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Towards a better understanding of carbon flow in the rhizosphere: a time-dependent approach using carbon-14

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Abstract Controversies exist in interpreting rhizosphere C flow obtained by different $^{14}CO_2$ labelling methods. However, there is a need for the standardisation of methods in order to be able to compare values obtained for different plants, different stages of development and different habitats. Perennial bromegrass (*Bromus erectus* Huds) grown in soils of different fertility was exposed to a $^{14}CO_2$ atmosphere for different periods of time: 1 h, 298 h and 78 days. The evolution of $^{14}CO_2$ in the soil was measured during and after labelling. The ^{14}C contents of plant and rhizosphere compartments were then estimated. The time-sequence of the rate of $^{14}CO_2$ evolution after 1 h of labelling, indicated a maximum after around 20 h, followed by an exponential decrease. When expressed as a percentage of net ^{14}C assimilation, root-soil respiration accounted for 14% and 18% in the nutrient-poor and nutrient-rich soils, respectively. Integration of the hourly values over several days showed that the dynamics of the evolution rate were similar for the 298-h and 78-day experiments, thus indicating that rhizosphere C flow was dominated by newly assimilated C. This was confirmed by the proportions of below-ground ^{14}C, measured for roots, respiration and soil, which were not significantly affected by the labelling regime. The differences were, however, found to be significant between the two types of soils. The conclusion was that the conditions for plant growth during labelling were more important than the length of time of labelling, and that this explained the discrepancies in the literature-cited values. A succession of short-term ^{14}C labelling of plants at different development stages followed by an allocation period of about 1 week is proposed to give a reliable estimation of the dynamics of C flow in the rhizosphere.

Key words *Bromus erectus* · Carbon flow · Carbon-14 labelling · Root exudation · Respiration

Dedicated to Prof. K. Vlassak on the occasion of his 65th birthday

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Introduction

Plant C released by growing roots is a major energy source for soil microorganisms. Even if this has always been emphasised in studies dealing with the rhizosphere, it has never been properly quantified, although it has important ecological implications. In fact, it is hardly possible to discriminate between plant and microbial metabolites. The measured production of CO_2 in the rhizosphere includes both a root and a microbial component. Estimates of C losses, by exudation or rhizodeposition – including sloughed root cells and tissues (Whipps and Lynch 1985) – and respiration in the rhizosphere range from 10% to 40% of plant-assimilated C (Van Veen et al. 1991). The range of values is an indication of the uncertainty of the estimates. This is the result of methodological difficulties due to the complexity of the system. So far, there is no conventional method of estimation allowing meaningful comparisons between studies done with different plants, at different development stages and subjected to different environmental factors.

Different labelling techniques have been used according to the objectives of a particular study, the two extremes being pulse and continuous labelling. It is currently accepted that the difference between both is in the distribution of the label within the plant (Lynch and Whipps 1990). Continuous labelling homogeneously labels the plant, while in pulse labelling, the labile C pool dominates (Meharg and Killham 1988) and none of the pools are labelled homogeneously (Meharg 1994). In fact, for pulse labelling, the first assessment will depend

on the time when partitioning is measured. Since estimation of the amount of labelled C in roots and soil requires harvests that should be sequential and destructive, the time-sequence of events occurring in the rhizosphere can only be indirectly ascertained by following the dynamics of labelled CO_2 evolution. Furthermore, an overall C balance can be estimated for the root and soil compartments. Theoretically, labelled C flow, resulting from pulse labelling, reflects the fate of C instantaneously assimilated. If the observations are maintained long enough, they should include all fractions, labile or not, providing that they are in detectable amounts when involved in respiration. When labelling is maintained during a longer period of time – one to several days – measurements will give integrated values of the rhizosphere processes which involve the assimilated C during this period. With continuous labelling, all plant C is homogeneously labelled and all the labelled material measured during respiration and in soil originates from the plant.

Comparisons between labelling techniques (Lynch and Whipps 1990; Van Veen et al. 1991; Meharg 1994) have indicated that controversies persist in interpreting rhizosphere C flow. They are related to the length of time during which the plants are exposed to $^{14}CO_2$. In fact, comparisons always refer to studies done by different authors, employing different procedures, different materials and under different conditions. One attempt was made to compare plants that received either a single pulse or a series of pulses during 8 consecutive days in the same study (Meharg and Killham 1988). Due to the specificity of the techniques involved, the results are, however, difficult to compare with those where plants were labelled for different lengths of time. The plant-labelling conditions may have played a major role in the observed discrepancies. Our aim in this study was to investigate differences in the estimation of the fate of C translocated to the root of perennial bromegrass (*Bromus erectus* Huds), obtained following the exposure of plants to ^{14}C for different durations: 1 h, 2 weeks and 11 weeks. The assumption was that the length of labelling should not interfere with the interpretation of rhizosphere C flow, as long as the plant growth conditions during and after the labelling period are carefully controlled. Moreover, measurement of changes with time, according to phenological stages or external conditions, from a series of experiments of short duration can reveal more information than experiments with continuous labelling. A comparison was also made between plants grown in two different types of soils, a recognised factor that affects the proportions of C flowing into each rhizosphere compartment (Merckx et al. 1987).

Materials and methods

Plant culture

Perennial bromegrass was grown in pots outside, during two consecutive years under the Mediterranean climate of the south of France. Seedlings obtained after 2 days germination of seeds in Petri dishes were transplanted in plain culture pots (3 plants pot^{-1}) holding 1 kg soil and buried in the ground. Two types of soil were used: (1) the A1 horizon of a decarbonated brown soil whose characteristics were: pH 6.8, total N 0.35%, total C 5.3%, qualified as fertile soil; and (2) the AB horizon of a siliceous brown soil with pH 6.7, total N 0.09%, total C, 1.25%; classified as poor soil. Both were chosen for their absence of carbonates, which could have interfered with the respiration measurements.

Plant labelling with $^{14}CO_2$

Three modes of labelling were applied in this study: long term, 78 days; mean term, 12 days; and short term, 1 h; all of them using the same equipment. At different periods of growth, according to each experiment, randomly selected pots were individually transferred to PVC plastic containers specially designed for labelling and respiration measurements (Warembourg et al. 1982). The soil atmosphere was enclosed by a tightly fitting partition above the soil, using a cover in which holes allowed the plants to emerge, after which glass wool holding a ring of silicone rubber (Dow Corning) was positioned around the stems. Great care was taken not to immerse the shoot bases into the silicone in order to allow the development of tillers. There were four holes along the side of the containers: one inlet port for aeration; one outlet port for collection of soil CO_2; one for the addition of water, one for the insertion of a water measuring device. Containers were immersed in water to check for leaks. The various openings were closed with rubber stoppers, except one that was used to raise the pressure by the slow introduction of air. By blowing gently, leaks were indicated by bubbling. The plant containers were then placed in a greenhouse under artificial light (600 mol photon m^{-2} s^{-1}) 2 days before labelling. Labelling was done indoors in a closed chamber according to a method similar to that described by Warembourg and Kummerov (1991). The equipment had been slightly changed to cope with the plant material and the type of exposure. The labelling chamber was tightly fixed on top of the plant containers, which were immersed in a temperature-regulated water bath. Only the aerial plant parts were exposed to $^{14}CO_2$. The CO_2 concentration inside the chamber was controlled and maintained at 350 ml l^{-1} using an infra-red gas analyser (IRGA; ADC, Hoddesdon, Hertfordshire, England) connected to a CO_2 source (Na_2CO_3) by a solenoid valve, which allowed the carbonate solution to drop into a H_2SO_4 solution thus allowing the CO_2 to evolve. This CO_2 was then labelled with ^{14}C at a predetermined specific activity. This was regulated by a differential ionisation chamber (CEA Cadarache, France) connected to a ^{14}C source (commercial solution of ^{14}C-Na_2CO_3) by another solenoid valve which controlled the addition of the labelled solution to the flask containing the H_2SO_4. When labelling exceeded 1 day, a bypass circuit operated by a timer was used during the night. It allowed the absorption, on sodalime, of the excess CO_2 (above the fixed 350 ml l^{-1} concentration) evolved during plant respiration. This was controlled by the IRGA. This dual control of CO_2 and radioactivity was very efficient and maintained a constant specific activity throughout the labelling period. The temperature inside the chamber and the photoperiod were maintained according to the ambient temperature prevailing at the time of each exposure. At the end of the $^{14}CO_2$ exposure period, unlabelled air was provided inside the chamber until the end of the respiration measurements. The plants were then harvested.

Sample analysis

During and after the labelling periods, the $^{14}CO_2$ evolved from each soil container was trapped using the classic aeration train method. With the help of a peristaltic pump, outside air, freed of CO_2 by passing through a sodalime column, was flushed into the top of each culture unit. The respired CO_2 was absorbed in 0.1-N or 0.2-N NaOH solution, automatically replaced at time intervals (every 2 to 4 h) using an automatic sample collector. The CO_2

content was measured by titration after aliquots were taken to determinate radioactivity by scintillation counting.

At the end of the chase period, the plant shoots were clipped and the corresponding roots washed free of soil and the water collected. After drying, the root fragments remaining in the soil were removed by hand under a microscope. All materials (plant and soil) were dried at 70 °C, weighed and ground. The C and ^{14}C contents were respectively determined using combustion and scintillation methods (Bottner and Warembourg 1976). Washed water was also analysed for ^{14}C content by scintillation counting.

Experimental procedure

The plants were sown in September in two consecutive years in the two types of soil. A long-term labelling experiment was undertaken in the first year. In order to seal the plant base properly with silicone rubber, the plants were only brought inside and prepared for labelling when the leaves reached 3 cm length (25 days). Six culture units (three for each soil) were labelled at the same time for 78 days at a specific activity of 3.4 kBq mg^{-1} C. The soil water content was adjusted to 80% soil capacity when the tension reached 50 kPa. The soil temperature was maintained at 17 °C. Respiration was measured during 83 days. Throughout the experiment, the containers were periodically checked for leaks. This was done by immersing the shoot bases into water and applying gentle pressure in the aeration train. Afterwards, water was removed from the chamber base. The second year, two series of labelling were undertaken. Six culture units (three for each soil) holding 64-day-old plants were labelled for 12 days at a specific activity of 5.8 kBq mg^{-1} C, and the chase period after labelling was maintained for 2 days. Eighty six-day-old plants (three culture units per soil) were labelled for 1 h at 2 mg l^{-1} at a specific activity of 33.6 kBq mg^{-1} C and the chase period was 92 h. Soil water and temperature were the same as during the first year experiment. Soil respiration was measured every 4 h for the long- and mean-term experiments, every 2 h for the short-term one.

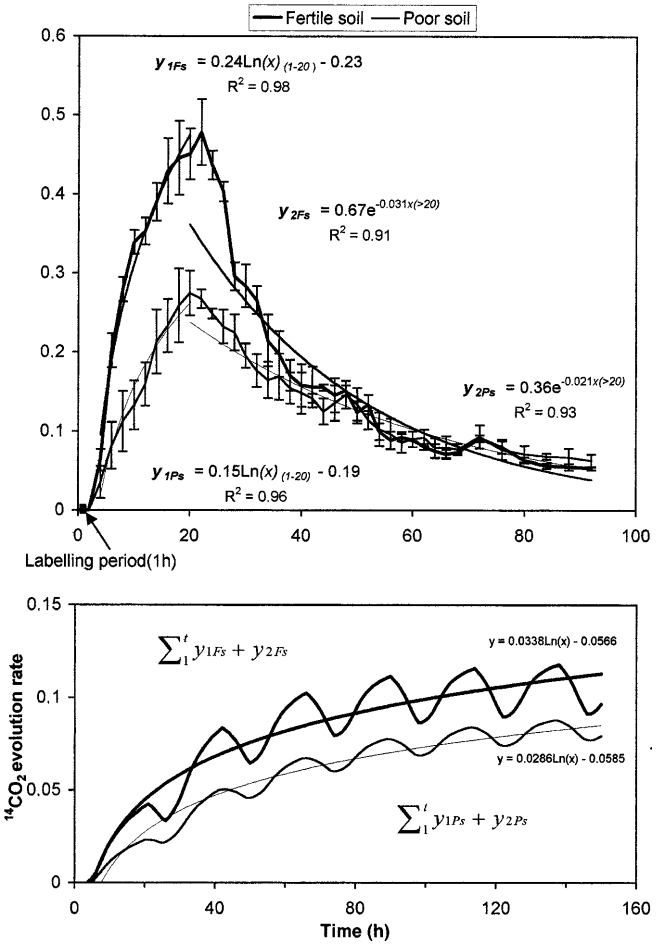

Fig. 1 **a** Rates of $^{14}CO_2$ evolution (expressed in % of net ^{14}C assimilation) from soils of different fertility after 1 h of labelling of plant shoots with $^{14}CO_2$ at mid-day. Values are means of three replicates; *bars* represent (±MD). **b** Simulated rates of $^{14}CO_2$ evolution using the equations given in Fig. 2a and a constant rate of ^{14}C assimilation for 8 h day^{-1}

Results

In order to correct for differences in plant sizes and photosynthetic rates among treatments, rates of $^{14}CO_2$ evolution in the soils were calculated on the basis of total ^{14}C recovered from plants, soil respiration and soil (^{14}C net assimilation), for each culture unit (three plants). Only the results of the short-term labelling experiment (Fig. 1) indicated a true percentage of the total assimilated ^{14}C evolved as $^{14}CO_2$ since the labelling period lasted only 1 h and the chase period started afterwards. For the mean-term and long-term labelling experiments (Figs. 2, 3), the method of estimation of the total recovered ^{14}C being destructive, $^{14}CO_2$ evolution rates were expressed (respectively per hour and per day) as a fraction (%) of total ^{14}C recovered at the end of the measurement period.

Analysis of below-ground $^{14}CO_2$ evolution

After 1 h of labelling, the time which elapsed before the labelled C appeared as $^{14}CO_2$, in measurable amounts in the soil of the culture units, was about 2 h (Fig. 1). The rate of $^{14}CO_2$ evolution then increased and reached a maximum around 20 h after exposure of the shoots to $^{14}CO_2$. There was a significant difference in proportions measured in the fertile and nutrient-poor soils. During this period, the rate was significantly expressed ($P<0.01$) by a logarithmic expression:

$$y1 = A\text{-log-}(t)\text{–}B, \quad (1)$$

where A was the constant rate increase, B the initial rate at time, $t=1$. Afterwards, the respiratory activity declined and the amount of $^{14}CO_2$ lost per unit of time showed an exponential decrease significantly described ($P<0.01$) by the expression:

$$y2 = Ae^{-kt}, \quad (2)$$

where y was the hourly rate of $^{14}CO_2$ production, A the initial rate and k the rate constant. The integral function of Eq. 2 made it possible to account for the total amount of $^{14}CO_2$ lost in the rhizosphere during the decreasing phase. The new expression was an exponential:

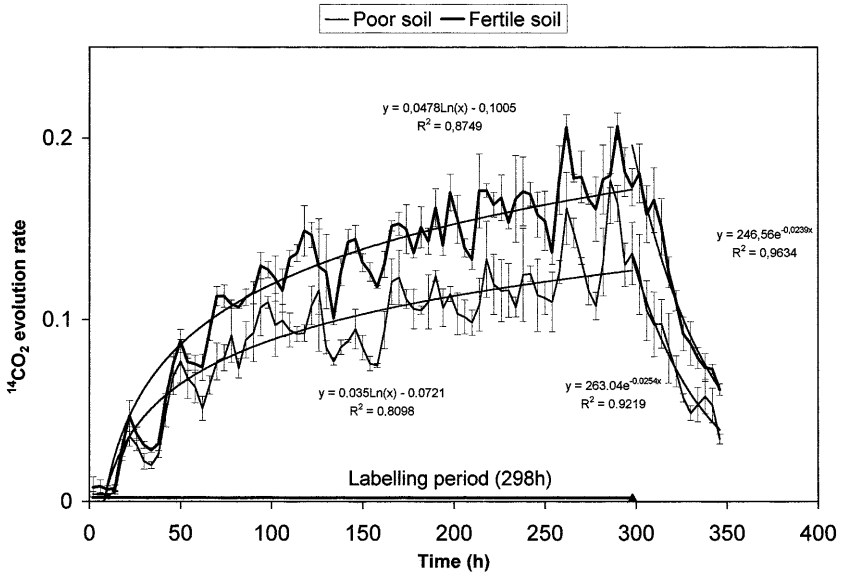

Fig. 2 Rates of $^{14}CO_2$ evolution (expressed in % of net ^{14}C assimilation) from soils of different fertility after 298 h of labelling of plant shoots with $^{14}CO_2$. Values are means of three replicates; *bars* represent ±MD

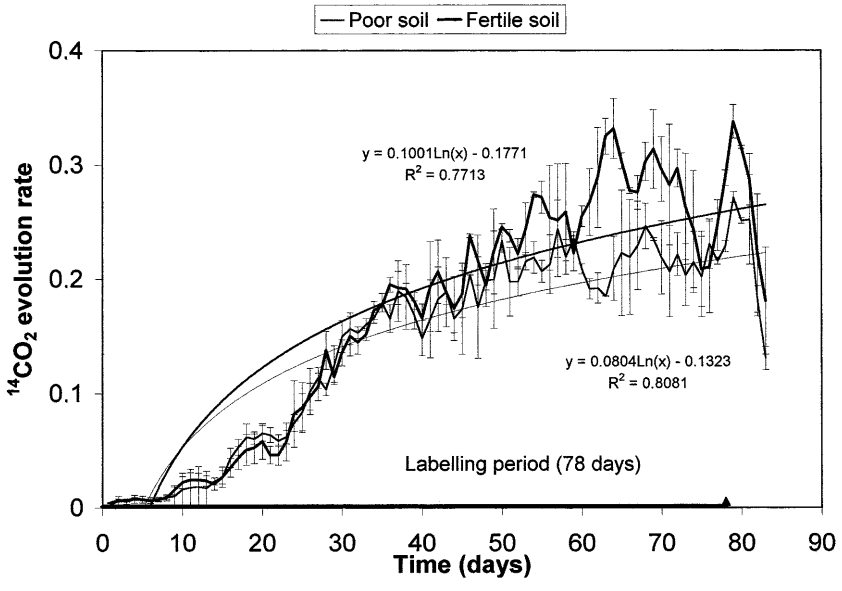

Fig. 3 Rates of $^{14}CO_2$ evolution (expressed in % of net ^{14}C assimilation) from soils of different fertility after 78 days of labelling of plant shoots with $^{14}CO_2$. Values are means of three replicates; *bars* represent ±MD

$$y=(A/k)e^{-kt}, \tag{3}$$

where A/k was the initial amount and k the rate constant of the loss. The loss after 20 h was thus calculated and a comparison was made between the measured values of below-ground $^{14}CO_2$ evolution and the values calculated using the model (Table 1). Without any change in the respiration process after the period of measurement, the predicted total $^{14}CO_2$ efflux amounted to 17.72% and 14.24% of the ^{14}C recovered, respectively, in the fertile and nutrient-poor soils. The measured values were well described by the model, which indicated that more than 80% of the ^{14}C lost by respiration occurred during the first 92 h.

A simulation was made of the $^{14}CO_2$ evolution rate following 6 days of ^{14}C assimilation using the 1-h model and the expression:

$$\sum_{1}^{t} y1+y2, \tag{4}$$

to describe the integration of the hourly values. The time-sequence followed a logarithmic type of expression (Fig. 1b). During the first few days the rate showed a sharp increase, then it levelled off. Day and night fluctuations were associated with the periods of assimilation.

During a labelling period of 12 days the $^{14}CO_2$ evolution rate (Fig. 2) rose sharply for about 100 h, then more slowly with a pattern that could be significantly described ($P<0.01$) by a logarithmic expression:

$$y=A\text{-log-}(t)\text{–}B, \tag{5}$$

where A was the rate constant and B the initial rate. The shape of the curve was very similar to the one ob-

Table 1 Comparison between measured and estimated values (using the model described in Fig. 1) of the $^{14}CO_2$ efflux in the rhizosphere of plants labelled for 1 h with $^{14}CO_2$. Measured values are means of three replicates ± SD

Time	20 h		92 h		Total
	Measured	Estimated (y_1)	Measured	Estimated (y_1+y_2)	Estimated (y_1+y_2)
Fertile soil	3.00 ± 0.59	2.98	10.87 ± 0.90	11.76	14.24
Poor soil	5.9 ± 0.45	6.1	15.38 ± 1.28	16.47	17.72

tained by the integration model described while using the 1-h-labelling values (Fig. 1b). This confirmed that rhizosphere C flow was mainly a short-term process. In the model (Fig. 1b), the ^{14}C net assimilation rate per hour was maintained at a constant value throughout the simulation period, whereas the measured values, obtained during the 12-day experiments (Fig. 2), took into account increasing assimilation rates associated with plant growth during the measurement period. This may explain the differences in the slopes described by the last part of the experimental and simulated curves. Throughout the measurement period, fluctuations were associated to day and night changes, but also to changes in the soil water content. There were also differences between plants grown on fertile and nutrient-poor soils, the latter showing less rhizosphere activity when expressed on the basis of total ^{14}C assimilation. At the end of the labelling period, the $^{14}CO_2$ evolution rate decreased with an exponential pattern similar to that described for the 1-h experiment.

The time-sequence of $^{14}CO_2$ evolution rates, obtained during a labelling period of 78 days starting with 25-day-old plants, followed a sigmoid-shaped curve (Fig. 3). The rate rose slowly for the first 15 days, more sharply the following 30 days, and more slowly thereafter. Altogether it was significantly described ($P<0.01$) by a logarithmic type of curve similar to that described for the 12-days labelling experiment. However, the slopes of the two experiments could not be compared since both the units of time and $^{14}CO_2$ evolution rate were different. Differences were also recorded in the rates of respiration between plants grown in the two types of soils.

^{14}C distribution

The distribution of ^{14}C in plant and soil components was estimated for each experiment at the end of the respiration measurements (Table 2). Comparison was difficult, as each experiment referred to a different length of time and period of plant development, but some trends did arise from these figures. When expressed in percentage of assimilated C, significantly more respiration was measured in the rich soil than in the nutrient-poor soil, even though the proportion of C translocated below-ground was not higher in the former. Respiration in the rhizosphere accounted for 11–16% of the C assimilated by plants growing in the nutrient-poor soil versus 13–22% for plants growing in the nutrient-rich soil. There were also differences between labelling regimes. The percentage in respiration was always higher for the 12-day labelling than for the 1-h labelling experiment. This might have occurred because respiration was not completed in the latter, as was shown above. Difficulties in sampling the roots and variations in extracting labelled roots of different ages, after each labelling regime, may also have contributed to some differences in proportions of ^{14}C recovered in roots and soils. When ^{14}C distribution was expressed on the basis of below-ground ^{14}C, the percentages were more affected by the type of soil than by the length of the labelling period (results of ANOVA, Table 2).

Table 2 Distribution of ^{14}C in plant-soil compartments following labelling of different duration and soil measurements of evolution of $^{14}CO_2$ by plants grown on different soils. Values are means of three replicates ± SD. Results given by two-way ANOVA. *ns* Not significant ($P>0.05$)

	Fertile soil			Nutrient-poor soil			Soil	Labelling
Labelling period	1 h	298 h	78 days	1 h	298 h	78 days		
Compartments	% of net ^{14}C assimilation							
Shoots	37.61 ± 2.06	30.82 ± 2.58	44.05 ± 0.42	33.64 ± 6.45	29.43 ± 5.01	44.76 ± 0.98	ns	**
Below-ground	62.39 ± 2.06	69.18 ± 2.58	55.95 ± 0.42	66.36 ± 6.45	70.57 ± 5.01	55.24 ± 0.98	ns	**
Roots	38.99 ± 3.39	36.21 ± 2.50	31.04 ± 1.59	45.41 ± 10.33	45.74 ± 2.35	35.85 ± 0.60	*	ns
Soil Respiration	15.38 ± 1.28	21.39 ± 0.75	13.19 ± 0.73	10.87 ± 0.90	15.66 ± 1.2	12.90 ± 1.20	***	***
Soil	8.02 ± 0.05	11.58 ± 0.99	11.74 ± 1.90	10.08 ± 3.78	9.18 ± 1.89	6.49 ± 0.82	ns	ns
	% of below-ground ^{14}C							
Roots	62.49 ± 3.5	52.30 ± 2.03	55.46 ± 2.43	68.43 ± 4.94	64.87 ± 1.45	64.90 ± 0.061	***	ns
Soil Respiration	24.65 ± 2.95	30.92 ± 0.31	23.56 ± 1.14	16.38 ± 1.30	22.19 ± 0.83	23.33 ± 1.76	**	ns
Soil	12.85 ± 1.91	16.77 ± 1.74	20.98 ± 3.56	15.19 ± 4.31	12.94 ± 2.04	11.78 ± 1.69	*	ns

*** $P<0.001$, ** $P<0.01$, * P

Discussion

The dynamics of ^{14}C soil respiration after 1 h of labelling indicated that allocation and use of current C in the below-ground system was at a maximum 20–24 h after assimilation, and that 80% of the efflux was completed by day 4. Several workers using either pulse (Cheng et al. 1993; Swinnen et al. 1994) or short-term (a few hours) (Warembourg and Paul 1973; Kucey and Paul 1982; Warembourg and Roumet 1989) labelling have reported similar results. The time-course of the $^{14}CO_2$ evolution curves might have been different with different gas diffusion characteristics of the soils used and different experimental conditions, but their shapes were identical. ^{14}C recovered from soil respiration originated partly from root respiration and partly from microbial use of root ^{14}C deposited into the soil following the exposure period. Experiments with gnotobiotic cultures (Warembourg and Billes 1979) and microsymbionts (Kucey and Paul 1982; Warembourg and Roumet 1989) have demonstrated that root C is readily available to rhizosphere organisms a few hours after assimilation, and that the maximum activity is recorded within 1 or 2 days. As discussed by Harris and Paul (1991), at the asymptote, it was assumed that the labelled C had achieved a stable distribution within the microbial biomass, and that further release resulted from maintenance and turnover rather than growth.

When plants were exposed to $^{14}CO_2$ for a longer period, the $^{14}CO_2$ evolution rate increased sharply until around day 4, and then it rose more slowly. The initial increase during the first 100 h was attributed to the progressive changeover between unlabelled and labelled C in root and microbial processes of the rhizosphere. According to the 1-h experiment, this was the time required for the evolution of 80% of the unlabelled C assimilated before the beginning of exposure as $^{14}CO_2$. After about 100 h most of the C came from newly assimilated C. The slow rise observed afterwards could be attributed to: (1) an increase in activity due to plant growth, and therefore an increase in the amount of C reaching the below-ground parts of the plants; and (2) the additive effects of the time-sequence of respiratory activities associated with each unit of translocated ^{14}C (as were measured after 1 h of labelling). The proportion of ^{14}C released by respiration after 12 days of labelling was slightly higher than that measured after 1 h, thus indicating that the chase period was not long enough in the latter. When corrected by the exponential model, the values were very close. For the 78-day experiment, which can be compared to continuous labelling, the $^{14}CO_2$ evolution rate followed a pattern similar to a growth curve. It first increased slowly, when the plants were young, then rose more sharply afterwards, and levelled off when the plants were around 80 days old (55 days after labelling). The last part of the curve was similar to the one described for the 12-day experiment. Published $^{14}CO_2$ respiration curves following long-term or continuous labelling of perennial plants with $^{14}CO_2$ are rather scarce. During 7 weeks continuous labelling of *Festuca pratensis* L., (Johansson 1991) found that the rate of $^{14}CO_2$ evolution increased throughout the experiment, and the ratio of labelled C in soil-root respiration to dry weight of the shoots was constant. The corresponding percentage distribution of below-ground ^{14}C was 28% in respiration, 63% in roots and 9% in soil, which was in accordance with the results obtained in this study. Percentage distribution in soil respiration, obtained in other long-term studies of plants at vegetative stages (generally the tillering stage) ranged between 21% (Barber and Martin 1976) and 39% (Whipps 1984; Merckx et al. 1985) for annual crops, and was estimated to be 28% in the perennial blue grama (Dormaar and Sauerbeck 1983).

Surprisingly, this study has shown that there were no significant differences in the proportions of below-ground ^{14}C recovered in soil-root respiration following exposure of the plants to $^{14}CO_2$ during increasing periods of time. In the light of literature on rhizosphere C flow following either pulse or continuous labelling (Meharg and Killham 1988; Lynch and Whipps 1990; Meharg 1994) these results were unexpected. They indicated that the bulk of microbial substrates liberated by the growing roots of plants in a vegetative stage of development (as in the present study) was liberated and utilised less than a week following C assimilation. Turnover and microbial degradation of simple root structures such as root cap cells and root hairs, known to be rather fast (Moore and McClelen 1983; Fusseder 1987), must have contributed to the first week of CO_2 efflux. More stable compounds may have appeared later and throughout the plant development. They were, however, not in significant proportions as to modify the overall CO_2 efflux measured over long periods of time. They were included in the asymptotic part of the exponential described for the 1-h labelling experiment.

The mean proportions of labelled C recovered from soil-root respiration of short-term or pulse-labelled plants in a vegetative stage of growth varied from 4.7% to 16% of below-ground ^{14}C for wheat, and from 23% to 25% for barley (Gregory and Atwel 1991; Swinnen et al. 1995). The values did not differ from those reported for continuously labelled plants, i.e. 8–23% (Barber and Martin 1976; Whipps 1984; Merckx et al. 1985) and 8–26% (Barber and Martin 1976; Whipps 1984) for wheat and barley, respectively. The ranges were, however, important due to methodological differences. The values were not systematically lower, as studies which compared pulse and continuous labelling (Lynch and Whipps 1990; Meharg 1994) would have suggested. With plant development, changes occur in C partitioning, especially for annuals during the reproduction stage (Martens 1990; Gregory and Atwel 1991; Swinnen et al. 1994), and decaying roots contribute to $^{14}CO_2$ efflux that will not be considered when using pulse- or short-term labelling techniques. One may also suggest that the material released in the rhizosphere

following root decay will not have the same ecological implications for microbial activities as the day by day release of less complex substrates.

This study also showed that soil type was a major factor affecting plant C partitioning. Significantly more C was allocated to root growth in the nutrient-poor soil, which confirmed a known strategy of plants living in low-nutrient habitats (Coleman et al. 1983), but proportionally less C was dissipated following respiration in the rhizosphere. This was in agreement with other studies which indicated that soil fertility and soil texture greatly influence the dynamics of root exudation, and the size and activity of the microbial biomass in the rhizosphere (Merckx et al. 1985, 1987).

In conclusion, the exposure of plants to $^{14}CO_2$ for increasing periods of time and under very carefully regulated conditions indicated that rhizosphere C flow during the vegetative stages of bromegrass growth was dominated by newly assimilated C. Temporal differences in the proportions associated to the activity of the rhizosphere microflora were related to changes in the proportions reaching the roots and not to changes in the origin of available substrates. It can be suggested that, for pot experiments, a succession of short-term exposures of plants at different stages of development to ^{14}C, followed by a chase period of around 1 week for respiration measurements, are appropriate for the estimation of the dynamics of the "rhizosphere effect": the C flow from the plant that is of importance to the rhizosphere microflora.

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