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Effect of nitrogen fertilisation on below-ground carbon allocation in lettuce

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Abstract: The aims of this study were to investigate the effect of nitrogen (N) fertilisation on the below-ground carbon (C) translocation by lettuce and the CO_2 efflux from its rhizosphere. Two N fertilisation levels (80 and 160 kg N ha^{-1}) and two growth stages (43 and 60 days) were tested. ^{14}C pulse labelling of shoots followed by ^{14}C monitoring in the soil, roots, microbial biomass and CO_2 efflux from the soil was used to distinguish between root-derived and soil organic matter-derived C. The ^{14}C allocation in the below-ground plant parts was 1.5–4.6 times lower than in the leaves. The total quantity of C translocated into the soil was much lower than in the case of cereals and grasses, amounting to 120 and 160 kg C ha^{-1} for low and high N respectively. N fertilisation diminished the proportion of assimilated C translocated below ground. About 5–8% of the assimilated C was respired into the rhizosphere. Root-derived CO_2 (the sum of root respiration and rhizomicrobial respiration) represented about 15–60% of the total CO_2 efflux from the planted soil. Two peaks were measured in the $^{14}CO_2$ efflux: the first peak (4–5 h after labelling) was attributed to root respiration, whilst the second peak (12 h after labelling) was attributed to microbial respiration of exudates. Twelve days after labelling, 0.15–0.25% of the assimilated C was found in the microbial biomass. The higher microbial activity in the lettuce rhizosphere doubled the soil organic matter decomposition rate compared with unplanted soil.

Keywords: carbon allocation; CO_2; ^{14}C pulse labelling; lettuce; modelling; priming effect; rhizodeposition; root respiration

INTRODUCTION

Plant residues are the main source of organic carbon in the soil. Many investigations have been carried out to estimate both the quantity and quality of above- and below-ground plant residues, their decomposition in the soil[1–3] and their humification.[4] In contrast, there is a lack of knowledge about carbon (C) input into the soil via root exudates during plant growth. Methodological difficulties and problems such as the rapid microbial decomposition of both exudates and fine dead roots are responsible for this deficiency. The CO_2 from exudates is released from the soil along with that derived from decomposing soil organic matter (SOM), and these two sources cannot be differentiated.

Pulse[5,6] or continuous labelling of above-ground plant parts has frequently been used to distinguish between humus-derived and root-derived CO_2 and to quantify rhizodeposition.[7–9] Pulse labelling, compared with continuous labelling, has the advantage of being easier to handle, provides more information on the recent photosynthate distribution at specific developmental stages of plants, and can be used for kinetic investigations of $^{14}CO_2$ evolution from the soil. The results obtained by pulse labelling correspond to the relative distribution of assimilated C at the moment of labelling. The distribution of labelled C does not correspond to the distribution of total unlabelled C in different plant parts, but rather to the product of total C in the plant part and its growth rate at the moment of labelling. In the case of continuous labelling, the total amount of assimilated C is known. In addition, the distribution of labelled C corresponds to the distribution of total C, as long as it was applied from first leaf emergence to harvest time (the specific ^{14}C activity or ^{13}C abundance is equal in all plant parts; isotopic effects are not considered here). Therefore continuous labelling is particularly appropriate for the estimation of the amount of total C transferred by the plant into the soil and below-ground pools during the labelling period. Continuous labelling is also useful for the differentiation of root-derived and SOM-derived CO_2.

Continuous labelling requires special equipment for exposing the plants over a long period to $^{14}CO_2$ with constant ^{14}C specific activity or to $^{13}CO_2$ with ^{13}C enrichment. In addition, the air temperature and moisture conditions must be controlled inside the labelling chamber. These facilities are expensive and limited to a few places in the world.

Experiments using C isotopes showed that, on average, about 10–17% of the gross C assimilation of

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plants was respired from the rhizosphere within a few days of assimilation.[6,8,10] The different components of rhizodeposition have been quantified in arable[11,12] and grassland[13,14] soils. Related knowledge about the rhizodeposition of vegetable crops is relatively poor and, generally, root mass is measured after washing out the soil from samples.[15,16] This procedure does not consider either exudates or root lysates and, in addition, significant amounts of fine roots and root hairs are lost during the washing procedure.

Most investigations on below-ground C translocation have been on cereals and pasture plants.[6,17] Consequently, there is a lack of information about vegetable crops. When compared with arable crops, most vegetables are very intensive crops and are characterised by higher plant matter production per unit time and unit area. Therefore differences in the distribution of assimilates compared with arable crops may be expected. For this reason, our investigations were carried out with lettuce, a typical representative of leaf vegetables that is harvested during the vegetative stage and characterised by a good response to mineral nitrogen (N) fertilisation. The aims of this research were to compare the distribution of assimilates in lettuce with that found in arable crops and, in so doing, to identify the components of rhizodeposition, quantify the C translocated into the soil, estimate the soil CO_2 efflux and, finally, evaluate any effect on the turnover of soil organic matter.

MATERIALS AND METHODS

Experiment design

We studied the C rhizodeposition and root respiration of lettuce (*Lactuca sativa* L) grown on a sandy Gleyic Cambisol by means of $^{14}CO_2$ pulse labelling of shoots at two development stages (43 and 60 days), following the application of two N fertilisation levels (80 and 160 kg N ha^{-1}). The experiment was conducted under controlled laboratory conditions. Four replications of each variant were used.

Soil

The soil, collected from the Ap-horizon of a long-term experiment in Großbeeren (52°21′N, 13°17′E), south of Berlin, had been fertilised annually with 30 t ha^{-1} manure + NPK over the previous 13 years. It is classified as a sandy Gleyic Cambisol[18] with 4.6% clay, 9.9% silt, 88.5% sand, 1.45 g cm^{-3} soil density, 19.2% w/w field capacity, 4.5% w/w permanent wilting point, 0.75% organic C, 0.076% total N and a pH of 6.4. More information on the soil type, the long-term experiment, fertilisation and vegetable crop rotation is given by Richter.[19] After sampling, the soil was air dried, homogenised and sieved (<2 mm screen) prior to the experiment. Each pot for plant growing was filled with 2 kg of air-dried soil to a bulk density of 0.9 g cm^{-3}.

Growing conditions

One seed of lettuce (*Lactuca sativa* L, var Libusa RZ) was grown in each chamber (see below). The plants were grown at 27 ± 1 °C day temperature and 22 ± 1 °C night temperature, with a day length of 14 h and a light intensity of approximately 400 µmol m^{-2} s^{-1} at the top of the canopy. The soil water content of each chamber was measured gravimetrically and adjusted daily to about 70% of field capacity with deionised water. Nitrogen fertilisation was applied as KNO_3 aqueous solution. Two levels of N fertilisation were tested: low (26.6 mg N kg^{-1}) and high (53.2 mg N kg^{-1}) (corresponding to total fertilisation of 80 and 160 kg N ha^{-1} respectively). According to the growth covering N_{min} target values of the system named 'KNS' and used for timing and quantification of N fertilisation rates in commercial vegetable production in Germany, the N content of the total plant fresh matter (60 t ha^{-1}) of field-grown lettuce is about 100 kg ha^{-1}.[20] The plants for the first labelling were fertilised in two applications

	Days after sowing						
	35	*42*	*43*	*50*	*55*	*60*	*72*
Plant height (cm)	ND	12	ND	15	16	19	ND
Growth stage (BBCH code)[a]	13	15	15	17	19	41	42
Growing time	←	←	←	←	←		
N application (kg N ha^{-1})[b]	20/40	20/40					
$^{14}CO_2$ labelling in first stage			$^{14}CO_2$		X		
Growing time	←	←	←	←	←	←	←
N application (kg N ha^{-1})[b]	20/40	20/40		20/40		20/40	
$^{14}CO_2$ labelling in second stage						$^{14}CO_2$	X

ND, not determined.

[a] The BBCH code of vegetable crops is a detailed crop-specific description of the phenological growth stages within the general BBCH scale of agricultural plants and weeds.[46,47] The phenological development of vegetables is divided into principal growth stages; each growth stage is subdivided into secondary growth stages. A two-digit decimal code is assigned to each growth stage.

[b] Low/high N level.

X, end of the experiment (at which time the plants and soil are sampled).

Table 1. Plant height and growth stage at various days after sowing of lettuce subjected to ^{14}C labelling and N application

and received only 40 and 80 kg N in total. Half of the final N amount was chosen because of the small demand of young plants. The plants for the second labelling received the full level of N fertilisation in four applications during the experiment (Table 1).

To compare total unlabelled CO_2 evolution with and without lettuce, soil without plants was also incubated in the same pots, under the same conditions and with the same two levels of N fertilisation.

Chamber and labelling

The labelling apparatus consists of a two-compartment plexiglass chamber with a lower part (138 mm diameter, 150 mm height) for the soil and plant roots and an upper part (138 mm diameter, 300 mm height) for the shoots and $^{14}CO_2$ generation (see extended description of the labelling chamber by Kuzyakov *et al*[13,21]). The two parts are separated from each other by a plexiglass lid with a drill hole (diameter 8 mm) for plants. One day before labelling, each hole containing a plant was sealed with a two-component silicon paste, NG 3170 (Fa Thauer & Co, Dresden, Germany). The seal was tested for air leaks by pumping air into the container with a peristaltic pump, with a wash flash between the pump and the container. If there were no air bubbles in the wash flash, then the container was accepted as being airtight.

Labelled ^{14}C as $Na_2{}^{14}CO_3$ (580 kBq) aqueous solution was placed in a 2 ml Eppendorf micro test tube in the upper compartment of the chamber, then the chamber was closed. A 1 ml aliquot of 2.5 M H_2SO_4 was added to the $Na_2{}^{14}CO_3$ solution in the micro test tube through a Teflon pipe in the upper chamber part. This allowed complete evolution of $^{14}CO_2$ into the chamber atmosphere. Ten hours after labelling, the air from the upper chamber was pumped through NaOH solution to remove the unassimilated $^{14}CO_2$. The ^{14}C activity found in this NaOH trap was defined as '^{14}C not assimilated' (see below). Then the top of the chamber was removed. The plants were labelled on days 43 and 60 after sowing (Table 1). Plants in different chambers were used for the first and second labellings. Complete descriptions of the two-compartment chambers and details of the ^{14}C labelling procedure are given by Kuzyakov *et al.* [13,21]

Microbial biomass C and ^{14}C

The microbial biomass C was determined by a fumigation–extraction method.[22] Fumigated and non-fumigated soil samples were extracted with 0.5 M K_2SO_4 for 30 min (soil/K_2SO_4 solution 1:2) and filtered. An aliquot of K_2SO_4 solution was analysed for organic C using C/N analyser Dimatoc-100 (Dimatec Co, Essen, Germany). The ^{14}C of another aliquot was determined by scintillation counting. The additional oxidisable C and ^{14}C counts obtained from the fumigated soils were taken to represent the microbial C flush and converted to microbial biomass C using the relationship[23]

$$\text{microbial C} = 2.34 \times \text{C flush}$$

The same factor (*2.34*) was used for N and newly incorporated ^{14}C. Analyses of microbial biomass C and labelled ^{14}C were replicated four times for each soil–root column.

Chemical and ^{14}C analysis

During the experiment the total and labelled $^{14}CO_2$ respired from plant roots and micro-organisms was pumped from the root compartment into a vial containing 20 ml of 0.5 M NaOH. The NaOH trap was changed every 2 h immediately after ^{14}C labelling. This period increased during the experiment, and by the end of the experiment the NaOH trap was changed twice a day. In total, the NaOH trap was changed 30 times during 12 days of monitoring of the $^{14}CO_2$ efflux. The quantity of CO_2-C derived from the root compartment and absorbed in NaOH was estimated in one aliquot (10 ml) by titration with 0.2 M HCl against phenolphthalein.[24]

Twelve days after each ^{14}C labelling, the shoots, roots and soil were destructively sampled. The soil was mixed and divided into three aliquots. The roots were separated from the soil by washing one subsample through a 0.5 mm sieve. The second and third subsamples were analysed for microbial biomass C and for chemical and ^{14}C parameters. Leaves, washed roots and soil were dried at 60 °C. The organic C content of dried soil and plant samples was estimated by a C–N analyser (Carlo Erba, Milan, Italy) on duplicate samples.

The ^{14}C activity of the NaOH solution was measured by scintillation counting (β-Spectrometer Tri-Carb 2000CA, (Canberra Packard Co Ltd, Frankfurt/M, Germany) using the scintillation cocktail Rothiscint 22x (Roth Company, Karlsruhe, Germany). The mixture ratio between the cocktail and the NaOH was 7:1. All ^{14}C measurements were made after the decay of chemiluminescence.

The ^{14}C activity of soil and plant samples (1 and 0.2 g respectively) was determined after combustion (2 min) in an Oxidiser-Unit (Model 307, Canberra Packard). The CO_2 produced was directly trapped in the scintillation cocktail Permafluor E^+ (Canberra Packard), followed by the measurement of ^{14}C activity. All ^{14}C measurements were standardised to absolute ^{14}C activity using a quench curve corresponding to the method of Two-Channel Ratio of Extended Standard (tSIE, Canberra Packard). The quench curve was produced using NaOH solution as a quencher. The efficiency of radioactivity measurement was between 88 and 90%.

Calculations and statistical analysis

The ^{14}C activity, the C efflux from the soil, and the C allocation in different pools were expressed as a percentage of the total assimilated ^{14}C. The total

assimilated ^{14}C ($^{14}C_{ass}$) was defined by the expression

$$^{14}C_{ass} = {}^{14}C_{in} - {}^{14}C_{nass} - {}^{14}C_r \quad (1)$$

where $^{14}C_{in}$ is the ^{14}C input activity, $^{14}C_{nass}$ is the non-assimilated ^{14}C, and $^{14}C_r$ is the remaining ^{14}C activity in the Eppendorf micro test tube.

The C amount for different flows has been calculated using the equation

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

where C^{flow}_{amount} is the C amount in the investigated compartment (ie roots, C allocated below ground, root-derived CO_2) ($g\,m^{-2}$), C_{shoots} is the C amount in the shoots ($g\,m^{-2}$), $^{14}C_{flow}$ is the percentage of ^{14}C in the investigated flow (% of assimilated ^{14}C) and $^{14}C_{shoots}$ is the ^{14}C content in the shoots at 8 days after labelling (% of assimilated ^{14}C).

For all calculations the amount of C in the shoots (C_{shoots}) was chosen as reference. This selection was made because the shoot mass and the ^{14}C incorporation in the shoots can be measured more accurately compared with all other compartments of the system (roots, CO_2, soil, etc). The C pools and C fluxes per pot were calculated per ha by considering the soil surface area of the experimental pot and the density of 10 plants per m^2.[25] The parameters C^{flow}_{amount}, $^{14}C_{flow}$ and $^{14}C_{shoots}$ were taken from the experimental results and were different for each development stage and N fertilisation rate investigated. We calculated with this method the total below-ground C translocation and the amount of rhizosphere-derived CO_2. Saggar *et al*[26] and Kuzyakov *et al*[13,21] have previously used the same method for the estimation of total below-ground C translocation. Comparison of this calculation method based on ^{14}C pulse labelling with a more precise method based on the ^{13}C natural abundance shows the same results for the estimation of root-derived CO_2.[27] The pulse labelling calculation method allows only a rough estimation of the C amount passed through each flow, because the parameters in eqn (2) are not constant during plant development. As a result, this calculation method can be used only after complete ^{14}C distribution in the plant and the achievement of the stage near to equilibrium. Warembourg and Estelrich[28] have shown that an allocation period of about 1 week is sufficient to give a reliable estimation of the dynamics of C flow in the rhizosphere. However, our results for C flow amounts calculated on the basis of pulse labelling must be interpreted with care because of the interpolation between labelling periods.

The priming effect in the rhizosphere soil was calculated as the difference in CO_2 efflux derived from microbial decomposition of SOM between containers with and without lettuce. The CO_2 efflux derived from decomposition of SOM in containers with lettuce was calculated as the difference between the total CO_2 efflux and the calculated root-borne CO_2 according to the ratio in eqn (1).

The ^{14}C data as percentages of total assimilated carbon and the allocation of C in different pools were analysed by variance (MANOVA) to determine the statistical significance of N fertilisation and plant growth stage. The dependent variables were the above- and below-ground ^{14}C, the total C translocated below ground, and the $^{14}CO_2$ efflux from the soil. The significance of differences was determined by an LSD test with $p \leqslant 0.05$.

RESULTS AND DISCUSSION

Below-ground C translocation of lettuce

Lettuce assimilated more than 95% of the supplied $^{14}CO_2$ during the first 10 h of exposure, and assimilates were quickly translocated within the plant. The $^{14}CO_2$ in the root compartment of the chamber was detected 1 h after $^{14}CO_2$ labelling. Fast translocation of assimilated carbon into the roots, followed by respiration as CO_2, has also been estimated for winter wheat and rye,[5] wheat and barley,[10] maize,[29] ryegrass[13,21] and soybean.[30]

According to the ^{14}C activity, the incorporation of carbon into the leaves was 1.5–4.6 times higher than below-ground translocation (Fig 1). The total C amount in shoots and roots is presented in Table 2. At day 60 the incorporation of carbon into the leaves was on average about two times higher than that of the below-ground portion. Between days 43 and 60 the proportion of assimilated ^{14}C found in the leaves decreased from 36.3 to 20.6% irrespective of N level, and in the roots increased from 1.5 to 3.6% and from 1.5 to 5.1% at high and low N levels respectively. In contrast, Swinnen *et al*[8] estimated an increasing C

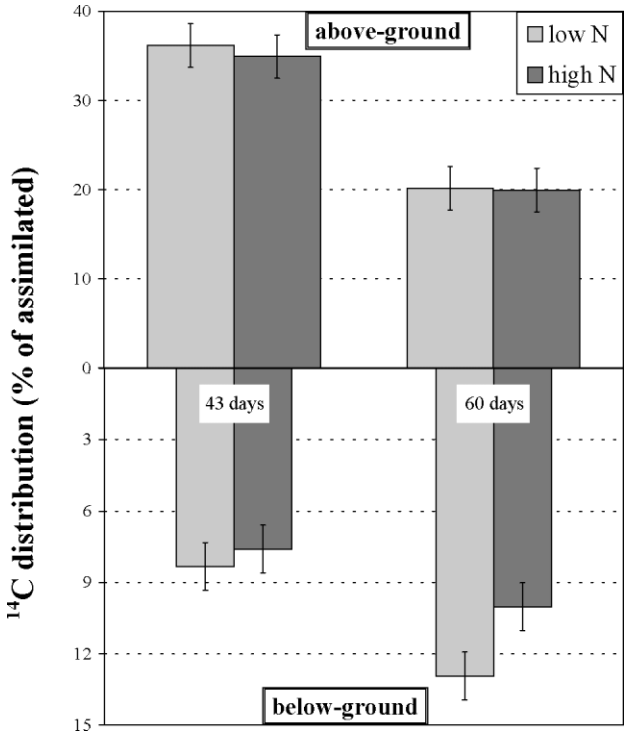

Figure 1. Effect of plant age and N fertilisation on relative distribution of photosynthetic assimilates by lettuce.

Table 2. Effect of plant age and N fertilisation on above-ground and below-ground biomass of lettuce (g C m^{-2})

	43 days		60 days		
	Low N	High N	Low N	High N	LSD 5%
Shoots	15.26	15.33	40.70	52.94	3.07
Roots	4.35	4.13	10.65	13.86	1.25

incorporation into the leaves and a decrease into the roots during the growth of wheat. Translocation dynamics similar to the results of this study was found for ryegrass growth.[13] Both the ryegrass[13] and the lettuce in this study were harvested during the fast vegetative growth stage. In contrast, most cereals were harvested at the end of plant growth, and generally in the reproductive stage. For this reason, the measured differences in the C translocation pattern between lettuce and cereals such as wheat may be related to the stage of growth at which the plants were labelled and harvested.

Our results suggest that the influence of nitrogen fertilisation on the relative carbon incorporation into the leaves was not significant by day 43. Although the absolute amount of C translocated into the roots was higher at high N (see below), the relative C incorporation into the roots was higher in the low-N treatment than at the high N level. This difference was only significant for the second growth period of lettuce (late $^{14}CO_2$ exposure; 5.1 and 3.6% of assimilated ^{14}C activity at low and high N levels respectively). The same effect of low N fertilisation rates has been observed for arable crops[31] and pasture plants.[21,28,32] The N depletion in the soil leads to an increased below-ground translocation of assimilates for additional root growth (relative to assimilated C), as well as for the energy necessary for N acquisition by the roots and for nutrient mobilisation from the SOM (see below).

A higher proportion of total assimilated C translocated into the soil was measured at the low N level. However, the plants at the high N level have a higher total productivity and assimilation intensity compared with the plants at the low N level. As a result, the low-N-fertilised plants led to a smaller release of plant C input into the soil than that of high-N-fertilised plants.

Total C input into the soil by lettuce

Traditionally, methods for estimating the quantity of root-derived organic C in the soil are characterised by the loss of the smallest roots, root tips and root exudates during the washing procedure. Labelling with ^{14}C allows the estimation of the total quantity of C translocated from the plant into the soil. We calculated the C input into the soil before root washing. Pulse labelling allowed only a rough assessment of ^{14}C translocation during plant growth, because the patterns of assimilate distribution and the specific ^{14}C activity of the different plant pools can change (see above). This should be taken into consideration when extrapolating the amounts of C translocation calculated using pulse labelling.

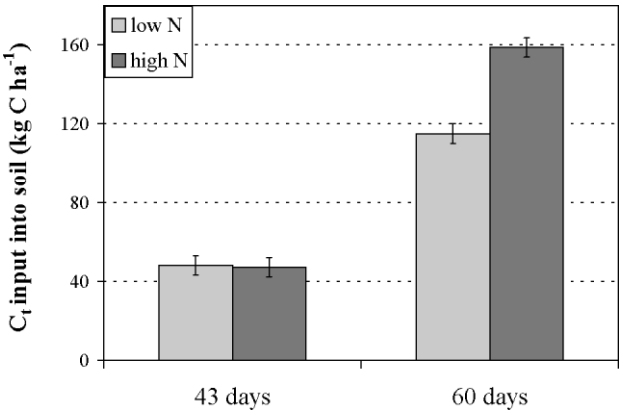

Figure 2. Total carbon input into soil by lettuce as a function of plant age and N fertilisation rate.

We calculated that lettuce translocated about 120–160 kg C ha^{-1} into the soil during the 60 day cultivation period (Fig 2). A decrease in the relative C translocation by increased N fertilisation (Fig 1) was overcompensated by the higher total plant matter production of fertilised plants. The result was that plants fertilised with the high N level translocated more C into the soil compared with plants grown at the lower N level (Fig 2). This corresponded to the 1.3 times higher total above-ground mass production of plants fertilised with 160 kg N ha^{-1} than that of plants fertilised with 80 kg N ha^{-1}.

According to our calculation, the amount of C translocated into the soil was 7–10 times lower in lettuce (120–160 kg C ha^{-1}) than in arable crops (200–2900 kg C ha^{-1} $year^{-1}$).[6,17,33] This is caused by the short growing period of lettuce in our experiment (only 60 days) and the relatively low plant matter production, as well as the different C translocation patterns. Ruehlmann and Ruppel[34] calculated the C translocation into the soil by field-grown vegetables using a model describing long-term changes in the soil organic matter content. The estimated values this model gave for vegetables with a mean growing period of 120 days per year was a translocation of 1400–2900 kg C ha^{-1} $year^{-1}$ over a period of 25 years. These values are similar to those of arable crops mentioned above.

The second labelling with calculation of the C input into the soil was made 60 days after sowing, 12 days before the end of the experiment. Each lettuce plant had a dry mass of 10 and 13 g at low and high N levels respectively, corresponding to 400 and 520 kg C ha^{-1} in the above-ground plant matter. The amount (120–160 kg C ha^{-1}) calculated as translocated into the soil was about 23% of the net fixed C and was independent of the N treatment. In the long-term field experiment with vegetables it was observed that between 23 and 38% of the net fixed C was translocated into the soil.[34] Both the amount and the proportion of C translocated into the soil increased when the soil texture became coarser; and the soil

organic matter content increased. Probably both effects are the result of a higher microbiological activity in sandy soils than in more heavily textured soils.[35,36]

Total CO_2 efflux from the soil

The carbon dioxide efflux from a planted soil consists of the following components:

(1) root-derived CO_2, which includes the contribution of root respiration and the CO_2 produced by micro-organisms decomposing exudates, sloughed root cells and fine roots;
(2) SOM-derived CO_2, which includes the CO_2 resulting from the oxidation of SOM that is the basal respiration of the bare soil, and that derived from the decomposition of SOM due to increased microbial activity in the rhizosphere soil.

It is generally difficult to quantify the individual components of the total CO_2 efflux from a cropped soil. The coupling of ^{14}C labelling of plants, simulation of C fluxes, and comparison of the CO_2 efflux from planted and unplanted soils could be one way of solving this problem. We found that 5–8% of the assimilated C was respired in the rhizosphere over 12 days after assimilation, and this percentage corresponded to a daily CO_2 efflux from the soil ranging from 0.44 to 2.20 kg C-CO_2 ha^{-1} day^{-1}. From 15 to 60% of the total CO_2 efflux from the planted soil was root-derived (Fig 3), corresponding to the sum of root respiration and microbial decomposition of roots and exudates. Consequently, the proportion of lettuce-derived CO_2 in the total CO_2 efflux was comparable with that of other arable plant species.[13] It must be considered that the humus-derived CO_2 was relatively low in this particular experiment, because the content of organic carbon in the soil was low (0.75%).

Separating root respiration and microbial respiration of rhizodeposits

The estimation of the aforementioned individual components of rhizosphere CO_2 is limited by the methods available. Only a few experiments have tried to separate the contribution of root respiration from the rhizomicrobial respiration *in situ*.[5,13,37,38] Most root exudates are easy decomposable and contribute to the C turnover in the soil, especially by increasing microbial activity in the rhizosphere soil. In contrast, CO_2 originating from root respiration cannot be utilised by 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 is part of the total CO_2 evolution from the soil and makes the determination of CO_2 due to the microbial decomposition of organic substances difficult.

Root and rhizomicrobial respiration were separated by using a model describing the CO_2 efflux from the rhizosphere, based on the assumption that different components of the total CO_2 efflux predominate during different periods after pulse labelling.[13,21] The model, assumptions and parameter estimation were described in detail earlier.[13,39] The most rapid process is the CO_2 efflux from root respiration, which predominates on the first day after labelling (Fig 4). The CO_2 evolved due to microbial respiration of root exudates occurs later than root respiration owing to the sequence of processes involved, including root exudation, uptake of root exudates and then decomposition by micro-organisms. This hypothesis is supported by the data in Fig 4: two peaks of the $^{14}CO_2$ efflux were measured during the first day after labelling. The microbial respiration of root exudates comprised the main source of root-derived $^{14}CO_2$ at 12 h after labelling and predominated during the second and fifth days. The microbial respiration of dead roots is a slow process and, at the time scale used in this study, its contribution to the total $^{14}CO_2$ efflux from the soil may be considered negligible in the first few days after pulse labelling. It was a predominant source of $^{14}CO_2$ after the fifth day (Fig 5) when the decomposition of exudates is finished.

The small increase in the $^{14}CO_2$ efflux intensity at 4 h after the $^{14}CO_2$ input by the first labelling (both N levels) is not significant and may be interpreted as an artefact (Fig 4). However, the maximum of the $^{14}CO_2$

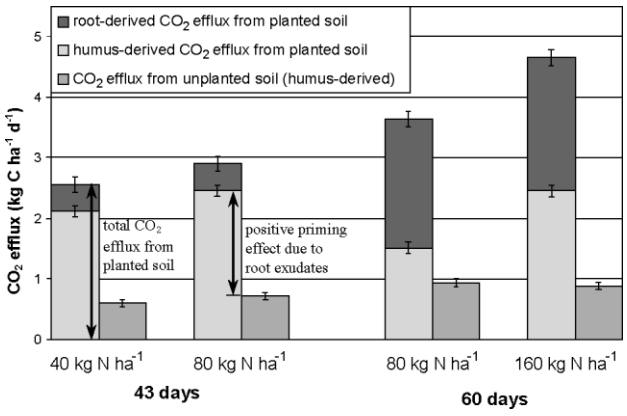

Figure 3. Partitioning of total CO_2 efflux from planted soil into root-derived and humus-derived CO_2 efflux and priming effect.

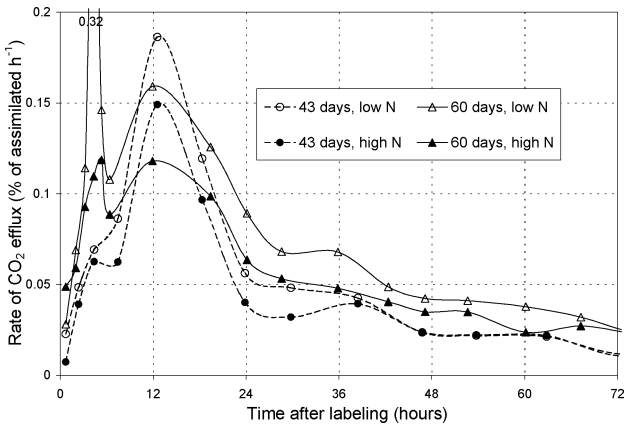

Figure 4. Rate of $^{14}CO_2$ efflux from soil as a function of plant age and N fertilisation.

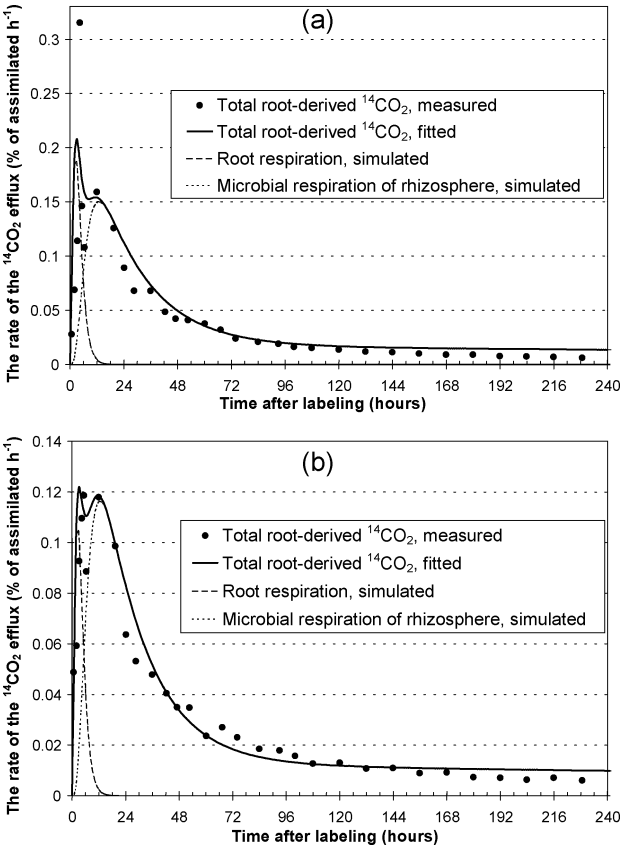

Figure 5. Partitioning of $^{14}CO_2$ efflux from soil into root respiration and microbial respiration of rhizodeposits by using modelling approach:[23,24] (a) low N fertilisation rate; (b) high N fertilisation rate.

efflux between 3 and 6 h after the second labelling is clear and significant. As such, we interpreted the first maximum of the $^{14}CO_2$ efflux as the peak from root respiration, and the second as the peak from rhizomicrobial respiration. It is interesting that the peak of low-N-fertilised lettuce is much higher than that of high-N-fertilised lettuce. It corresponds to the increased loss of C by the roots at the low N fertilisation level. Warembourg and Billes[40] have reported a similar behaviour of the $^{14}CO_2$ efflux from the soil with wheat, and Nguyen *et al*[29] with maize. However, the first peak in the $^{14}CO_2$ efflux occurred earlier in our experiment with lettuce, in comparison with the experiments with wheat[40] and maize,[29] because of the forced ventilation of the soil in the containers.

The modelling approach based on different stages of $^{14}CO_2$ efflux after ^{14}C pulse labelling allowed the separation of $^{14}CO_2$ derived from root respiration and microbial respiration of exudates without soil sterilisation or other treatments. However, this approach can be used only after pulse labelling. The fitted curve (Fig 5) and the separation of root respiration from microbial respiration of root exudates and dead roots confirm the assumptions. Only the data for the second labelling are shown in Fig 5.

In order to separately calculate the total C amount passed through root respiration and root exudation, the cumulative CO_2 amounts passed through root respiration and microbial respiration by utilisation of root exudates were calculated by Runge–Kutta integration. The integration was performed only until day 10 after labelling, ie until the last measurement of CO_2 efflux from the soil. The calculated root respiration ranged from 0.94 to 0.53% and the microbial respiration of root exudates and dead root debris accounted for 8.3–6.0% of the assimilated ^{14}C for low and high N levels respectively. Thus the estimated contribution of root respiration to the total $^{14}CO_2$ efflux from the rhizosphere fluctuated from 8 to 10%, which is smaller than reported earlier.[5,21] However, these previous experiments lasted only 4 h and 8 days respectively, compared with 12 days for the current study. According to the assumption that root respiration finishes 1 day after labelling, prolonging the $^{14}CO_2$ monitoring period increases the contribution of microbial respiration and decreases the percentage of root respiration.

Incorporation of rhizodeposits into microbial biomass

The microbial decomposition of easily available organic substances in the soil is a very fast process, and it is more rapid in the rhizosphere than in the bulk soil because of a larger and more active microbial biomass. In addition, the concentration of easily available substrates is higher in the rhizosphere than in the bulk soil. The high microbial activity in the rhizosphere may lead to the lower absorption of exudates on clay surfaces and organic colloids than in the bare soil. Therefore the exudates in the rhizosphere are less physically protected than in the bulk soil. In this experiment it was not possible to measure the dynamics of microbial activity, microbial biomass and ^{14}C incorporation throughout. For this reason, analyses were carried out at the end of each $^{14}CO_2$ monitoring period after harvesting, 55 and 72 days after sowing (Table 1).

Microbial biomass C increased 2–3 times at the second harvest (Fig 6(a)) and corresponded to the higher amount of roots on day 72. Higher N fertilisation also led to an increase in microbial biomass at the second harvest. The microbial biomass C was enhanced from 48 to 73 mg C kg^{-1} in the soil by the addition of 80 kg N ha^{-1}. This was most likely due to the increased below-ground C translocation in lettuce (see above), which corresponded to the increase in root mass at the high N fertilisation rate. Similar increases in biomass at higher levels of N fertilisation were found in the rhizosphere of maize,[41] but not for wheat.[42]

In contrast, the proportion of ^{14}C in the microbial biomass was higher at the lower N fertilisation rate (Fig 6(b)). This different behaviour of microbial biomass C and microbial biomass ^{14}C during plant growth was significant during the second labelling period. The higher microbial ^{14}C content corresponded with the higher ^{14}C total below-ground translocation of less fertilised plants (Fig 1). Addi-

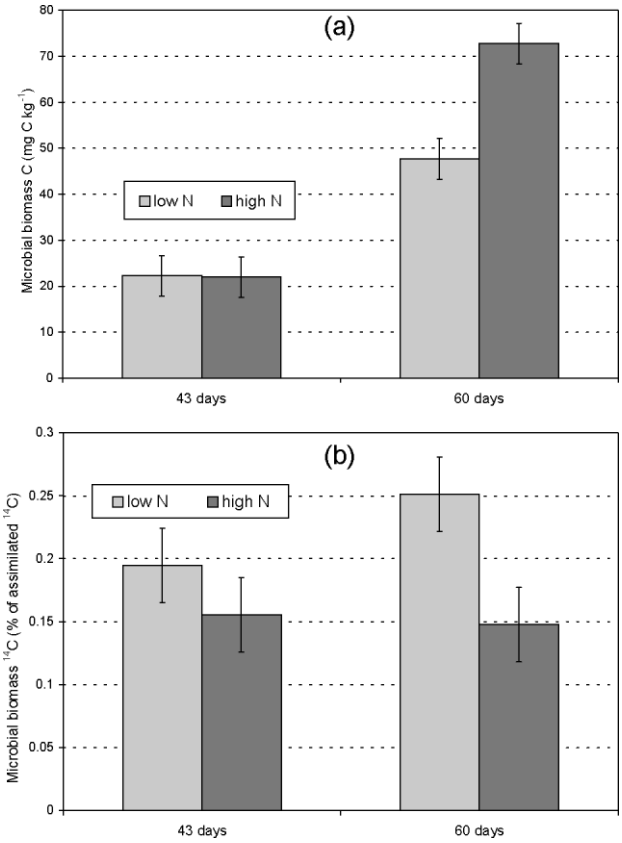

Figure 6. (a) Soil microbial biomass C and (b) plant-assimilated C incorporated into soil microbial biomass as a function of plant age and N fertilisation.

tionally, the higher microbial biomass in highly fertilised soil (Fig 6(a)) may have led to more complete microbial utilisation of labelled exudates. Ladd *et al*[43] pointed out that faster turnover rates would result in lower microbial ^{14}C accumulation. The increased ^{14}C incorporation into the microbial biomass by N fertilisation measured in some other studies was estimated using continuous labelling techniques,[41,42] in contrast to the pulse labelling used in our study. The plants fertilised with the high N level grew faster, had a larger leaf area and assimilated more CO_2 compared with plants grown at the low N level. Therefore the total C translocated into the rhizosphere by high-N-fertilised plants is higher and the dilution of labelled ^{14}C introduced into the system only once by unlabelled C is also higher. This can lead to the decrease in ^{14}C content of the microbial biomass.[41,42]

C priming effects due to rhizodeposition

Along with the direct contribution of the roots to the total CO_2 efflux from the soil, an indirect priming effect due to increased microbial activity in the rhizosphere can lead to additional microbial decomposition of the SOM. As mentioned before, microbial biomass and microbial activity are higher in the rhizosphere than in the bulk soil or the bare soil.[5,7] The increased microbial activity in the rhizosphere may either accelerate or retard the decomposition of SOM. Acceleration can result from additional nutrient mobilisation from the soil organic matter, while retardation can occur because of the competition for limited nutrients (eg N) between plant roots and micro-organisms in the rhizosphere or because of the microbial biomass switching from the decomposition of SOM to the decomposition of easily available root exudates. These kinds of interactions between SOM turnover and decomposition of root exudates belong to the phenomenon known as a priming effect.[44] Knowledge of the interactions between roots and micro-organisms, especially in the rhizosphere, is limited and seldom reflected in the literature.[11,13,45]

We estimated the priming effect by calculating the difference in the humus-derived CO_2 efflux from cropped and non-cropped soils (Fig 3). The humus-derived CO_2 efflux from soil with lettuce was calculated as the difference between the total CO_2 efflux and the root-derived labelled $^{14}CO_2$ (Fig 3). Cropping the soil with lettuce led to a 1.5–3.0 times faster decomposition of SOM (Fig 3), and this additional CO_2 efflux corresponded to 0.5–1.8 kg CO_2-C ha^{-1} day^{-1}, depending on the age of the lettuce and the level of N fertilisation. Assuming a C/N ratio of 10 in the different fractions of the soil organic matter, the net N mineralisation ranged from 0.05 to 0.18 kg N ha^{-1} day^{-1}; quantities that can be sufficient to satisfy the lettuce N needs, since we have estimated that the mean daily N demand of young lettuce plants is about 0.14 kg N ha^{-1} day^{-1}. Priming effects can be real or apparent.[44] The most important mechanism for real priming effects is the acceleration of soil organic matter turnover due to increased activity or amount of microbial biomass.[44] Isotopic exchange, pool substitution and different uncontrolled losses of mineralised N from the soil are responsible for apparent N priming effects.[44] In this study a real priming effect was measured, because it occurs owing to increased microbial activity in the rhizosphere compared with soil without plants. The additional C mineralisation due to the priming effect was low because of the low SOM content in the soil used and the small amount of C deposition with young lettuce. In comparison, the positive priming effect of other plant species characterised by a well-developed root system and grown on soils rich in organic matter was much higher than that calculated in our study. Kuzyakov *et al*[13] estimated a positive priming effect of 6 kg N ha^{-1} day^{-1} for perennial ryegrass. Helal and Sauerbeck[7] estimated a decrease of 4–7% in the SOM content during the growth of maize, due to root-affected microbial decomposition. In contrast, measurements showed slow decomposition of SOM by cultivation of wheat using the ^{13}C natural abundance method.[11] These contrasting results may have been caused by the use of different methods, and it may be worthwhile to compare both methods in the same investigation so as to more accurately evaluate the priming effect with respect to plant nutrient supply.

CONCLUSIONS

The observed results show that the total amount of C translocated by lettuce into the soil is much lower than in the case of cereals and grasses. About 5–8% of the assimilated C was respired as CO_2 in the rhizosphere during 10 days after assimilation. This corresponded to a daily CO_2 efflux from the soil of 0.44–2.20 kg C ha^{-1} day^{-1}, depending on the growth stage and N fertilisation rate. Root-derived CO_2 represented about 15–60% of the total CO_2 efflux from the planted soil. Two peaks were measured in the $^{14}CO_2$ efflux: the first peak (4–5 h after labelling) was attributed to root respiration, whilst the second peak (12 h after labelling) was attributed to microbial respiration of exudates. Twelve days after labelling, 0.15–0.25% of the assimilated C was found in the microbial biomass. The higher microbial activity in the lettuce rhizosphere doubled the soil organic matter decomposition rate compared with unplanted soil. The lettuce plants provoked a real priming effect that reached 0.05–0.18 kg N ha^{-1} day^{-1} depending on the plant age, with a tendency to be enhanced by higher N fertilisation.

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REFERENCES

1 Klimanek EM, Umsetzungsverhalten von Ernterückständen. *Arch Acker- Pflanzenbau Bodenkunde* **34**:559–567 (1990).
2 Rochette P, Angers DA and Flanagan LB, Maize residue decomposition measurement using soil surface carbon dioxide fluxes and natural abundance of carbon-13. *Soil Sci Soc Am J* **63**:1385–1396 (1999).
3 Whitmore AP, Describing the mineralization of carbon added to soil in crop residues using second-order kinetics. *Soil Biol Biochem* **28**:1435–1442 (1996).
4 Kuzyakov Y, The role of amino acids and nucleic bases in turnover of nitrogen and carbon in soil humic fractions. *Eur J Soil Sci* **48**:121–130 (1997).
5 Cheng W, Coleman DC, Carrol CR and Hoffman CA, *In situ* measurement of root respiration and soluble C concentrations in the rhizosphere. *Soil Biol Biochem* **25**:1189–1196 (1993).
6 Kuzyakov YV, Tracer studies of below-ground carbon translocation from atmosphere by plants. Review. *Eurasian Soil Sci* **34**:28–42 (2001).
7 Helal HM and Sauerbeck D, Carbon Turnover in the Rhizosphere. *Z Pflanz Bodenkunde* **152**:211–216 (1989).
8 Swinnen J, Van Veen JA and Merckx R, ^{14}C pulse-labelling of field-grown spring wheat: an evaluation of its use in rhizosphere carbon budget estimations. *Soil Biol Biochem* **26**:161–170 (1994).
9 Swinnen J, Van Veen JA and Merckx R, Carbon fluxes in the rhizosphere of winter wheat and spring barley with conventional *versus* integrated farming. *Soil Biol Biochem* **27**:811–820 (1995).
10 Gregory PJ and Atwell BJ, The fate of carbon in pulse labelled crops of barley and wheat. *Plant Soil* **136**:205–213 (1991).
11 Cheng W, Measurement of rhizosphere respiration and organic matter decomposition using natural ^{13}C. *Plant Soil* **183**:263–268 (1996).
12 Merckx R, den Hartog A and Van Veen JA, Turnover of root-derived material and related microbial biomass formation in soils of different texture. *Soil Biol Biochem* **17**:565–569 (1985).
13 Kuzyakov Y, Ehrensberger H and Stahr K, Carbon partitioning and below-ground translocation by *Lolium perenne*. *Soil Biol Biochem* **33**:61–74 (2001).
14 Meharg AA and Killham K, Carbon distribution within the plant and rhizosphere in laboratory and field grown *Lolium perenne* at different stages of development. *Soil Biol Biochem* **22**:471–477 (1990).
15 Froehlich H, Die Bodendurchwurzelung seitens verschiedener Gemüsearten. *Arch Gartenbau* **4**:389–417 (1956).
16 Köhnlein J and Vetter H, *Ertragsrückstände und Wurzelbild: Menge und Nährstoffgehalt der auf dem Acker Verbleibenden Reste der Wichtigsten Kulturpflanzen*. Parey, Hamburg (1953).
17 Kuzyakov Y and Domanski G, Carbon input by plants into the soil. Review. *Z Pflanz Bodenkunde* **163**:421–431 (2000).
18 FAO-UNESCO, *Soil Map of the World*. UNESCO, Rome (1990).
19 Richter KH, Grundlagen umweltschonender Bodennutzungsstrategien im nordostdeutschen Tiefland. Abschluβbericht des interdisziplinären DFG-Projektes Ri 640. *Ökologische Hefte Landwirtschaftlich-Gärtnerischen Fak Humboldt-Univ Berlin* **11**:1–228 (1999).
20 Lorenz HP, Schlaghecken J, Engl G, Maync A and Ziegler J, *Ordnungsgemäße Stickstoffdüngung im Freiland-Gemüsebau nach dem 'Kulturbegleitenden N_{min} Sollwerte (KNS)-System'*. Ministerium für Landwirtschaft, Weinbau und Forsten, Rheinland-Pfalz, Mainz (1989).
21 Kuzyakov Y, Kretzschmar A and Stahr K, Contribution of *Lolium perenne* rhizodeposition to carbon turnover of pasture soil. *Plant Soil* **213**:127–136 (1999).
22 Vance E, Brookes PC and Jenkinson DS, An extraction method for measuring soil microbial biomass C. *Soil Biol Biochem* **19**:703–707 (1987).
23 Ross DJ, Estimation of soil microbial C by fumigation–extraction method: influence of seasons, soils and calibration with the fumigation–incubation procedure. *Soil Biol Biochem* **22**:295–300 (1990).
24 Black CA, *Methods of Soil Analysis. Part 2*. American Society of Agronomy, Madison, WI, pp 1562–1565 (1965).
25 Vogel G, *Handbuch des Speziellen Gemüsebaus*. Eugen Ulmer, Stuttgart (1996).
26 Saggar S, Hedley C and Mackay AD, Partitioning and translocation of photosynthetically fixed ^{14}C in grazed hill pastures. *Biol Fert Soils* **25**:152–158 (1997).
27 Kuzyakov Y and Cheng W, Controls of rhizosphere respiration and organic matter decomposition by photosynthesis and diurnal fluctuation. *Soil Biol Biochem* **33**:1915–1925 (2001).
28 Warembourg FR and Estelrich DH, Towards a better understanding of carbon flow in the rhizosphere: a time-dependent approach using carbon-14. *Biol Fert Soils* **30**:528–534 (2000).
29 Nguyen C, Todorovic C, Robin C, Christophe A and Guckert A, Continuous monitoring of rhizosphere respiration after labelling of plant shoots with $^{14}CO_2$. *Plant Soil* **212**:191–201 (1999).
30 Warembourg FR, Montagne D and Bardin R, The simultaneous use of $^{14}CO_2$ and $^{15}N_2$ labelling techniques to study the carbon and nitrogen economy of legumes grown under natural conditions. *Physiol Plant* **56**:46–55 (1982).
31 Johansson G, Below-ground carbon distribution in barley (*Hordeum vulgare* L) with and without nitrogen fertilization. *Plant Soil* **144**:93–99 (1992).
32 Powell CE and Ryle GJA, Effect of nitrogen deficiency on photosynthesis and the partitioning of ^{14}C-labelled leaf assimilate in unshaded and partially shaded plants of *Lolium tenulentum*. *Ann Appl Biol* **90**:241–248 (1978).
33 Lynch JM and Whipps JM, Substrate flow in the rhizoshere. *Plant Soil* **129**:1–10 (1990).

34 Ruehlmann J and Ruppel S, Kalkulation der Rhizo-C-Deposition in gemüsebaulich genutzten Böden eines Dauerversuches am Standort Großbeeren, in *Rhizodeposition und Stoffverwertung. 10. Borkheider Seminar zur Ökophysiologie des Wurzelraumes*, Ed by Merbach W, Wittenmayer L and Augustin J, Teubner, Stuttgart, pp 127–132 (2000).
35 Franko U, Modellierung des Umsatzes der organischen Bodensubstanz. *Arch Acker- Pflanzenbau Bodenkunde* **41**:527–547 (1997).
36 Müller T, The microbial activity as a function of the soil clay content. *Mitteilungen Deutschen Bodenkundlichen Gesellschaft* **85**:569–570 (1997).
37 Helal HM and Sauerbeck D, Short term determination of the actual respiration rate of intact plant roots, in *Plant Roots and Their Environment*, Ed by McMichal BL and Person H, Elsevier, Amsterdam, pp 88–92 (1991).
38 Swinnen J, Evaluation of the use of a model rhizodeposition technique to separate root and microbial respiration in soil. *Plant Soil* **165**:89–101 (1994).
39 Kuzyakov Y and Domanski G, Model for rhizodeposition and CO_2 efflux from planted soil and its validation by ^{14}C pulse labelling of ryegrass. *Plant Soil* **239**:87–102 (2002).
40 Warembourg FR and Billes G, Estimating carbon transfers in the plant rhizosphere, in *The Soil–Root Interface*, Ed by Harley JL and Russel RS, Academic Press, London, pp 183–196 (1979).
41 Merckx R, Dijkstra A, den Hartog A and Van Veen JA, Production of root derived material and associated microbial growth in soil at different nutrient levels. *Biol Fert Soils* **5**:126–132 (1987).
42 Liljeroth E, Van Veen JA and Miller HJ, Assimilate translocation to the rhizosphere of two wheat lines and subsequent utilization by rhizosphere microorganisms at two soil nitrogen concentrations. *Soil Biol Biochem* **22**:1015–1021 (1990).
43 Ladd JN, Amato M, Grace PR and Van Veen JA, Simulation of ^{14}C turnover through the microbial biomass in soils incubated with ^{14}C-labelled plant residues. *Soil Biol Biochem* **27**:777–783 (1995).
44 Kuzyakov Y, Friedel JK and Stahr K, Mechanisms and quantification of priming-effects. Review. *Soil Biol Biochem* **32**:1485–1498 (2000).
45 Bottner P, Cortez J and Sallih Z, Effect of living roots on carbon and nitrogen of the soil microbial biomass. *Br Ecol Soc Spec Publ* **10**:201–210 (1991).
46 Bleiholder H, Van den Boom T, Langelüddecke P and Strauss R, Einheitliche Codierung der phänologischen Stadien bei Kultur- und Schapflanzen. *Gesunde Pflanzen* **41**:381–382 (1989).
47 Feller C, Bleiholder H, Buhr L, Hack H, Heß M, Klose R, Meier U, Strauß R, Van der Boom T and Weber E, Phänologische Entwicklungsstadien von Gemüsepflanzen. I. Zwiebel-, Wurzel-, Knollen- und Blattgemüse. *Nachr Deutschen Pflanzenschutzdienstes* **47**:3–19 (1995).