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Model for rhizodeposition and CO_2 efflux from planted soil and its validation by ^{14}C pulse labelling of ryegrass

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Abstract

A model for rhizodeposition and root respiration was developed and parameterised based on ^{14}C pulse labelling of *Lolium perenne*. The plants were grown in a two-compartment chamber on a loamy Haplic Luvisol under controlled laboratory conditions. The dynamics of $^{14}CO_2$ efflux from the soil and ^{14}C content in shoots, roots, micro-organisms, dissolved organic carbon (DOC) and soil were measured during the first 11 days after labelling. Modelled parameters were estimated by fitting on measured ^{14}C dynamics in the different pools. The model and the measured ^{14}C dynamics in all pools corresponded well (r^2=0.977). The model describes well $^{14}CO_2$ efflux from the soil and ^{14}C dynamics in shoots, roots and soil, but predicts unsatisfactorily the ^{14}C content in micro-organisms and DOC. The model also allows for division of the total $^{14}CO_2$ efflux from the soil in $^{14}CO_2$ derived from root respiration and $^{14}CO_2$ derived from rhizomicrobial respiration by use of exudates and root residues. Root respiration and rhizomicrobial respiration amounted for 7.6% and 6.0% of total assimilated C, respectively, which accounts for 56% and 44% of root-derived $^{14}CO_2$ efflux from the soil planted with 43-day-old *Lolium perenne*, respectively. The sensitivity analysis has shown that root respiration rate affected the curve of $^{14}CO_2$ efflux from the soil mainly during the first day after labelling. The changes in the exudation rate influenced the $^{14}CO_2$ efflux later than first 24 h after labelling.

Introduction

During the last few decades, important progress has been made in the study of rhizosphere processes. The application of carbon isotope techniques has improved the understanding of carbon (C) partitioning in the rhizosphere. They have also provided more realistic means of quantifying the sources of CO_2 efflux from the soil, as compared to the methods based on the root-washing procedure or observations of root growth. Labelling of plants with carbon isotopes (especially ^{14}C) is widely used to quantify the extent of rhizodeposition as it allows for differentiation between root-derived organic C and native soil organic C (Buyanovsky et al., 1994; Kuzyakov and Domanski, 2000; Kuzyakov et al., 1999; Whipps, 1990).

In relation to investigations on CO_2 evolution from planted soil, three sources of soil-derived CO_2 can be established: (1) root respiration; (2) microbial respiration of root exudates, lysates, sloughed-off root cells, root hairs and dead roots (all are described as rhizodeposits), and (3) microbial respiration of soil organic matter (SOM) (Kuzyakov and Domanski, 2000). The separation and quantification of these sources contributing to CO_2 efflux from the soil remains the most important problem in calculations of C balance and C turnover in soils.

Nutrient solution cultures (Groleau–Renaud et al., 1998; Helal and Sauerbeck, 1991; Heulin et al., 1987; Hodge et al., 1996; Meharg and Killham, 1991), soil sterilisation (Barber and Martin, 1976, Martin, 1977;

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Merbach et al., 1990; Merbach and Ruppel, 1992) and fumigation techniques (Helal and Sauerbeck, 1991) connected with ^{14}C or ^{13}C labelling were used in the 1970s and 1980s for the separation of root respiration from microbial respiration. The results of these studies have shown that investigations based on artificial environments for the roots like hydroponic cultures or sterile soils give unreal figures for C partitioning. This makes them unsuitable for the prediction of C flows under natural conditions.

During the last few years, special efforts have been made to divide root-derived CO_2 into CO_2 from root respiration and from microbial respiration of root-borne substances during plant growth on non sterile soils. Beside pulse and continuous labelling with C isotopes, other techniques have been developed. The first method, called isotope dilution, is based on the addition of a solution of unlabelled glucose to the soil and simultaneous ^{14}C pulse labelling (Cheng et al., 1993, 1994, 1996). The method assumes that added glucose does not disturb any processes taking place in the rhizosphere and that the dilution of $^{14}CO_2$ coming up from the soil is in a simple and proportional relationship with the amounts of added glucose. The latter gives the possibility to calculate the ratio of root respiration–rhizomicrobial respiration. Using this method, Cheng et al. (1993) have shown that root respiration of 3-week-old wheat plants accounted for about 41% of the root-derived CO_2. Main shortcoming of the isotope dilution method is that the adequate ratio of root respiration–rhizomicrobial respiration can be registered only for a short time (about 4–5 h) after supplying the soil with glucose. After this time, micro-organisms begin to grow faster due to additional portion of available C, they produce more CO_2 from up taken glucose and the ratio is no longer adequate.

Another approach involves the differences in ^{13}C discrimination ($\delta^{13}C$) by C3 and C4 plants (Cheng, 1996; Qian et al., 1997; Rochette andFlanagan, 1998). Cultivation of C3 plants on soil previously grown with C4 plants (Cheng, 1996) or vice versa (Qian et al., 1997; Rochette andFlanagan, 1998) and monitoring the $\delta^{13}C$ values of CO_2 coming from the soil allows separation of the humus-derived CO_2 from root-derived CO_2. This modified method can be easily used under field conditions (Rochette and Flanagan, 1998). In addition, it allows the estimation of the changes in decomposition rate of soil organic matter in presence of roots.

Swinnen (1994) has developed a method based on the addition of ^{14}C-labelled model rhizodeposits to soil. This approach assumes that the $^{14}CO_2$ efflux from soil with added model rhizodeposits corresponds to rhizomicrobial respiration in the soil with applied above-ground labelling of plants and is compared with the $^{14}CO_2$ efflux from the soil with pulse labelled plants (Swinnen, 1994). The difference between the variants is an estimate of the root respiration. According to this method, the contribution of root respiration of 30-day-old wheat and barley to the total root-derived CO_2 was between 89% and 95%. These results seem to be too high considering the high concentration of exudates in the rhizosphere (Cheng et al., 1993) and oppose many other investigations about partitioning of $^{14}CO_2$ efflux (Cheng et al., 1993; Helal and Sauerbeck, 1989; Kuzyakov et al., 1999).

The next method used for separation of root respiration from rhizomicrobial respiration is based on the kinetics of $^{14}CO_2$ efflux from soil after pulse labelling (Kuzyakov et al., 1999, 2001a; Swinnen et al., 1994a,b). This method assumes that $^{14}CO_2$ generated by roots respiration appears earlier than $^{14}CO_2$ derived from micro-organisms using the rhizodeposits. This delay is due to the time necessary for the synthesis of exudates, for the exudation and secretion processes and for the uptake and utilisation of rhizodeposits by micro-organisms (Warembourg and Billes, 1979). The kinetics method has shown that root respiration and rhizomicrobial respiration of growing *Lolium perenne* amounted to 41% and 59% of root-derived CO_2 efflux from the soil, respectively (Kuzyakov et al., 1999). The contribution of root respiration varied from 17% to 61% of total CO_2 efflux from the soil depending on *Lolium* age. These results agree well with those of Cheng et al. (1993), although these two approaches are based on different assumptions (Cheng et al., 1993; Kuzyakov et al., 1999, 2001a).

In previous publications (Kuzyakov et al., 1999, 2001a), we used a simple simulation model of below-ground C flows and root-derived CO_2 efflux from the soil. Most of the parameters of C flows between the C pools were taken from literature. Only the parameters responsible for root respiration and exudation were fitted according to our experimental results. Meanwhile, the experiment with continuous monitoring of the $^{14}CO_2$ activity after pulse labelling of maize (Nguyen et al., 1999) and of lettuce (Kuzyakov et al., 2002 unpublished) confirms the hypothesis of the time delay in the appearance of $^{14}CO_2$ respired by micro-organisms in comparison to $^{14}CO_2$ from root respiration and showed two maxima of the $^{14}CO_2$ efflux intensity. These and our unpublished results on

kinetics of $^{14}CO_2$ efflux after pulse labelling have led us to a further development of the model and to its parameterisation. This work aims at developing and verifying the model of rhizodeposition and root-derived CO_2 efflux from the soil. In addition, a special experiment was designed to measure the dynamics of ^{14}C in each pool described by the model. This allows the estimation of the model parameters by fitting the measured ^{14}C dynamics in each pool of the plant–soil–CO_2–system.

Materials and methods

Soil

The soil, a loamy Haplic Luvisol (FAO-UNESCO, 1990), was taken from the top 10 cm (Ap horizon) of a long term field experimental station Karlshof of the University of Hohenheim (5 km to the South of Stuttgart). Wet soil was passed through a 5-mm sieve to remove the stones and big roots, air dried and mixed. The electrostatic method was used to remove small roots and plant remains (Kuzyakov et al., 2001b). The soil contains no $CaCO_3$ and has the following characteristics: pH 6.0, C_t 1.2%, N_t 0.13%, clay 20%, silt 8%. The bottom part of each pot was filled with 1.55 kg of air dried soil to the final density of 1.06 g cm^{-3}.

Growing conditions

Seeds of ryegrass (*Lolium perenne* L., spec. Gremie) were germinated on moist paper for 5 days. Nine non-vernalized seedlings of *Lolium* were grown in each container. The distance between every two plants was about 3.5 cm. The plants were grown at 27±1 °C day and 22±1 °C night temperatures with a day-length of 14 h and 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 60% of the available field capacity (24% of d.w.). Plants were not supplied with any fertilisers.

Chamber and labelling

For ^{14}C labelling, we used the two compartment Plexiglas chambers described in detail previously in Kuzyakov et al. (1999, 2001a). Briefly, the airtight chambers used for labelling are divided into two compartments by a perforated lid to allow plant growth. The holes with plants were sealed with Silicone rubber NG 3170 (Fa. Thauer & Co., Dresden) one day before labelling. The sealing was checked to ensure that it was airtight. 450 kBq of ^{14}C as $Na_2{}^{14}CO_3$ solution was put in a 2 cm^3 Eppendorf micro test tube placed in the upper compartment of the chamber. The chamber was then closed and 1 cm^3 of 5 N H_2SO_4 was added to the $Na_2{}^{14}CO_3$ solution in the micro test tube through a pipe on the upper chamber part. The efficiency of $^{14}CO_2$ evolution varied between 70% and 95% and was considered in the calculation of assimilated ^{14}C amounts.

The labelling of the all plants took place at the beginning of tillering (plant heights 15 cm, 43 days after sowing). This stage has been chosen because most plants reveal the maximal intensity of root growth and rhizodeposition during tillering (Kuzyakov et al., 1999; Swinnen et al., 1994a). Each chamber was labelled separately. Separate labelling of plants in each container and long labelling periods decrease the natural differences in the intensity of ^{14}C assimilation by plants between the chambers and keep the variability between the replications small. Plants were allowed to assimilate $^{14}CO_2$ for 6 h. Thereafter, flushing of the upper compartment started to remove the remaining unassimilated $^{14}CO_2$ and to trap it in NaOH. Then the upper part of the chamber was opened. Unassimilated $^{14}CO_2$ trapped in NaOH and the ^{14}C activity in the Eppendorf micro test tube were subtracted from the input of ^{14}C activity. The result was assumed to be the total amount of assimilated ^{14}C. Hence, all pools are presented in per cent of total assimilated ^{14}C, and all flows are presented in per cent of total assimilated ^{14}C per hour.

During the experiment, CO_2 evolving from the compartments containing soil and roots was trapped in 20 cm^3 of 0.5 N NaOH solution by continuously pumping (100 cm^3 min^{-1}) with a membrane pump. Air cycling through the bottom compartments was started immediately after labelling. NaOH solutions were changed every hour immediately after labelling. This period was lengthened during incubation and the NaOH solution was changed four times during the last two days. Frequent changes of the NaOH solution were necessary to achieve increased time-resolution of $^{14}CO_2$ evolution, especially during first two days after the labelling.

To compare total unlabelled CO_2 evolution from the soil with and without *Lolium perenne*, the soil was also incubated in the same pots and under the same conditions. Containers used as controls were covered with lids without holes and did not receive $^{14}CO_2$ for

labelling. The CO_2 evolution from the soil of these containers was measured in the same way and steps as in containers with plants.

Sample analysis

The plants were destructively harvested 6, 12, 24, 48, 96, 192 and 264 h after the labelling. At harvest, each plant was cut at the base, the lid of the bottom compartment was opened and each root–soil column pulled out. Roots were carefully removed from the soil by handpicking. The soil adhering to the roots was accepted as rhizosphere soil and was removed by washing with deionized water. Shoots, washed roots and soil were dried at 60 °C.

Microbial biomass C was determined by a fumigation-extraction method (Ross, 1990; Vance et al., 1987). 5 g of fumigated and non-fumigated soil samples (two replicates from each sample) were extracted with 15 cm^3 0.5 M K_2SO_4 for 30 min and filtered through ashless filter paper '589^3 Blue ribbon' (Schleicher & Schuell GmbH, Germany). An aliquot of K_2SO_4 solution was analysed for organic C with Dimatoc-100 and for ^{14}C activity 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):

$$\text{Microbial C} = \text{C flush} * 2.34.$$

The ^{14}C activity in the K_2SO_4 extract without soil fumigation and ^{14}C activity found in water remaining after root washing were accepted as ^{14}C in DOC and were used for parameter fitting.

Dry samples of shoots, roots and soil were ground in a ball mill (Retsch, Germany) prior to analysis for total carbon and ^{14}C content. Total C content in soil, shoots and roots was measured with a C-analysator (Carlo Erba) using 0.1 g of soil samples and 0.02 g of plant material samples. Radioactivity of shoots, roots and soil samples was measured with the scintillation cocktail Permafluor E^+ (Canberra Packard) by a Liquid Scintillation Counter Tri-Carb 2000CA (Canberra Packard) after combustion of 1 g of soil sample or 0.2 g of plant sample within an oxidiser unit (Canberra Packard, Model 307).

Total content of C–CO_2 collected in NaOH solution was measured by titration with 0.2 N HCl against phenolphthalein after addition of 0.5 N $BaCl_2$ solution (Black, 1965). ^{14}C in CO_2 collected in the NaOH solution and ^{14}C in water obtained from root washing was measured on 1 cm^3 aliquots added to 10 cm^3 scintillation cocktail Rothiscint-22x (Roth Company, Germany) after the decay of chemiluminiscence (for NaOH). The ^{14}C counting efficiency was about 89% and the ^{14}C-activity measurement error did not exceed 2%. The absolute ^{14}C-activity was standardised by addition of NaOH solution as quencher to the scintillation cocktail and using a two-channel ratio method of extended standard (tSIE).

$^{14}CO_2$ effluxes from the soil were measured with three replications for each harvesting time. Because of the 7 harvesting periods, the first points of $^{14}CO_2$ monitoring (6 h after labelling) are measured with 21 replications and the last (264 h) with three. ^{14}C measurements of shoots, roots and soils were conducted with three replications. ^{14}C measurements in soil micro-organisms were conducted with six replications.

Assumptions

The model is a simplification of C translocation and partitioning within the plants. We assumed that the plant biomass did not significantly change during the whole period of $^{14}CO_2$ monitoring until the end of C allocation. Influence of plant growth (reverse transport of ^{14}C-labeled compounds from the roots to the shoots) on partitioning processes was not included in the model. The model does not consider the diurnal changes in assimilation, translocation and respiration activity. All ^{14}C flows in the model were described by first-order kinetics (Van Veen et al., 1985). We rejected the use of other kinetics types used in some other models (Darrah, 1996) because they did not significantly change the C flows, although they need additional parameters, which makes experimental estimation difficult. At time t=0 (immediately after $^{14}CO_2$ generation) the whole labelled C is located in the atmosphere and is unassimilated. All C flows are described in per cent of total assimilated ^{14}C. Since one simulation step is equal to 1 h, all rate parameters are given in h^{-1}.

The model

The model was developed to describe the processes of below-ground C partitioning, especially for the separation of CO_2 evolved by root respiration and rhizomicrobial respiration after the $^{14}CO_2$ pulse labelling of plants. The model consists of twelve C pools (Fig. 1):

CO_2 – $^{14}CO_2$ that was introduced into the upper chamber part at the beginning of the labelling; it is equal to 100% of ^{14}C activity at $t = 0$;

Glucose – ^{14}C photosynthetically assimilated in leaves;

GluShot and GluRoot – ^{14}C distributed between the shoot and the below-ground components after assimilation (labile pool);

Shoot and Root – ^{14}C incorporated in the shoot and root tissues after partitioning (stable pool);

ShoCO_2 – $^{14}CO_2$ evolved by shoot respiration;

Exudate – ^{14}C in easily available organic substances released by roots and micro-organisms into the soil;

Biomass – ^{14}C incorporated in soil micro-organisms by utilisation of exudates and root residues;

Humus – ^{14}C incorporated in soil organic matter as a result of humification of microbial biomass, exudates and root residues;

RootCO_2 – $^{14}CO_2$ evolved by root respiration;

BioCO_2 – $^{14}CO_2$ evolved by rhizomicrobial respiration.

The pools described above can be combined in main compartments (Figure 1):

Atmosphere encompasses all the CO_2 sources and includes the subcompartment **rhizosphere respiration** (here it is equal to root-derived CO_2), which comprises of CO_2 derived from root respiration and CO_2 derived from rhizomicrobial respiration. In our model there is no exchange between root-derived CO_2 and the atmosphere, because in the experiment, these compartments were separated from each other (see 'Chamber and labelling').

Shoot gathers photoassimilates (*Glucose*), GluShoot and the shoot tissue.

Root is made up of GluRoot and root tissue.

Soil includes exudates, microbial biomass and humus substances.

Briefly, the C flows in the model occur as follows: $^{14}CO_2$ injected into the atmosphere is assimilated photosynthetically by plants and tracer amount in the atmosphere diminished:

$$\frac{d\mathrm{CO_2}}{dt} = -\mathrm{ar} \cdot \mathrm{CO_2}, \tag{1}$$

where *ar* is assimilation rate and CO_2 is the amount of carbon dioxide in the atmosphere. The meanings of the symbols used in this and following equations are also presented in Figure 1 and summarised in Table 1. The assimilation rate (ar) is very high (Anten, 1995) so that after 2 h, the most part of offered CO_2 is converted to glucose. Glucose is partitioned between the shoot and all below-ground components:

$$\frac{d\mathrm{Glucose}}{dt} = \mathrm{ar} \cdot \mathrm{CO_2} - s \cdot \mathrm{Glucose} - (1 - s) \cdot \mathrm{Glucose}, \tag{2}$$

where s is the partitioning factor between shoots and below-ground components. The relation (s) between shoot and all below-ground compartments corresponds to the sum of ^{14}C amounts experimentally found in the upper and bottom container pools after the ^{14}C partitioning was completed.

The assimilates remaining in the leaves are rapidly used for shoot respiration, for shoot growth and for slow long-term shoot respiration:

$$\frac{d\mathrm{GluShoot}}{dt} = s \cdot \mathrm{Glucose} - \mathrm{sg} \cdot \mathrm{GluShoot} - \mathrm{sr} \cdot \mathrm{GluShoot}, \tag{3}$$

where sg is the shoot growth rate, sr is the rapid shoot respiration.

Assimilates translocated to below-ground are used for root respiration, exudation and root growth:

$$\frac{d\mathrm{GluRoot}}{dt} = (1 - s) \cdot \mathrm{Glucose} - \mathrm{rr} \cdot \mathrm{GluRoot} - \mathrm{rg} \cdot \mathrm{GluRoot} - \mathrm{exu} \cdot \mathrm{GluRoot}, \tag{4}$$

where rr is the root respiration, rg is the root growth, and exu is the exudation.

Root respiration contributes directly to $^{14}CO_2$ efflux from the soil, while exudates and roots are used by rhizosphere micro-organisms (*Biomass*) at different rates. One part of C used by micro-organisms is respired and makes up the $^{14}CO_2$ efflux from the soil. Another part of C incorporated in micro-organisms can be secreted as exoenzymes or other organic substances and flows in the exudate pool:

$$\frac{d\mathrm{Biomass}}{dt} = \mathrm{rm} \cdot \mathrm{Root} + \mathrm{em} \cdot \mathrm{Exudate} + \mathrm{hm} \cdot \mathrm{Humus} - \mathrm{br} \cdot \mathrm{Biomass} - \mathrm{bh} \cdot \mathrm{Biomass} - \mathrm{be} \cdot \mathrm{Biomass} \tag{5}$$

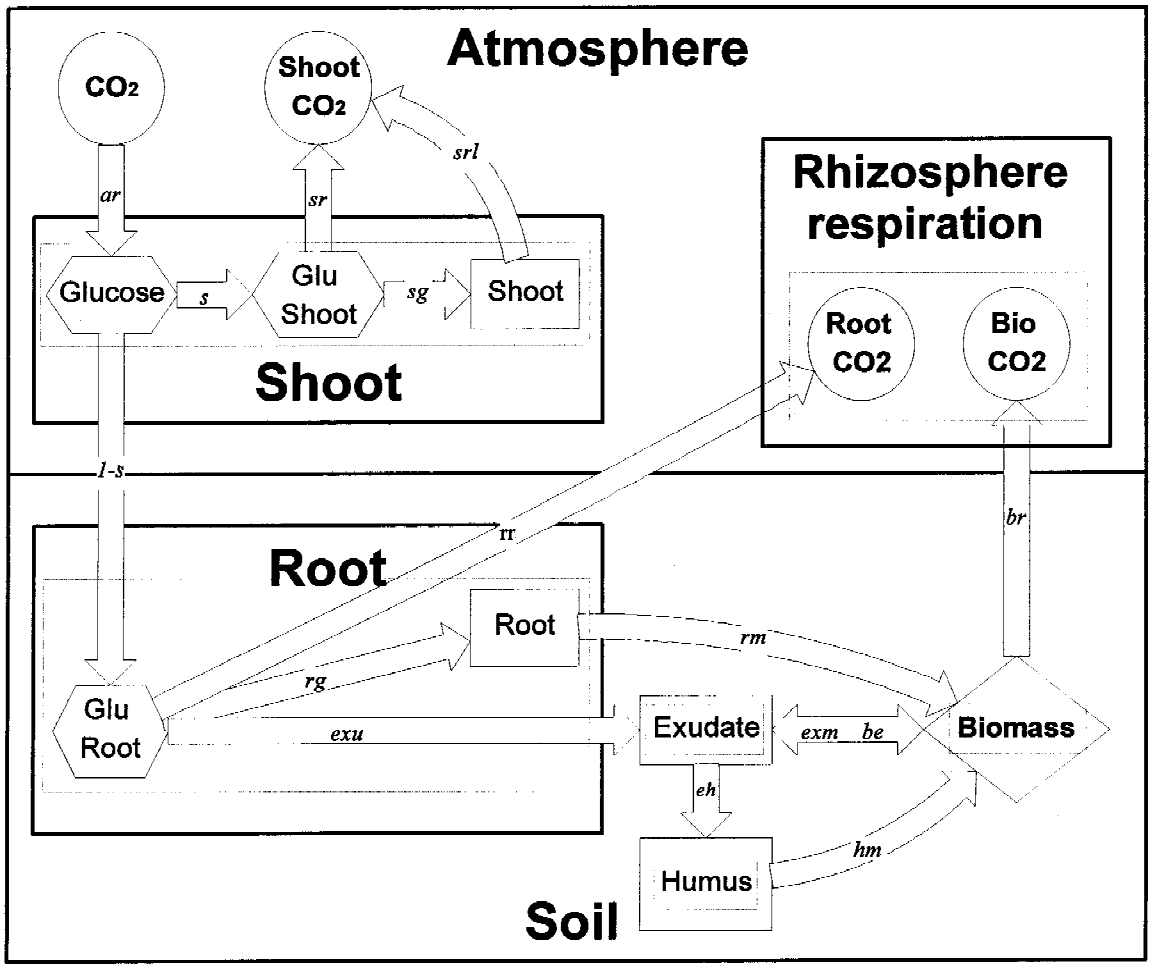

Figure 1. Schematic representation of the C pools and C flows in the rhizodeposition model.

where rm is the root mineralisation, em is the exudate mineralisation, hm is the humus mineralisation, br is the biomass respiration, bh is the biomass humification, be is the biomass exudation.

A small part of exudates can be chemically incorporated in humus substances (Bondietti et al., 1972; Dashman and Stotzky, 1982; Kuzyakov and Galitza, 1993; Müller–Wegener, 1982):

$$\frac{d\text{Humus}}{dt} = \text{bh} \cdot \text{Biomass} + \text{eh} \cdot \text{Exudate} - \text{hm} \cdot \text{Humus}, \quad (6)$$

where eh is the exudate humification.

$$\frac{d\text{Humus}}{dt} = \text{bh} \cdot \text{Biomass} \quad (7)$$

The end product of ^{14}C transformations within a plant–soil–atmosphere system is CO_2. Therefore, the $^{14}CO_2$ efflux from the soil (root-derived CO_2, RCO_2) represents two main sources: root respiration and rhizomicrobial respiration:

$$\frac{d\text{RCO}_2}{dt} = \text{br} \cdot \text{Biomass} + \text{rr} \cdot \text{GluRoot} \quad (8)$$

Parameterisation

In the previous version of the model (Kuzyakov et al., 1999), most parameters were taken from literature. The rate constants used for calculations in this version of the model were fitted according to the ^{14}C dynamics in all pools measured in one experiment. We used the following general approaches for the parameterisation

Table 1. Modelled ^{14}C flows (parameters) and their values obtained from the simulation of experimental data

Parameter	Value[a]	Pools for fitting (according to Fig. 1)
Assimilation rate (ar)	0.59	CO_2, Root, Shoot
Ratio: shoot/root (s)	0.51	Root, Shoot
Shoot growth rate (sg)	0.045	Shoot, GluShoot
Short-term shoot respiration (sr)	0.04	Shoot, GluShoot 0–48 hours
Long-term shoot respiration (srl)	0.001	Shoot, GluShoot after 48 hours
Exudation rate (exu)	0.016	DOC, CO_2 efflux, Biomass
Exudate mineralisation (em)	0.06	DOC, Biomass, CO_2 efflux after 48 hours
Exudate humification (eh)	0.04	DOC, Biomass, Humus
Root growth (rm)	0.039	Root
Root respiration (rr)	0.01	Root, CO_2 efflux 0–48 hours
Root mineralisation (rm)	0.0012	Root, CO_2 efflux after 5 days
Biomass respiration (br)	0.012	Biomass, DOC, CO_2 efflux
Biomass humification (bh)	0.016	Biomass, Humus
Biomass exudation (be)	0.0035	Biomass, DOC
Humus mineralisation (hm)	0.0004	Biomass, Humus, CO_2 efflux after 5 days

[a]All parameters are given in hour^{-1}, except *s*.

of the model. The relationship between the rates of two or more C flows originating from one source corresponds to the relationship between the measured ^{14}C totals passing through each flow. The rate constant of each flow was fitted according to the ^{14}C dynamics in the pools. At first, we fitted each parameter separately to achieve best accordance between the measured and fitted dynamics of ^{14}C in the responsible pool. Later, the fitting occurred in parameter pairs by minimising the least squares using the Marquardt method. Table 1 presents the parameters and their values after the best harmony with experimental values used in the model. The pools used for the fitting of the model parameters are also presented.

Results and discussion

^{14}C *in shoot*

The first destructive sampling of labelled plants took place after 6 h at which time the maximum ^{14}C content in the shoots was measured. It reached 40% of total assimilated ^{14}C (Figure 2). Here it is important to note that the ^{14}C measured in the shoots at each sampling includes the ^{14}C used later for shoot tissue and for shoot respiration. Part of the ^{14}C measured in the shoots will be translocated below-ground too (Keutgen et al., 1995; Swinnen et al., 1994a). The ^{14}C content in the shoots dropped down after the maximum in two steps. The first step showed a rapid decrease in ^{14}C content (Figure 2). It was connected with an intensive short-term utilisation of the new assimilates for energy and with the export of assimilates from source leaves to the roots. This occurred during the first two days after the labelling. The second phase of ^{14}C decrease was much slower than the first one. It was mainly connected with maintenance respiration of ^{14}C incorporated into the tissues. Kuzyakov et al. (2001a) investigated the dynamics of shoot respiration and have shown that $^{14}CO_2$ losses from shoot respiration were at a maximum during the first 24 h after the labelling. They reached 17% of the total assimilated ^{14}C. Later, the losses of fixed ^{14}C were much smaller. In the current study, shoot respiration was not measured because of two reasons. Firstly, long-term covering of plants for collection of $^{14}CO_2$ respired by shoots increases the temperature and humidity in the chamber. This can lead to a disturbance of processes such as photosynthesis, respiration and upward transport of water with nutrients and assimilates, thus changing the C allocation. Secondly, additional equipment is required to maintain constant input of unlabelled CO_2 to the upper chamber necessary for photosynthesis during the whole measurement period.

The model parameters responsible for rapid and slow shoot respiration and shoot growth rate were fitted on the measured ^{14}C content in the shoots. The simulated ^{14}C dynamics in the shoots correspond well

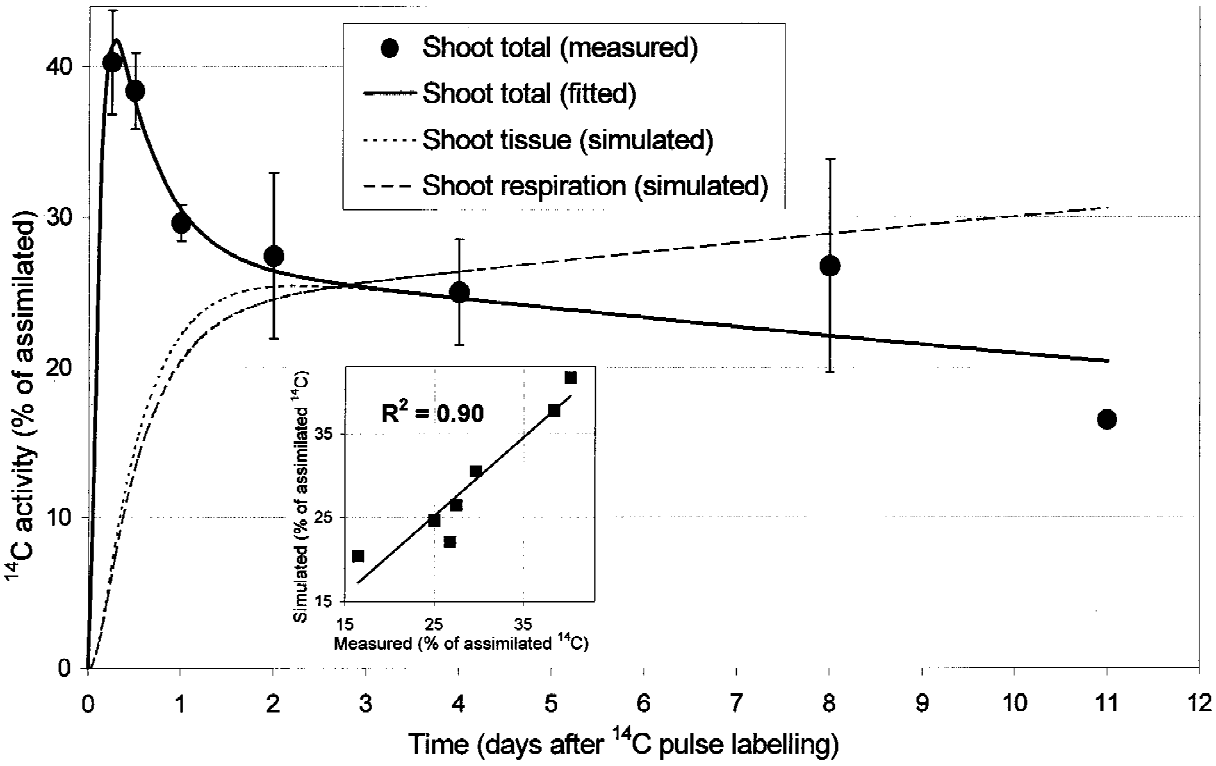

Figure 2. Measured (points, means ± SD) and fitted (solid line) ^{14}C dynamics in the shoots of *Lolium perenne*. The dotted line represents the simulated ^{14}C dynamics in the shoot tissue. The dashed line represents the simulated ^{14}C dark respiration of shoots.

to the measured ones (Figure 2, inset, R^2=0.90). The model allows simulation of the ^{14}C dynamics in both shoot pools: in shoot tissue and in assimilates utilised by rapid shoot respiration to produce $^{14}CO_2$ (Figure 2). Separate measurements of ^{14}C in these shoot compartments were too intensive for this study. The $^{14}CO_2$ amount respired during rapid shoot respiration corresponds to the difference between the upper curve (total ^{14}C in the shoots) and the bottom dotted curve (simulated amount of ^{14}C incorporated in the shoot tissue). The simulated amount of $^{14}CO_2$ respired by shoots is equal to 30% of total assimilated ^{14}C in 11 days after the labelling. The last value corresponds well to the shoot respiration of 76-days-old *Lolium perenne* measured earlier (Kuzyakov et al., 2001a).

^{14}C in roots

In general, the ^{14}C dynamics in roots (Figure 3) were similar to the ^{14}C dynamics in shoots. They can be described with the same three steps. Firstly, a rapid increase in the ^{14}C content in the roots occurred where the maximum has been reached (42.5%). Then a rapid decline (until 2nd day) in the ^{14}C content was observed as a result of root respiration and root exudation. The last phase, later than 2nd day, showed a slow decrease in the ^{14}C content, connected with exudation and decomposition of root hairs.

In contrast to the shoots, the maximum of ^{14}C content in the roots was reached at the second sampling time (12 h), that is 6 h later than in the shoots. This delay can be explained by the time that is necessary for the transportation of assimilates to the roots. In many studies, the first appearance of labelled $^{14}CO_2$ from the soil was measured soon after the labelling (Cheng et al., 1993; Gregory and Atwell, 1991; Kuzyakov et al., 1999, 2001a; Nguyen et al., 1999; Swinnen et al., 1994a; Warembourg et al., 1982).

Unlike the shoots, the ^{14}C-labelled assimilates transported to the roots are not only used for respiration and for the build up of root tissues, but they are also exuded as organic substances into surrounding soil. Because of the complexity of the soil and the strict connections between roots and the surrounding soil, it is much more difficult to follow the decreases in ^{14}C content caused by root respiration and exudation

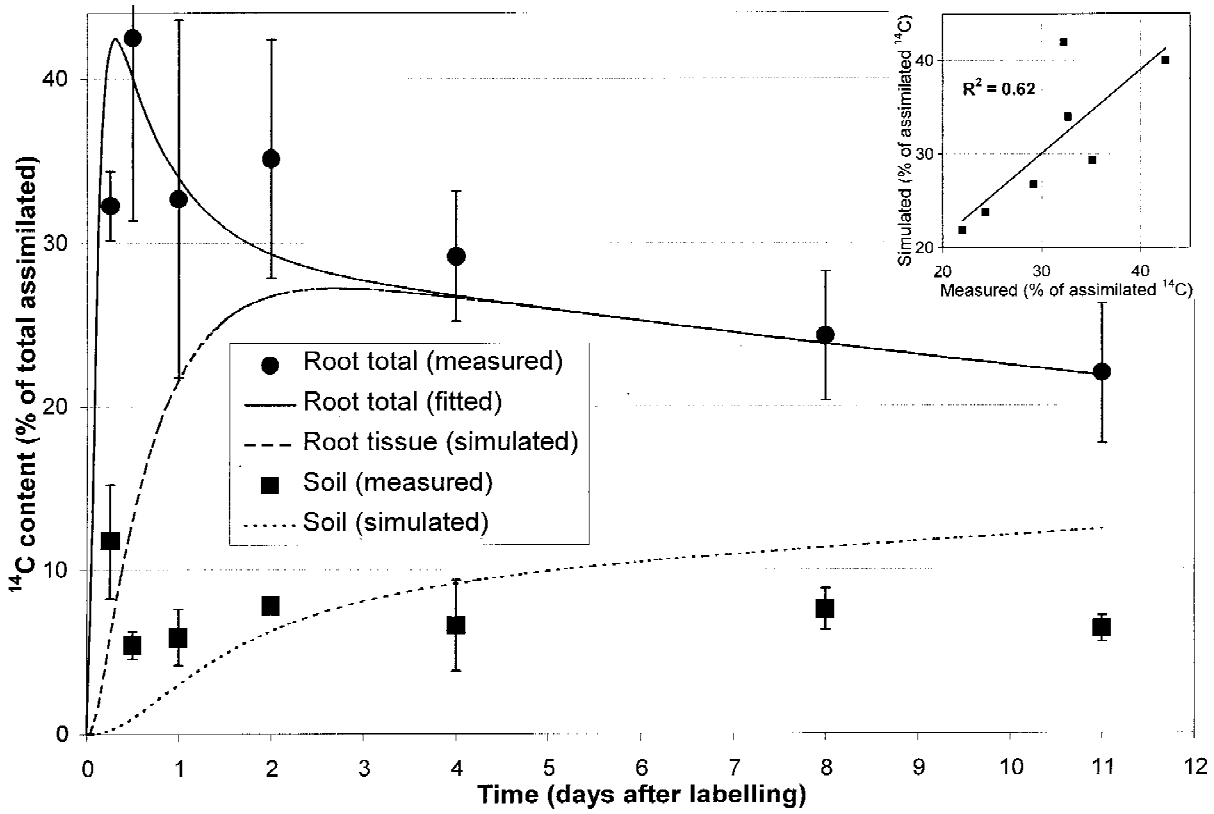

Figure 3. Measured (points, means ± SD) and fitted (solid line) ^{14}C dynamics in the roots of *Lolium perenne* and in the soil. The dotted line represents the simulated ^{14}C dynamics in the root tissue.

and separate them from each other, than it was possible to do for ^{14}C dynamics in the shoots.

The simulated ^{14}C dynamics in the roots describe well the measured data (r^2=0.62). The fitting of the parameters occurred as follows: The first rapid decline of the ^{14}C was fitted by changing the rates of root respiration. On the second day after labelling, the parameters responsible for exudation and biomass respiration were changed to achieve the best harmony between simulated and measured data. After 4–5 days, the most parts of labelled exudates were mineralised and the microbial decomposition of roots was responsible for the decline in ^{14}C content in the roots (Figure 3). The simulated incorporation of photoassimilates in the root tissue (Figure 3, dotted line) has nearly the same dynamics as for the shoots (compare Figures 2 and 3). As shown by the shoot/root ^{14}C partitioning ratio (parameter *s*), about 48% of assimilated C were translocated below-ground. This value is slightly higher than is usually reported by other authors for both cereals and grasses, including studies conducted on *Lolium perenne* (Kuzyakov et al., 1999; Meharg and Killham, 1990; Palta and Gregory, 1998; Swinnen, 1994; Swinnen et al., 1994a; Whipps, 1990). About one half of below-ground translocated C was lost from the roots during 11 days after labelling, due to root respiration, exudation and root decomposition. About one third of below-ground allocated C was respired to CO_2. The remainder, about one sixth, remained in the soil as incorporated C in soil organic matter or adsorbed C on clay minerals.

^{14}C dynamics in soil

The ^{14}C content in the soil after the labelling of plants represents the C amount transferred into soil and stored in it for a longer time period. It means that the exudates and root residues will be humified and transformed to some fractions of soil organic matter (SOM). However, the investigations on the SOM turnover are very limited in experiments of short duration. Because our study took only 11 days, we were not able to chase both incorporation of ^{14}C released

from roots into SOM and microbial mobilisation of ^{14}C from SOM. The measured ^{14}C content in the soil was about 11% of assimilated C at the beginning and 8% on the 11th day (Figure 3). But the simulated ^{14}C content in the soil increased after the ^{14}C pulse.

Two main causes can be responsible for the contrast in the measured and modelled ^{14}C content in the soil. The first one can have a technical background. Shortly after the labelling, most of the ^{14}C would be transferred to the root hairs and fine roots (Lacointe et al., 1995). These can be easily broken when removing the roots from the soil as well as by root washing. They cannot be separated from the soil and contribute greatly to the measured ^{14}C soil pool. The second reason is connected with the dynamics of incorporation of low molecular organic substances into the SOM and clay minerals. Fast incorporation of ^{14}C from low molecular organic substances into the SOM and clay minerals was shown in some previous studies (Bondietti et al., 1972; Dashman and Stotzky, 1982; Kuzyakov and Galitsa, 1993; Müller–Wegener, 1982) and the incorporation of 3–30% of low molecular organic substances into SOM can precede the microbiological decomposition. The simulation of ^{14}C incorporation into SOM in our model does not include such a fast step that could be a reason for observed disagreement between measured and simulated data.

$^{14}CO_2$ efflux from soil

The total amount of ^{14}C in CO_2 efflux from the soil was 13.6% of assimilated C during the 11 days after the labelling. It was about one third of the below-ground translocated C. These results correspond well to other studies on cereals (Cheng et al., 1993; Merbach et al., 1990; Swinnen 1994; Swinnen et al., 1994a, 1995a) and perennial grasses (Kuzyakov et al., 1999, 2001a; Meharg and Killham, 1990; Warembourg et al., 1990; Zagal, 1994; reviewed by Kuzyakov and Domanski, 2000).

Two curves were used for the estimation of model parameters responsible for the CO_2 efflux. The curve of the cumulative $^{14}CO_2$ efflux from the soil (Figure 4, top) was used to adjust parameters responsible for the total amount of respired $^{14}CO_2$. These parameters describe the relationship between the amounts of ^{14}C incorporated in the root tissue and ^{14}C respired by roots and rhizosphere micro-organisms using the exudates. The curve for the intensity of $^{14}CO_2$ efflux (Figure 4, bottom) was used to adjust the parameters responsible for the dynamics of root respiration rate, exudation rate, root decomposition rate and biomass respiration rate. Using only one curve to estimate several parameters was possible because different parts of the curve are responsible for the different parameters (see below for detailed description). The fitted curves describe well both the cumulative $^{14}CO_2$ efflux from the soil and its intensity (R^2= 0.998 and R^2 = 0.94, respectively).

^{14}C in micro-organisms and exudates

The C incorporation in micro-organisms from root-borne organic substances has seldom been measured in the investigations of C translocation and partitioning by plants. Although the biggest part of below-ground translocated C passes through soil microbial biomass and is used by it, only few works have been devoted to investigating the allocation of root-derived C in micro-organisms (Nicolardot et al., 1994; Qian et al., 1997; Saggar et al., 1994; Van Ginkel et al., 1997) and we have found only one paper on the dynamics of ^{14}C in soil microbial biomass after pulse labelling of plants (Rattray et al., 1995).

We have measured the ^{14}C content in soil micro-organisms 7 times after the ^{14}C pulse labelling of plants (Figure 5). It enabled us to get more information on the utilisation of rhizodeposits by rhizosphere micro-organisms. Two maxima of the ^{14}C content in soil micro-organisms were measured. The first one appeared at the first sampling time and the second was detected 48 h after the labelling. Only the second ^{14}C maximum can be simulated using the chosen model approach (Figure 5). The model was not able to describe the dynamics of ^{14}C in micro-organisms that occurred during the first 12 h. There are two reasons for the appearance of the first maximum. The plants release the exudates with low molecular weight very soon after the assimilation and they need a longer time to synthesise the high molecular substances like polysaccharides, etc., coming out in the second maximum. This approach was suggested earlier by Warembourg and Billes (1979) for $^{14}CO_2$ efflux from the soil. The second reason can have a technical background: Six hours after the labelling, an important part of ^{14}C activity is located in root hairs and fine roots. It was not possible to remove them completely from the soil during the preparation of soil samples for fumigation. Therefore, a substantial part of fine roots is destroyed by fumigation and organic substances containing ^{14}C come out into the K_2SO_4 extract.

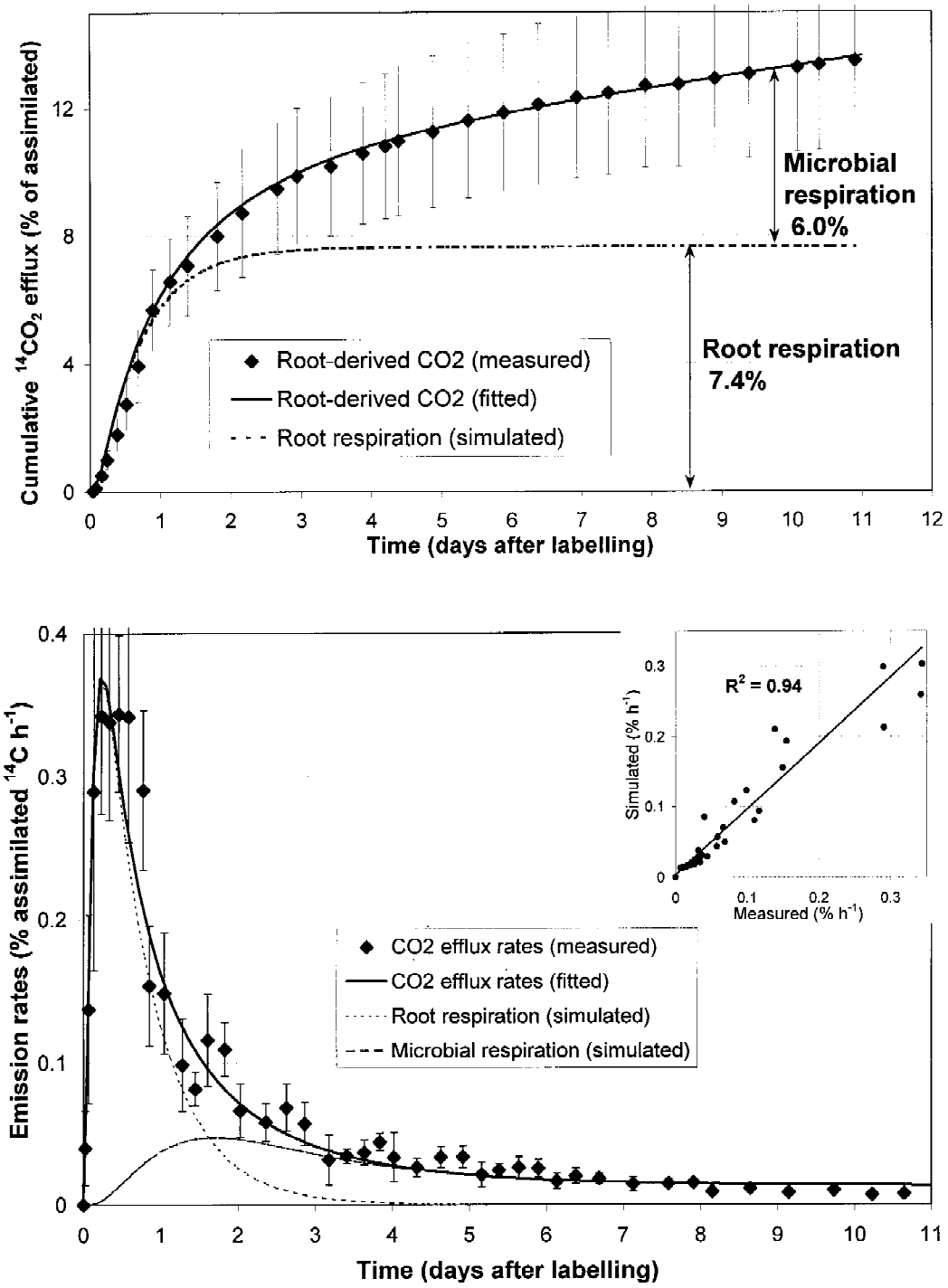

Figure 4. Measured (points, means ± SD) and fitted (solid line) $^{14}CO_2$ efflux from the soil (top: cumulative $^{14}CO_2$ efflux, bottom: $^{14}CO_2$ efflux dynamics), and simulated separation of total $^{14}CO_2$ efflux in root respiration and microbial respiration of exudates.

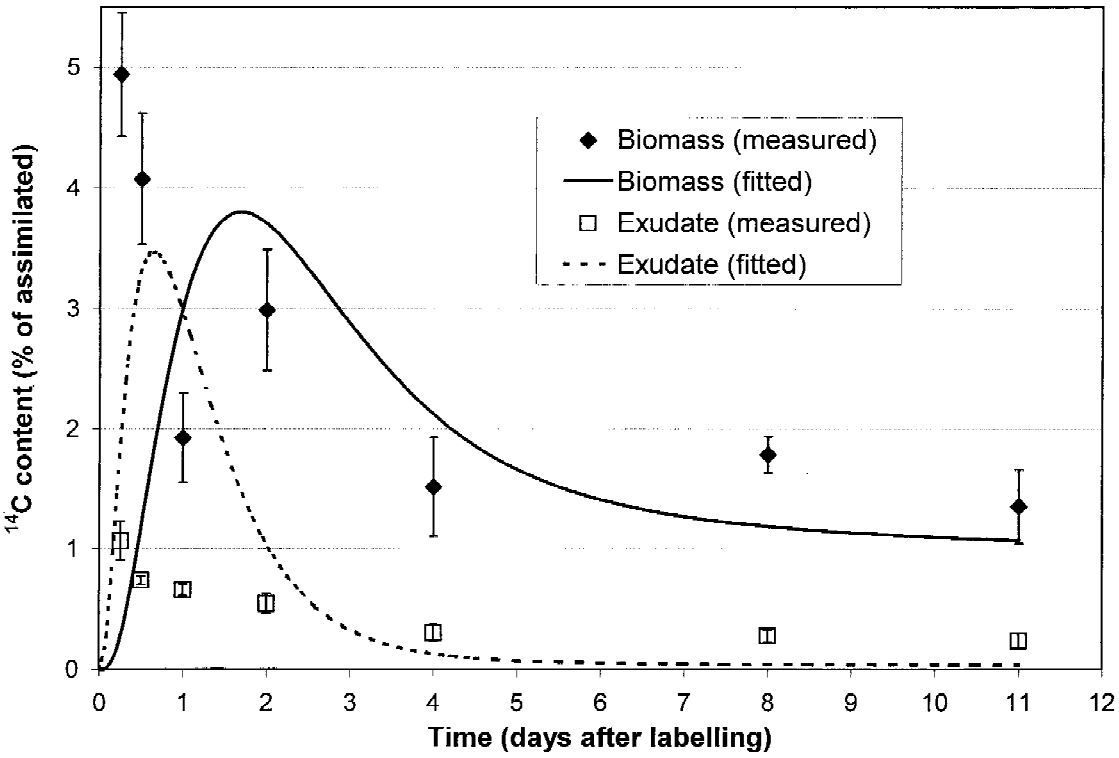

Figure 5. Measured (points, means ± SD) and fitted (lines) ^{14}C dynamics in soil micro-organisms and exudates (DOC).

The simulation of ^{14}C dynamics in micro-organisms (Figure 5) has given the worst results in comparison with the simulation of ^{14}C dynamics in shoots, roots and respired CO_2. According to r^2=0.12, only a small percent of observed variability of ^{14}C found in micro-organisms can be described by the model. Unsatisfactory agreement between measured and simulated ^{14}C dynamics in micro-organisms can be connected with the deficits of the model as well as with the method used for biomass estimation. Thus, a different Kc factor (2.34) was used to describe ^{14}C and C_t incorporation in soil micro-organisms (Van Ginkel et al., 1997). The Kc factor for rhizosphere micro-organisms can differ from that of bulk soil because of the active growth processes and faster turnover rates. No special experiments were conducted here for the determination of the Kc factor for rhizosphere micro-organisms.

In order to estimate ^{14}C flows through exudates, we have accepted that the sum of ^{14}C in K_2SO_4 extract obtained from the root free soil and ^{14}C in the aqueous solution obtained from root washing corresponds to the total ^{14}C in DOC (exudates). As is shown in Figure 5, we observed a slow decrease of the ^{14}C amount in DOC from 1% of assimilated C at the first sampling point to about 0.23% at day 11 after the labelling. The amounts of ^{14}C in DOC are 5 times smaller than ^{14}C content in micro-organisms at each measurement. Therefore, the acquisition of exudates by micro-organisms is faster than microbial respiration. The maximum of the ^{14}C in easily available rhizodeposits was measured shortly after labelling and there was no second maximum. This supports the second hypothesis described above that technical problems are responsible for the appearance of the first ^{14}C maximum in microbial biomass.

Changing of model parameters by fitting the simulated curve to measure ^{14}C content in exudates did not give a better accordance. The model greatly overestimated the amounts of measured ^{14}C-labelled DOC shortly after the labelling but underestimated it 5 days after, although the R^2 is satisfactory (R^2=0.56). When all C flows in the model were directed only to CO_2 efflux from the soil, the simulated ^{14}C content in exudates disappeared very quickly. This contradicted the experimental data. The introduction in the model of additional C flows from biomass to exudates and from humus to biomass (see Figure 1) slowed down the

consumption of ^{14}C in exudates. This change in the model significantly improved the simulated curve, especially for the points after 5 days, but it was still not enough to describe well the ^{14}C dynamics in exudates. Both the lack of knowledge about fast C flows in the rhizosphere and the strong simplification used in the model lead to the differences between simulation and measured data. Inclusion of two or more microbial biomass pools in the model could improve the agreement between modelled and measured data, although experimental estimation of the model parameters will be more difficult.

Sensitivity analysis of the model

The sensitivity analysis of the model is the investigation on the effect of changing parameters on the simulated results. The parameters describing root respiration and the exudation rates had the greatest influence on the dynamics of CO_2 efflux from the soil. Therefore, we were interested in their effect on the curve of $^{14}CO_2$ efflux from the rhizosphere. The sensitivity analysis was done separately for root respiration rate and for exudation rate. The values obtained from fitting (Table 1) were chosen as the basis values for sensitivity analysis. Then each value was changed in 10 steps from 0.001 to 0.0229 h^{-1} for root respiration rate and from 0.0001 to 0.16 h^{-1} for exudation rate. As a result, we obtained the simulation curves of $^{14}CO_2$ total efflux from the soil for each parameter (Figurr 6).

Although the root respiration rate and the exudation rate did not differ essentially (Table 1), both parameters affect the $^{14}CO_2$ efflux curve during different periods. The sensitivity analysis of the root respiration rate has shown (Figure 6, top) that root respiration affected the curve of CO_2 efflux only during the first 48 h after the labelling. This effect was strongest during the first 12 h. Simulated root respiration (dotted line) from the ^{14}C pulse was completed after 48 h. The changes in the exudation rate affected the total CO_2 efflux later than 24 h after the ^{14}C pulse chase (Figure 6, bottom). The maximal effect of exudation rate on rhizosphere respiration occurred about 48 h after the ^{14}C pulse. The curve of total ^{14}C efflux from the rhizosphere was negligibly affected by change in exudation rate during the first 24 h from labelling. It changes the maximum appearing on the 6th hour by only 4% of the value.

Results presented here have shown that root respiration and exudation affect strongly the main curve of $^{14}CO_2$ efflux dynamics from the soil. The changes in the root respiration rate have greater effect on $^{14}CO_2$ efflux than the changes in exudation rate. In the extreme case, it could lead to two separate peaks of the $^{14}CO_2$ efflux from the soil as reported by Warembourg and Billes (1979) and Nguyen et al. (1999).

Separating root respiration and microbial respiration of rhizodeposits

As described in the 'Introduction', there are many technical difficulties in the experimental separation of root respiration from microbial respiration of rhizodeposits in an intact soil. Our objective was to use a simulation model to overcome these difficulties. In previous steps, we have estimated all model parameters on the basis of measured ^{14}C dynamics in different pools. Therefore, these parameters are validated for growth of a 43-day-old *Lolium perenne* on a loamy Haplic Luvisol under the used experimental conditions. The correspondence of the model to the measured data in all pools was good (r^2=0.977). The sensitivity analysis has shown that parameters responsible for root respiration and microbial respiration of rhizodeposits affect the simulated $^{14}CO_2$ efflux curve at different times after the labelling. These pre-requisites allow the use of the simulation of the intensity of root respiration and of rhizomicrobial respiration for separation of these CO_2 sources and for estimation of total C amount passed through each of these processes (Figure 4, bottom). The total C amount passed through each of these processes corresponds to the area under each simulated curve. An integration of each function was done to calculate separately the total root respiration and root exudation. The calculated root respiration and the microbial respiration of available rhizodeposits and dead root residues amounted to 7.6% and 6.0% of the total assimilated C, respectively. So, the contribution of root respiration and rhizomicrobial respiration to the root-derived CO_2 was equal to 56% and 44%, respectively. It is important to note that the integration of the rhizomicrobial respiration curve was conducted only for 11 days after the labelling. This relation is close to our previous research with *Lolium* using the simple version of the model and it is near to those of Cheng et al. (1993) found for 3-week-old wheat plants growing on an arable sandy soil using the isotope dilution method.

The use of the model

There are only few models that describe the C flow from the plant into the rhizosphere soil following C

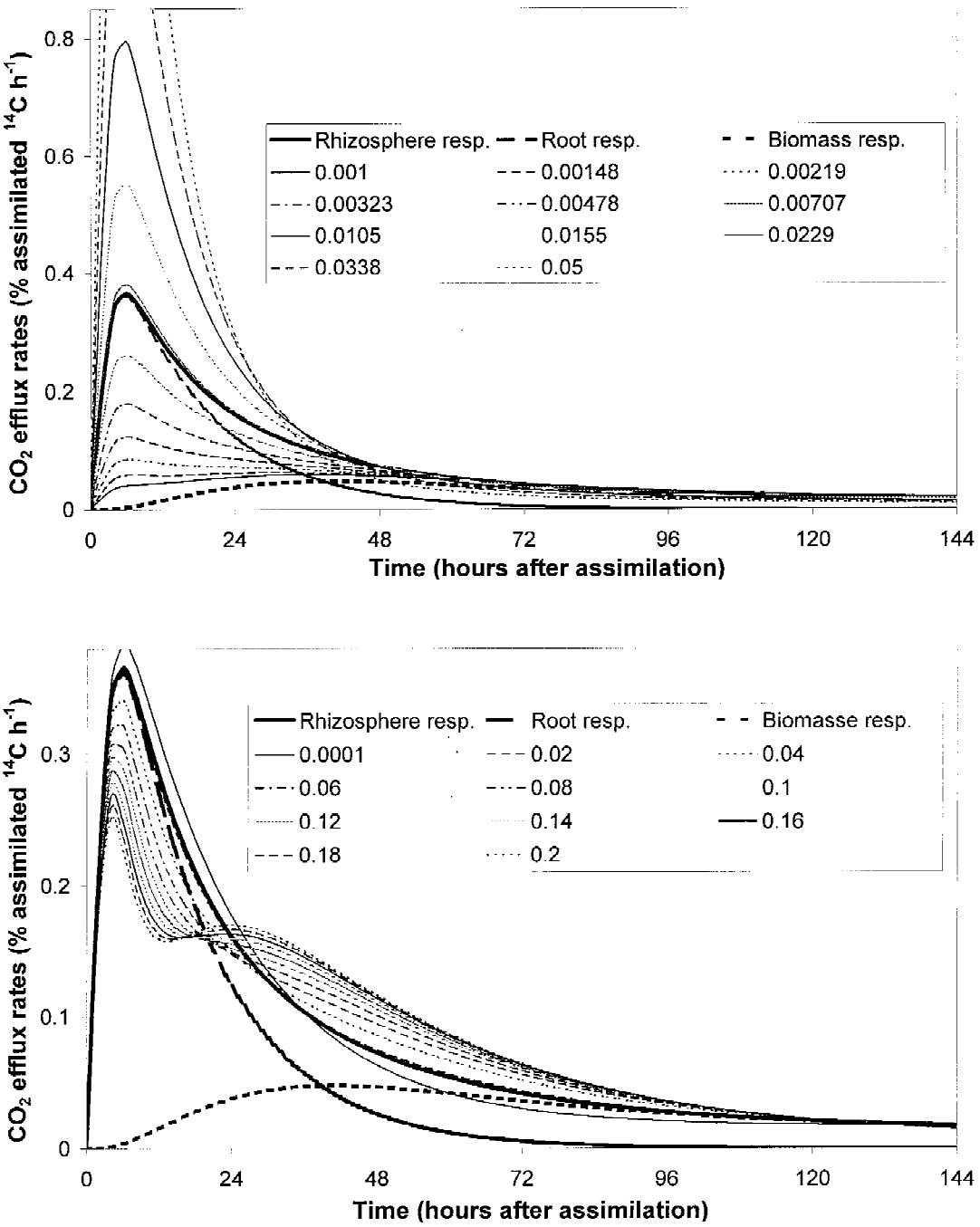

Figure 6. Sensitivity analysis of the model: Effect of root respiration rate (top) and exudation rate (bottom) on the dynamics of total $^{14}CO_2$ efflux from the soil. *Rhizosphere resp.* show the fitted curve of $^{14}CO_2$ efflux calculated with parameters from Table 1. *Root resp.* and *Biomasse resp.* shows the simulated $^{14}CO_2$ efflux (parameters are presented in Table 1) originated from root respiration and microbial respiration by decomposition of exudates, respectively. The numbers in the legends show the values of root respiration rate or exudation rate tested in simulations for sensitivity analysis (compare with Table 1).

utilisation by soil micro-organisms. The model of Darrah (1996) allows comparison of the rhizodeposition under sterile and nonsterile conditions at ambient and elevated $^{14}CO_2$ concentrations. The model of Newman and Watson (1977) is directed to microbial abundance in the rhizosphere depending on the distance to the root surface. Both models use the parameters taken from literature, mostly from the experiments in nutrient solutions.

Our model uses empirically determined parameters of C allocation in a plant–soil–atmosphere system. The described simulations were based on parameters obtained for 43-day-old *Lolium perenne* grown on a loamy Haplic Luvisol under laboratory conditions as described above. There is no reason why parameters for other plants and soils could not be used by the same model approach. However, special experiments are necessary to obtain or validate most of the parameters. The $^{14}CO_2$ efflux dynamics that are easy to measure can be used only for such parameters as root respiration rate, exudation rate and root mineralisation rate. If the parameters are known, they can be used later for prediction of C flow in similar experiments. The model allows to achieve important progress for the description and interpretation of root derived $^{14}CO_2$ efflux from the soil after pulse labelling.

The model can be developed further for the interpolation of a series of pulse labelling during the whole plant growth. For this model, development changes in the main pools like shoots and roots during the plant growth are necessary. In this case, a simulation of the C flows can be done not only in per cent of assimilated C like in this work, but in C mass units per area. This development can allow the prediction of C balance and turnover in fields considering the rapid C flows like exudation or root hair decomposition, which are usually not included in the traditional C balance of the soil.

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