

IN SITU MEASUREMENT OF ROOT RESPIRATION AND SOLUBLE C CONCENTRATIONS IN THE RHIZOSPHERE

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Summary—Rhizosphere respiration can consume up to 30% of total net photosynthetic production, principally from root respiration and from rhizo-microbial respiration using plant-derived substrates. Rhizosphere respiration has rarely been studied *in situ* because of technical difficulties in partitioning respiration between roots and their rhizosphere associates. We report a procedure to simultaneously measure root respiration, rhizo-microbial respiration and soluble C concentration in the rhizospheres of intact plants. This procedure involves: (1) $^{14}\text{CO}_2$ pulse-labelling and tracing of photosynthetically-fixed C released from the root-soil column as $^{14}\text{CO}_2$ under three concentrations of [^{12}C]glucose added to the soil; and (2) calculation of root respiration, rhizo-microbial respiration and soluble C concentrations in the rhizosphere.

In an experiment with 3-week old wheat plants, we found that root respiration and rhizo-microbial respiration contributed, on average, 40.6 and 59.4% of total rhizosphere respiration, respectively, and that the soluble C concentration in the rhizosphere averaged 667 mg C l^{-1} of soil water. This soluble C concentration was about 20–100 times higher than the previous estimates used in some rhizosphere models.

INTRODUCTION

The rhizosphere is characterized by very high numbers and activities of organisms. Concentrations of microbes in the rhizosphere can reach 10^{10} – 10^{12} g^{-1} of rhizosphere soil (Foster, 1988). Invertebrate numbers in the rhizosphere are at least two orders of magnitude greater than in the surrounding soil (Lussenhop and Fogel, 1991). This highly active system associated with plant roots is mainly supported by the C input from roots. The organic C-input by growing plant roots in soil can be divided into two main groups: (1) water-soluble exudates, such as sugars, amino acids, organic acids, hormones and vitamins, which leak from the root and (2) water-insoluble materials, such as cell walls, sloughed-off materials and mucilage. We mainly focus on current photosynthate allocated to water-soluble exudates in this study.

A number of studies have quantified C input into the soil by growing plants in growth chambers with a continuous supply of $^{14}\text{CO}_2$ in the air (Barber and Martin, 1976; Johnen and Sauerbeck, 1977; Whipps and Lynch, 1983; Whipps, 1984, 1987; Merckx *et al.*, 1985; Van Veen *et al.*, 1989; Martin, 1990; Liljeroth *et al.*, 1990; Kuikman *et al.*, 1991). In most of these experiments soil $^{14}\text{CO}_2$ has been treated as one category—rhizosphere respiration. Functionally, rhizosphere respiration consists of root respiration and microbial respiration utilizing root-derived materials (rhizo-microbial respiration). Total rhizo-

sphere respiration of annual plants has been estimated to be in the range of 20–80% of the plant C transfer to the below-ground system via roots (Martin and Kemp, 1986; Lambers, 1987; Lambers *et al.*, 1990; Whipps, 1990). These two functional categories of rhizosphere respiration have rarely been studied *in situ* because of technical difficulties in partitioning respiration between roots and their rhizosphere associates (Helal and Sauerbeck, 1991). The ecological implications of root respiration are different from those of root exudation. Root respiration is a direct release of photosynthetically-fixed C, whereas root exudation is a process through which photosynthetically-fixed C enters the C pool in the soil.

Although total rhizosphere respiration has been shown to vary greatly depending on the testing conditions and plant species (Whipps, 1984, 1990), very little is known about which portion of the rhizosphere respiration varies more in response to environmental changes. Some reports suggest that root exudation varies substantially depending on the soil and other environmental conditions, such as anoxia, mechanical force, water stress, nutrient status, temperature, pH and day-length (e.g. Barber and Gunn, 1974; Martin, 1977; Hale and Moore, 1979; Wiedenroth and Poskuta, 1981; Lee and Gaskins, 1982; Martin and Foster, 1985; Merckx *et al.*, 1987; Smucker and Erickson, 1987; Meharg and Killham, 1990).

Compared to photosynthesis, we know considerably less about root respiration and exudation. Most research has been performed by growing plants

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in nutrient solution or artificial soils under gnotobiotic conditions (Lambers, 1987). Due to the artifacts of this method, these values may bear little relevance to the reality of the complex structure and the functional relationships taking place in the soil.

Pulse labelling of plants with $^{14}\text{CO}_2$ or $^{11}\text{CO}_2$ and subsequent tracing has been used to investigate rhizosphere respiration as a whole because current photosynthate is the main C source for rhizosphere respiration (Meharg and Killham, 1988; Wang *et al.*, 1989). We have developed a novel procedure to simultaneously measure root respiration, rhizomicrobial respiration and soluble-C concentration in the rhizospheres of intact plants using ^{14}C pulse-labelling and tracing.

We hypothesized that adding [^{12}C]glucose to the rhizosphere immediately before pulse labelling of plant shoots should: (1) dilute the water-soluble ^{14}C -labelled C in the rhizosphere-rhizoplane; (2) result in a lower rate of $^{14}\text{CO}_2$ output from the root-soil component and a higher concentration of water-soluble ^{14}C -labelled C in the rhizosphere and bulk soil than the water addition control during the initial period (4–6 h) after labelling. This hypothesis was developed from the concept of 'isotopic trapping' (Wolf, 1964). In our study this hypothesis was tested and the concept of isotopic trapping was applied to measurements of root respiration, rhizomicrobial respiration and soluble-C concentration in the rhizosphere.

The concept of isotopic trapping has been used in several physiological studies (Drew and Smith, 1967; Geiger *et al.*, 1974). For example, to determine whether or not compound B is an intermediate in reaction from A to C, one runs the reaction with labelled A and unlabelled B. If B is truly an intermediate in the reaction, unlabelled B and labelled B will be incorporated into the end product proportionately, and thus labelled B will be left in the medium after the reaction is complete. Therefore, if any radioactivity is detected in B at the end of the reaction, B is an intermediate. We used labelled photosynthate in both root respiration and microbial respiration. However, root exudates act as the intermediates in the process of microbial respiration but not of root respiration. We added glucose as the unlabelled intermediate to dilute and trap the labelled exudates temporarily in the rhizosphere. Therefore, microbial respiration ($^{14}\text{CO}_2$ evolved from microbial utilization of exudates) must be inversely proportional to the glucose- ^{12}C concentration in the rhizosphere; whereas root respiration is independent of the glucose- ^{12}C concentration in the rhizosphere.

MATERIALS AND METHODS

Soil

Soil was taken from a ploughed agricultural plot, 0–15 cm layer, in the Horseshoe Bend Research Area

of the University of Georgia, Athens, Ga. The soil is a Hiwassee series (Typic Kanudult), a well-drained sandy clay loam (66% sand, 13% silt, 21% clay, pH 6) found on 0–2% slope. Organic C, total Kjeldahl N, and available P (dilute HCl– H_2SO_4 extractable) of the soil are 1.13, 0.11%, and $57.3 \mu\text{g g}^{-1}$ respectively. Its water holding capacity is 35% w/w. The soil was sieved (<2 mm), homogenized, and air-dried before use.

Growing plants

In experiments 1 and 2, PVC containers (5 cm i.d., 15 cm tall, capped bottom, with air inlet tubing at the top and air outlet tubing at the bottom) were made and used for growing wheat. Smaller PVC containers (3.2 cm i.d., 15 cm tall) were used in experiment 4. The containers were filled with 380 g (165 g for experiment 4) of air-dried soil. Then five winter wheat (*Triticum aestivum*) seedlings (<24 h old) were planted about 2 cm deep into each container. After emergence, plants were thinned to three (two in experiment 4) individuals per container, and grown under a metal halide-brilliance light (photosynthetic photon flux density = $490 \mu\text{E s}^{-1} \text{m}^{-2}$ at the mid-canopy) and 22°C constant temperature. Water content in each container was maintained at about 20% w/w by weighing and watering each day. The plants were 3 weeks old at the time of labelling.

In experiment 3, 300 ml Plexiglas culturing bottles (5 × 10 × 12 cm, with air outlet tubing at the top and air inlet tubing at the bottom) were used to grow rye. The bottles were first filled with 300 g of air dried soil. Three rye (*Secale cereale*) seedlings (<24 h old) were planted into each bottle. In each bottle only one plant was allowed to grow under the same lighting and temperature as in experiments 1, 2 and 4. The plants were 3 weeks old at the time of labelling.

Labelling

The labelling apparatus consists of (1) a Plexiglas chamber, (2) an air supply and dispensing system, (3) a $^{14}\text{CO}_2$ generating and infrared gas analyser loop and (4) two air mixing fans (Fig. 1). One day before labelling and testing, each plant container was sealed at the base of each plant with a mixture of Vaseline (petroleum jelly) and low melting point Paraffin (m.p. 55–55°C) in a proportion of 5:1 (w/w) (only melted Paraffin at 58°C was used for experiment 3). The seal was tested for air leaks by pumping air into the container and submerging the whole container in water. The soil water content of each container was measured gravimetrically and was brought to about 45% of its water holding capacity. In experiments 1–3, glucose solution or deionized water was added through the air inlet tubing of each container 1 h before ^{14}C -labelling to just reach a water content of 80% holding capacity and pre-set glucose concentrations via vacuum suction. In experiment 4, in order to obtain a more uniform substrate distribution a surplus amount (50 ml total) of glucose solution (or

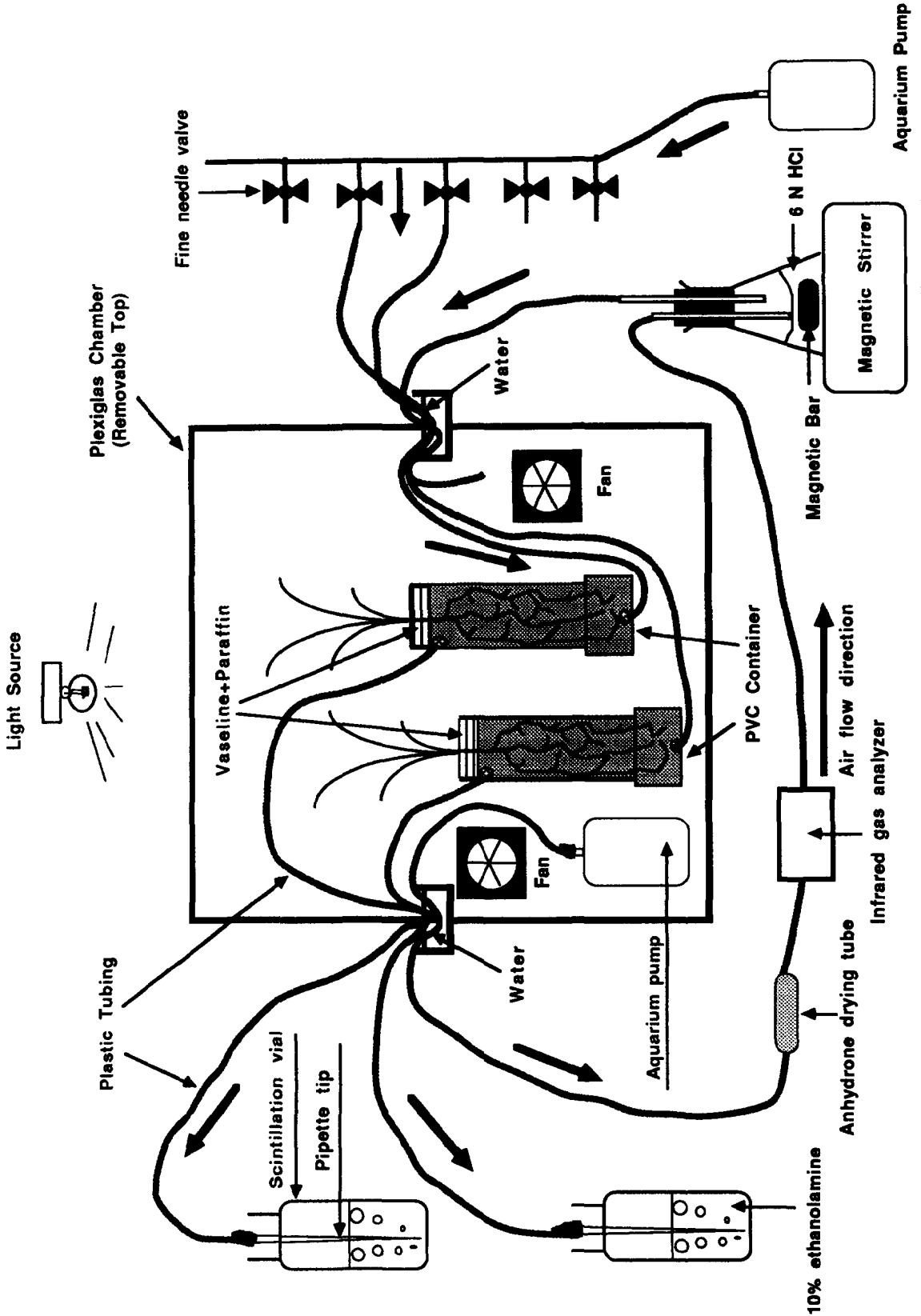


Fig. 1. Equipment configuration for ^{14}C pulse-labelling of plant shoots and subsequent tracing of below-ground $^{14}\text{CO}_2$ evolution.

water for the control) was added into each container via vacuum and the effluent was kept frozen for analysis of glucose content later. Air flows through all the containers were adjusted to the same rate of $50 \text{ cm}^3 \text{ min}^{-1}$. Immediately after all the plant containers were sealed into the chamber, labelling was begun by injecting $\text{NaH}^{14}\text{CO}_3$ solution into the acid flask connected in the $^{14}\text{CO}_2$ injection loop. The top of the chamber was removed after the labelling period of 20 min for experiments 1, 2 and 3, and 10 min for experiment 4.

Sample analysis

The $^{14}\text{CO}_2$ evolved from each container was trapped by continuously pumping room air ($50 \text{ cm}^3 \text{ min}^{-1}$ flow rate) through the root-soil column and bubbling in 3 ml of ethanolamine scintillation cocktail. The CO_2 trap was changed every 10 min. The cocktail was counted directly using a liquid scintillation counter (Beckman LS 3801).

By the end of the tracing period, each plant was cut at the base, each root-soil column was pulled out, and roots were hand-picked from the soil. Soluble ^{14}C in 20 g of fresh root-free soil was extracted in 50 ml of 0.5 M K_2SO_4 solution. The radioactivity in the extract was measured via liquid scintillation counting.

Roots, shoots and root-free soils were air-dried under a fumehood first and 60°C oven-dried later. Soil particles on the roots were washed off in beakers with tap water before roots were dried. Dry root, shoot and soil samples were pulverized in a ball mill before analysis for radioactivity. Radioactivities of the roots, shoots and soils were measured by liquid scintillation after combustion in an OX-300

Biological Oxidizer (R. J. Harvey Instrument Co., Hillsdale, N.J.).

RESULTS AND DISCUSSION

The elapsed time for the labelled-C to reach the roots and soil and appear as $^{14}\text{CO}_2$ from the container was *ca* 30–60 min. The rates of $^{14}\text{CO}_2$ evolution from the root-soil columns of water addition control, 1.3 and 6.7 g C l^{-1} of soil solution glucose addition treatments after a 10-min pulse labelling of shoots with $^{14}\text{CO}_2$ reached a maximum at about 2 h after the introduction of $^{14}\text{CO}_2$ to the shoots, then declined (Fig. 2). Several experiments produced similar results (data not shown). No subsidiary local maxima could be detected within this initial period of chasing (4–8 h). This suggested a mostly direct source-sink relationship between shoots and roots. As discussed by Harris and Paul (1991), the timing and shape of these curves might change with different settings of gas flow dynamics with each experimental design and gas diffusion characteristics of the soil used in the experiments. The temporal dynamics of $^{14}\text{CO}_2$ evolution from the root-soil column after a pulse labelling in our study was comparable with that produced by ^{14}C technique (Minchin and McNaughton, 1984; Dyer *et al.*, 1991; Freckman *et al.*, 1991) in general patterns but with a coarser resolution of timing.

Addition of [^{14}C]glucose solution to the root-soil column 1 h before pulse labelling of shoots with $^{14}\text{CO}_2$ substantially reduced the rates of $^{14}\text{CO}_2$ evolution from the root-soil columns and increased the amount of water-soluble ^{14}C in the soil to 3–12-fold compared to the water addition controls (Fig. 2 and Table 1). These results directly confirmed our original

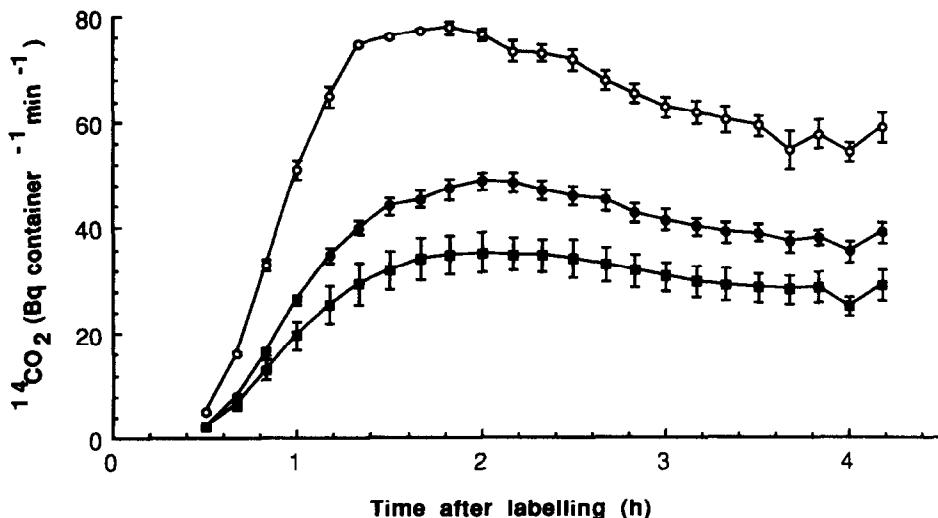


Fig. 2. Rates of $^{14}\text{CO}_2$ evolution from root-soil columns after 10 min labelling of shoots with $^{14}\text{CO}_2$ in the air (data from experiment 4). Treatments: water addition control (○); 1.3 g C glucose l^{-1} (●); 6.7 g C glucose l^{-1} (■).

Table 1. Effect of adding [¹⁴C]glucose to root-soil columns immediately before a pulse labelling of shoots with ¹⁴CO₂, evolution rates of the root-soil columns and soluble-¹⁴C concentrations in the soil. Numbers area means with standard errors in parentheses

Treatment	¹⁴ CO ₂ (kBq)	Soluble- ¹⁴ C (kBq)
Experiment 1 (wheat)		
Control (water only)	55.39 (6.14)	8.21 (0.59)
Glucose (2.9 mg C ml ⁻¹)	39.22 (5.25)	29.30 (2.00)
Experiment 2 (wheat)		
Control (water only)	86.36 (2.18)	2.04 (0.52)
Glucose (5.8 mg C ml ⁻¹)	50.43 (1.07)	25.16 (1.11)
Experiment 3 (rye)		
Control (water only)	12.88 (3.22)	ND*
Glucose (5.8 g C ml ⁻¹)	4.55 (1.07)	ND

*ND: not determined.

hypothesis and validated the isotopic trapping mechanism. Glucose addition did not significantly affect the C allocation pattern of the plants (Table 2). The below ground:above ground ratio of labelled-C was ca 1:4 for both water addition control and glucose addition treatment by the end of 4 h tracing. This ratio would be higher if the labelled-C lost during processing (6 h) could be accounted for. Results from solution culture studies (Williams *et al.*, 1991) using a split-root method and C tracers (¹⁴C and ¹¹C) also indicated that no significant effect on C allocation, root respiration and exudation could be detected 1 h after adding low concentrations of monosaccharide to the culturing solution.

Based on the isotopic trapping mechanism, rhizo-microbial respiration (¹⁴CO₂ evolved from microbial utilization of exudates) must be inversely proportional to the glucose-[¹²C] concentration in the rhizosphere; whereas root respiration is independent of the glucose-[¹²C] concentration in the rhizosphere. The following equation can be written for each concentration of glucose and a family of two equations can be established for two glucose concentrations added to the same experiment:

$$\frac{{}^{14}\text{CO}_2 \text{ with glucose} - \text{Root Resp.}}{{}^{14}\text{CO}_2 \text{ without glucose} - \text{Root Resp.}} = \frac{\text{Soluble C (g l}^{-1}\text{)}}{\text{Soluble C (g l}^{-1}\text{)} + \text{glucose C (g l}^{-1}\text{)}}$$

The rates of ¹⁴CO₂ evolution with or without glucose can be measured directly. The glucose C concentrations in the rhizosphere can be derived from calculations based on the rate of glucose uptake (determined in a separate study) in the root-soil column, the rate of water loss, and the amount of

glucose and water added at the beginning of the experiment (also see Table 3). The two unknown variables are the ¹⁴CO₂ evolution rate that is due solely to root respiration and the soluble C concentration in the rhizosphere of intact plants. By solving two equations in a family simultaneously, the values of the two variables can be determined.

There are several assumptions implicated in the equation: (1) adding glucose to the root-soil column does not produce short-term effects on plant physiology (such as changes of root respiration, exudation and C allocation) other than diluting the root exudates; (2) glucose (substrate added) is compatible with root exudates in terms of substrate specificity and has similar assimilation efficiency and absorption kinetics for rhizosphere microflora; (3) adding glucose to the root-soil column does not stimulate or suppress the microbial activities in the rhizosphere during the period of the experiment. The first assumption has been supported by the results obtained from our experiments (Table 2). This assumption may cover several processes such as glucose absorption by roots and osmotic effect due to glucose addition. In a solution culture study with barley, Williams *et al.* (1991) suggested that these effects on plant carbon partitioning and other physiological aspects were transient and minimum. The second assumption is inferred from the fact that a majority (about 60–90%) of root exudates is in the form of reducing sugars (Hale *et al.*, 1971; Rovira and Davey, 1974; Bokhari *et al.*, 1979; Krafczyk *et al.*, 1984), and that glucose has been used as a universal C source for soil microorganisms (Anderson and Domsch, 1978). The third assumption is inferred from the general belief that microbial growth in the rhizosphere is not limited by available C since the rhizosphere is rich in

Table 2. Distribution of ¹⁴C after 20 min of labelling and 4 h of tracing of 3-week old wheat. Numbers are means of three replicates. Numbers in each column with the same letter are not significantly different (*t*-test: $\alpha = 0.05$). Results obtained from experiment 2

Treatment	Root (%)	Shoot (%)	Below (%)*
Glucose (5.8 g C ml ⁻¹)	9.34a	79.81a	20.19a
(standard deviation)	0.58	1.52	1.52
Water only	9.42a	80.00a	20.00a
(standard deviation)	0.10	0.55	0.55

*Sum of ¹⁴C in roots, respiration and soil.

Table 3. Cumulative $^{14}\text{CO}_2$ evolved (0.5–4.2 h) in root–soil columns with two concentrations of glucose addition and water addition control, and calculated root respiration, rhizo-microbial respiration, and concentration of soluble-C in the rhizosphere of 3-week old wheat after pulse labelling of shoots (10 min) with $^{14}\text{CO}_2$. Mean glucose concentration was calculated based on the amount of glucose added and the uptake rate of 8.68 mg glucose container $^{-1}$ h $^{-1}$ obtained from a separate study under the same soil and plant conditions (data from experiment 4)

Mean glucose concentration (g C l $^{-1}$ soil water)	
Glucose I	1.19
Glucose II	6.78
$^{14}\text{CO}_2$ evolved (kBq container $^{-1}$) (mean \pm 1 SE)	
Water only	13.72 \pm 0.27
Glucose I	8.50 \pm 0.34
Glucose II	6.30 \pm 0.63
Root respiration (kBq container $^{-1}$) (% water addition)	5.57 40.61
Rhizo-microbial respiration (kBq container $^{-1}$) (% water addition)	8.15 59.39
Water soluble-C (mg C l $^{-1}$ soil water)	667

available C but poor in mineral nutrition (Helal and Sauerbeck, 1989). Fully validating these assumptions requires further separate studies specially designed for this purpose.

Using the data shown in Table 3 and the equation described previously, we computed root respiration rates, rhizo-microbial respiration rates and soluble C concentrations in the rhizosphere (Table 3). Root respiration and rhizo-microbial respiration contributed 40.6 and 59.4% of total rhizosphere respiration, respectively. Soluble C concentration in the rhizosphere of 3-week old wheat plants was 667 mg C l $^{-1}$ of soil water (Table 3).

All these calculations were based on data produced from one short (10 or 20 min) pulse of $^{14}\text{CO}_2$ assimilated into the plants. Therefore the values are all related to the current photosynthate and merely point measurements at a certain time and under certain conditions. They cannot be extrapolated to represent older C already incorporated into plant tissues before the experiment or younger C assimilated later.

The soluble-C value is very sensitive to changes of the $^{14}\text{CO}_2$ evolution rates from either the glucose treated or water additional control. A 5% increase of $^{14}\text{CO}_2$ evolution rate from the lower glucose-added (1.19 g C l $^{-1}$) treatment will result in an 80.8% increase in soluble-C value, and only 4% change in the split of total rhizosphere respiration between root respiration and rhizo-microbial respiration if other values remain unchanged. If the standard error associated with the $^{14}\text{CO}_2$ evolution rate of glucose-I in Table 3 is used in analysis, the 90% confidence interval is 0.29–1.292 for soluble-C and 35.6–43.6% for root respiration as a proportion of that in the water addition. This is mainly due to our experimental design which involved the use of two different

concentrations of glucose addition, about 2 and 10 times higher than the soluble-C concentration in the rhizosphere. We used this design to produce different $^{14}\text{CO}_2$ evolution rates from either the glucose treated or water additional control and relatively stable glucose concentrations in the root–soil columns. Inherently, high experimental accuracy is required to determine the concentration of soluble-C in the rhizosphere if this equation is to be used.

Using a selective inhibitor (*p*-hydroxy-mercuri-phenyl-sulphonate) to suppress rhizo-microbial respiration in conjunction with continuous ^{14}C -labelling, Helal and Sauerbeck (1991) have reported that root respiration contributed 24 and 16% of total rhizosphere respiration for bean and corn plants, respectively, and the remainder was rhizo-microbial. Due to the differences in experimental procedures it is difficult to relate their values to ours. Values of rhizo-microbial respiration estimated or extrapolated based on results from solution culture experiments (Warembourg and Billes, 1979; Minchin and McNaughton, 1984) are not comparable with ours because of methodological differences between the studies. Furthermore, all these values are probably condition-specific and phenology-dependent.

The soluble C concentration in the rhizosphere of these wheat plants was about 20–100 times higher than previous estimates used in rhizosphere models (Newman and Watson, 1977; Darrah, 1991a, b). Our results indicate a highly C-rich environment in the rhizosphere, and that serious limitations may be inherent in the rhizosphere models of Newman and Watson (1977) and Darrah (1991a, b) which were based on the assumption that soluble C supply was the sole controlling factor for microbial growth.

This procedure opens up new opportunities for research in the areas of root respiration physiology, plant–soil interactions and rhizosphere ecology. With this procedure we can now move some of our studies from mostly solution cultures to true soil conditions. This advancement is not only important to root physiology but also significant for studies of terrestrial C cycling (Van Veen *et al.*, 1991; Anderson, 1992).

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