilized plants, the ^{14}C decrease in the stubble is about 5.5% and 1.6% of recovered C, respectively, and the ^{14}C decrease in the roots is about 0.5% and 0.8%. Mackie-Dawson (1999) found a significant drop in the N content of the stubble of defoliated plants during regrowth compared with undefoliated plants. This demonstrates the retranslocation of N from the stubble into the newly grown leaves. In contrast to C retranslocation from the stubble found in this experiment for *L. perenne*, the decrease in labelled C in the roots during regrowth in *F. pratensis* was stronger than in the stubble (Johansson 1993). A similar effect was observed for green panic grass, where ^{14}C was translocated into newly grown shoots from the roots (Bushby et al. 1992). These contrasting results indicate that different pasture grass species preferably store C in different plant parts. Moreover, the cutting height above the soil surface can affect the subsequent retranslocation pattern: only few C reserves remain in plants cut close to the soil, and the contribution of C stored in the stubble therefore remains small compared to that stored in the roots.

Because more "old C" was retranslocated to the newly grown shoots in unfertilized plants, the decrease in ^{14}C in the stubble of the unfertilized plants exceeds that of fertilized plants. However, the same relationship for roots was not significant. Two weeks after defoliation, the small decrease in the total mass of roots and stubble of cut plants was lower than that of the uncut plants, although this decrease was not significant (Fig. 5). Another investigation on *Lolium* reported a significant decrease in root biomass and root diameter after defoliation compared to undefoliated plants (Mackie-Dawson 1999). The decrease in both total mass and ^{14}C in the root and stubble tissue reflects the retention of new assimilates in regrowing leaves and the exhaustion of available carbohydrates in roots and stubble (Chapin and Slack 1979); it also reflects the retranslocation of stored C reserves in newly grown shoots (Boot 1989) and a reduced total amount of assimilated C (Visser et al. 1997).

^{14}C incorporation into microbial biomass and DOC

Tracing the C assimilated by plants in different soil pools, including incorporation into microbial biomass, is a next step in the investigation of below-ground C allocation by plants. Only few studies have dealt with this issue.

The ^{14}C incorporation into microbial biomass in our experiment amounted to between 0.5% and 1.7% of recovered ^{14}C (Fig. 7) and confirms our previous results (Kuzyakov et al. 2001). These values correspond to about one-third of the ^{14}C soil residue. Martin and Merckx (1992) measured 38–42% of the ^{14}C soil residue in microbial biomass. Domanski et al. (2001) measured higher microbial ^{14}C incorporation, although the time after labelling was much shorter than that in our experiment. Tracing the ^{14}C assimilated by wheat in soil, Liljeroth et al. (1990) found 2.4–4.7% incorporation into microbial biomass; the higher values are explainable by the continuous ^{14}C labelling used in that study.

The differences between the treatments were not significant in the present study; the N fertilized plots tended to have less ^{14}C in the microbial biomass. Similar results

Table 1 Distribution of dry weight (*DW*) and plant-assimilated C in different particle size fractions and its relative enrichment (*RE*) with plant-derived C

	DW %	Uncut labelled		Cut labelled		Cut unlabelled	
		^{14}C (%)[a]	RE	^{14}C (%)	RE	^{14}C (%)	RE
Sand (63–250 and >250 µm)	5.03	39	7.90	50	10.60	54	10.71
Silt (2–63 µm)	71.2	15	0.21	35	0.48	13	0.18
Clay (0.1–2 µm)	16.8	13	0.77	14	0.80	26	1.54
Recovery (%)	93.0	70		99		93	

[a] ^{14}C distribution data are presented as percentages of ^{14}C activity in soil

were obtained earlier for *Lolium* (Kuzyakov et al. 2001) and for lettuce. No effect of defoliation on ^{14}C incorporated into microbial biomass and on the total ^{14}C of soil was measured 2 weeks after cutting. This result reflects the absence of differences in ^{14}C content in soil after the experiment (see above).

DOC extracted by K_2SO_4 aqueous solution using the extraction-fumigation method allows a rough estimation of the C readily available for rhizosphere microorganisms. The relatively constant amount of ^{14}C in the DOC of soil of different treatments is remarkable: ca. 1.2% for all treatments (Fig. 7). This constant amount depends on: (1) the equilibrium between the C input into the rhizosphere as root exudates and their utilization by microbial biomass (Domanski et al. 2001); (2) the fact that the fumigation-extraction method does not distinguish between the C from fine roots and especially root hairs and that from killed microbial cells; both C pools are extracted by K_2SO_4.

At the end of the experiment, xylanase, invertase and protease activities, involved in C and N cycling, were measured. The enzyme activities of planted soil were about 2.5 mg GE g^{-1} 24 h^{-1}, 4.9 mg GE g^{-1} 3 h^{-1}, and 210 µg Tyr g^{-1} 2 h^{-1} for xylanase, invertase, and protease, respectively; these values are similar to those of pasture and agricultural soils (Kandeler et al. 2001). The activities of xylanase (1.6 mg GE g^{-1} 24 h^{-1}) and invertase (4.0 mg GE g^{-1} 3 h^{-1}) of unplanted soil were significantly lower than those of the planted soil, whereas no differences were measured for protease activity. These enzyme activities were independent of cutting and N fertilization in ryegrass. These results pertain to 2 weeks after defoliation, when new shoots of the cut plants catch up with the shoots of the uncut plants. There may have been differences in the enzyme activities shortly after defoliation, when photosynthesis and below-ground C input were strongly reduced, but if so they disappeared after 2 weeks.

Distribution of assimilated C in different particle size fractions

Studies on the decomposition of organic substances in soil usually investigate the distribution of C in different particle size fractions (Nicolardot et al. 1992; Ladd et al. 1995; Stemmer et al. 1999). We were unable to find any studies tracing the plant-assimilated C in the particle size fractions or fractions of soil organic matter. This issue is especially interesting because the incorporation of C from exudates and root secretions in soil organic matter can differ from that of plant residues. Exudates comprising mostly low molecular weight organic substances (Merbach et al. 1999; Gransee and Wittenmayer 2000) are not only readily available for soil microorganisms, but they strongly participate in chemical reactions (Tanaka et al. 1995), as they can be chemically incorporated in soil organic matter (Kuzyakov and Galitsa 1993; Haider 1996), by being adsorbed on clay minerals (Dashman and Stotzky 1982).

At the end of the experiment the soil separated from roots was fractionated into particle size fractions (Table 1). Silt makes up the largest textural fraction – about 71% – of the soil originating from loess. The sand fraction amounts to about 5% and clay to about 17%. The residue of about 7% corresponds to the sum of the fine clay that was not collected by fractionation and losses. Although the silt makes up the highest proportion of the soil mass, it has minimal ^{14}C specific activity. The maximal enrichment with ^{14}C was observed for the sand fraction (Table 1). Similar results on the inclusion of plant-derived C were observed in a study on the long-term cultivation of maize by using ^{13}C natural abundance (Balesdent et al. 1987). Also Stemmer et al. (1999) found a higher incorporation of plant residues in the sand fraction. Based on the relative enrichment of different particle size fractions it can be concluded that the turnover of the sand fraction is most rapid. However, the high ^{14}C specific activity measured for sand can also be due to the methodology employed. In the fractionation procedure, sand is initially separated from other fractions by sieving. Therefore all organic fragments that cannot be separated during the sample's preparation remain on the sieves (250 µm and 63 µm) together with the sand fraction (Ladd et al. 1995). Naturally, the organic fragments derived from *Lolium* roots in our experiment and other plant residues in the experiments of other authors have a much higher ^{14}C specific activity than the actual sand fraction; nevertheless they are analysed together for ^{14}C in the fractionation method used. This calls for a methodological study examining the suitability of the particle size fractionation procedure for investigations on the incorporation of plant residues. An experiment on the incorporation of ^{14}C-labelled soluble organic compounds (Nicolardot et al. 1992; Ladd et al. 1995) or finely

ground plant residues could contribute to this. Cellulose will be incorporated like plant residues into sand and coarse clay fractions, although there is no maximum incorporation into the sand fraction in the case of soluble glucose (Nicolardot et al. 1992). A special pre-treatment such as electrostatic separation (Kuzyakov et al. 2001) or flotation of non-humified plant debris could improve the particle size fractionation procedure and its suitability for plant residue-incorporation studies.

Based on ^{14}C specific activity, the silt fraction has the longest turnover of soil organic C. The clay fraction has a higher ^{14}C specific activity than the silt fraction; therefore, organic C adsorbed on clay can be considered as younger. The relatively greater age of the organic matter associated with the silt fraction has been determined by radiocarbon dating (Anderson and Paul 1984). The stability of organo-mineral complexes, especially for the silt fraction, was also recorded for chernozemic soils originating from loess (Tiessen and Stewart 1983). The silt fraction is usually rich in highly aromatic organic compounds (Turchenek and Oades 1979), which are very resistant to mineralization, yielding longer turnover times. The clay fraction is usually considered to be very heterogeneous in terms of soil organic matter and its degradability. It usually contains components with high and low turnover rates. We conclude that root-derived C is preferentially incorporated in the coarse and fine-textured fractions, and less in the silt fraction. The longer incubation period of organic residues in soil could lead to a redistribution of C initially incorporated in sand and clay fractions.

There were no significant differences in ^{14}C distribution in particle size fractions in response to defoliation treatments. During regrowth of *F. pratensis*, Johansson (1993) observed no translocation in soil of C assimilated after cutting because the newly assimilated C was preferentially used for shoot regrowth. We can therefore conclude that most of the C found in the soil after the experiment was translocated before defoliation when all plants had the same treatment.

In conclusion, the portion of C respired by roots and released as exudates decreases by a factor of about 3 during plant development. Cut non-fertilized plants respired less C in the rhizosphere than the uncut plants and cut fertilized plants. About 80% of the root-derived CO_2 efflux originates from the C assimilated after the defoliation, although 20% originates from the C assimilated before cutting. N fertilization lowers the below-ground C losses (root respiration and exudation) during regrowth. The shoots are the main sink of assimilated C before and after defoliation. N fertilization leads to higher C incorporation into the shoot parts growing after defoliation compared with unfertilized plants. Less ^{14}C was incorporated into the roots of N fertilized plants. The RGR of roots and stubble is minimal and that of shoot parts cut by defoliation is maximal. Twelve percent of the ^{14}C was recovered in the newly grown leaves after regrowth; nevertheless, 4.7% and 2.4% of the ^{14}C in the new shoot parts were translocated from the root and shoot reserves of unfertilized and fertilized plants, respectively. Most of the C retranslocated into the new *Lolium* leaves originated from the stubble and not from the roots.

Between 0.5% and 1.7% of the recovered ^{14}C was found in the soil microbial biomass. Cutting and fertilization did not change the incorporation of ^{14}C in the microbial biomass nor xylanase, invertase, and protease enzyme activities. Tracing assimilated C in particle size fractions showed maximal incorporation in the sand and clay fractions.

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