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IS AVAILABLE CARBON LIMITING MICROBIAL RESPIRATION IN THE RHIZOSPHERE?

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Summary—It is widely known that the carbon availability in the rhizosphere is much higher than in the bulk soil. However, studies have failed to show whether microbial respiration in the rhizosphere is carbon-limited. Precise and timely measurements are lacking. We measured carbon availability index (basal respiration divided by substrate-induced respiration), and water soluble carbon in soils sampled at four spatial points (rhizoplane, rhizosphere, bulk soil and root-free soil) in the rhizosphere continuum in greenhouse and field experiments. Carbon availability index and water soluble carbon were inversely related to the relative distance from the root surface, with several times higher concentrations in the rhizoplane soils. Microbial respiration was not limited by available carbon in the rhizoplane and in the rhizosphere. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Many studies have quantified and characterized soluble root exudates in artificial growth systems (Rovira and Davey, 1974; Vancura and Stanek, 1975; Hale and Moore, 1979). Recently carbon input via roots into the soil system has been investigated in more realistic growth chamber systems using ^{14}C labelling techniques (Barber and Martin, 1976; Johnen and Sauerbeck, 1977; Martin, 1977; Whipps and Lynch, 1983; Whipps, 1984; Merckx *et al.*, 1985; Whipps, 1987; Liljeroth *et al.*, 1990; Kuikman *et al.*, 1991). Our knowledge of the rhizosphere carbon economy has considerably increased (Whipps, 1990). However, the issue of carbon availability or limitation on microbial activities in the rhizosphere remains to be fully investigated. The amount of available carbon has been assumed to be the only controlling factor for microbial growth in the rhizosphere by several rhizosphere models (Newman and Watson, 1977; Darrah, 1991a,b). Available carbon supply might not be the most limiting factor to microbial growth in the rhizosphere as suggested by Helal and Sauerbeck (1986). This is based on the fact that the conversion of plant carbon into microbial biomass in the rhizosphere is very low, i.e. 13%. The mineral nutrient supply in the rhizosphere may severely limit microbial growth and activities (Van Veen *et al.*, 1989). The actual concentration of soluble carbon in the rhizosphere can be two orders of magnitude higher than has been assumed in the rhizosphere models (Cheng *et al.*, 1993).

In this paper we report results from three small-scale experiments. The objectives of this study were: (1) to answer the question of carbon availability or limitation to microbial activity in the rhizosphere continuum from the rhizoplane to root-free soil; (2) to investigate the distributions of water-soluble carbon concentration in the rhizosphere continuum. To meet the first objective, we employed the concept of carbon availability index (CAI: the ratio of basal respiration to substrate-induced respiration) (Parkinson and Coleman, 1991). To obtain samples from the rhizosphere continuum, we used membrane columns to exclude roots in the first pot experiment, and membrane cells to enclose roots in the second pot experiment. For the purpose of comparison, we carried out the third experiment using soil blocks taken directly beneath field-grown maize plants.

MATERIALS AND METHODS

Membrane column experiment

Soil used in this experiment 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 well-drained sandy clay loam floodplain soil (siliceous thermic Rhodic Kanhapludult, 66% sand, 13% silt, 21% clay, pH 6 in water) 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%,

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and 57.3 $\mu g\ g^{-1}$, respectively. Its water holding capacity is 35% w/w. The soil was sieved (< 2 mm), homogenized, and air-dried before use.

Winter wheat (*Triticum aestivum*, Var: GA Gore) seeds were planted around a Versapor 3 μm membrane (Gelman Sciences Inc., Ann Arbor, MI 48106, U.S.A.) column (filled with 200 g soil) at the center of 20 cm dia pots filled with 3 kg of air-dried soil (Fig. 1(a)). The planted pots were placed in a plant growth chamber with a day–night cycle of 14 h day and 10 h night. The photosynthetic active photon flux at the top of the canopy was 800 μmol s^{-1}. Air temperature in the growth chamber was 22°C during day time and 14°C during night time. The pots were watered daily to 80% water holding capacity.

After 20 days of growth five replicate pots were destructively sampled for rhizoplane soil, rhizosphere soil, bulk soil and root-free soil. Root-free soil was taken from the center of the membrane column. Bulk soil was taken from the homogenized soil not attached to roots but within the rooting zone. Rhizosphere soil was the soil loosely attached to the root system, removed by hand shaking. The soil which was firmly attached to the root system and did not fall off after hand shaking was the rhizoplane soil, which was washed off in 100 ml deionized water in a beaker. During sampling, we took special precautions to keep the root system together as much as possible in order to minimize root damage.

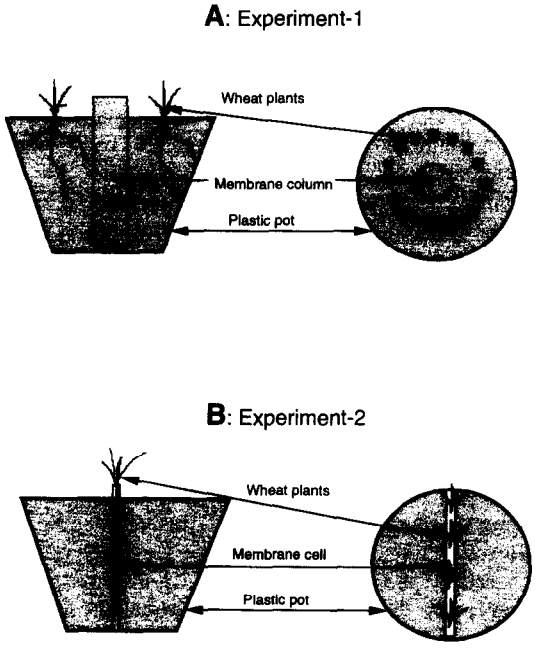

Fig. 1. Designs of membrane cells and columns. (**A** the membrane column experiment; **B** the membrane cell experiment.)

Basal respiration (**BR**) and substrate-induced respiration (**SIR**) of all soils were measured 45 min after the start of the destructive sampling using the measuring system of Cheng and Virginia (1993). Briefly, the measurements of CO_2 concentrations in the air flowing out of the incubating flasks were carried out every 20 min for 2 h after the samples were connected to the testing system. Both BR and SIR were measured at 22 ± 0.5°C with 10 g fresh soil in each incubating flask. The glucose concentration used in the SIR measurement was 30 mg glucose ml^{-1} of soil water. Water was added to the soil sample for BR measurement to bring the moisture content to the same level as the SIR. BR and SIR of rhizoplane soil was measured by pouring 30 ml of well-mixed slurry in a long test tube with an airstone dispensing air at the bottom of the tube. The amount of soil in the slurry was measured by filtration after the test. The total amount of soil washed off the root system of each replicate pot was determined by filtration after all tests were done, and ranged from 9.77 g to 13.54 g. The dry weight of the washed root system of each replicate pot ranged from 0.59 g to 0.86 g. The Carbon availability index (**CAI**) was obtained by dividing BR by SIR. Water soluble carbon was extracted immediately using 10 g of fresh soil with 30 ml of deionized water and 30 min shaking on a shaker. Water soluble carbon in the root washing suspension of rhizoplane soil was measured by filtering a 30 ml subsample of the suspension and the extracts were measured on a Shimadzu TOC analyzer.

Membrane cell experiment

The soil, the plant species, the growth conditions and all the measurements in this experiment were the same as in the membrane column experiment. The difference was the design of the membrane bags. In this experiment, membrane cells (see Fig. 1b) were used to keep plant roots inside the cells. Each membrane cell was filled with 50 g air-dried soil. There were four replicate pots in this experiment.

In this experiment, root-free soil was taken from the soil blocks 6 cm away from both sides of the membrane cell. Bulk soil was the thin (3 mm) soil layer contacted on both sides of the membrane cell. The rhizosphere soil was the soil loosely attached to the root system inside the membrane cell. The soil firmly attached to the root system and not loosened after hand shaking was the rhizoplane soil, which was washed off in 100 ml deionized water in a beaker. The total amount of soil washed off the root system of each replicate pot ranged from 3.73 g to 6.04 g. The dry weight of the washed root system of each replicate pot ranged from 0.45 g to 0.72 g.

Field maize experiment

Three soil blocks (30 × 30 × 20 cm) were taken from one plowed plot at the Horseshoe Bend Experimental Field, University of Georgia. Each of the soil blocks had a field-grown maize (*Zea mays*) plant (just after flowering) at the center. It took 10 min to transport the sampled soil blocks to the laboratory where the blocks were separated. The soil in the plot was identical to that described in the membrane column experiment. Rhizoplane, rhizosphere and bulk soils were separated in the same manner and time frame as described in the membrane column experiment. The amount of soil washed off the root system of each replicate block ranged from 2.08 g to 4.23 g. Due to the small amount of rhizosphere soil collected, BR of rhizoplane, rhizosphere and bulk soils were measured after 40 min incubation, then SIR was measured after adding glucose solution to the same sample used in BR measurement. Water-soluble C and CAI of rhizoplane, rhizosphere and bulk soils were measured by the same procedures described in the membrane column experiment.

There were five replicate pots in the membrane column experiment, four replicates in the membrane cell experiment, and three replicate blocks in the field maize experiment. The statistical procedure of the student's t-test was applied for mean comparisons.

RESULTS AND DISCUSSION

The manipulations in the membrane column experiment and the membrane cell experiment were successful. No roots were found either inside the membrane columns or outside them. The wheat plants were healthy and uniform.

In the two growth chamber experiments, both SIR and BR rates in the rhizoplane soils declined rapidly once the soils were separated from the root surface (Fig. 2), indicating other factors may have become limiting, but not available carbon since adding glucose did not either change the declining trend or increase the respiration rates. This may be caused by changes in physical, chemical and biological conditions unique to the rhizoplane due to the destructive sampling. Further studies are needed to fully understand this phenomenon.

The basal respiration rates of the rhizosphere soils from the two pot experiments declined linearly during the 2 h period of measurements, but the SIR rates in the rhizosphere soils remained stable. This indicated that available carbon in the rhizosphere soils was utilized and became limiting once these soils were separated from the roots. Our time-course data indicated that the amount of available carbon changed quickly after the destructive sampling. It is critical to carry out the measurements as quickly as possible if they are to be relevant to the undisturbed

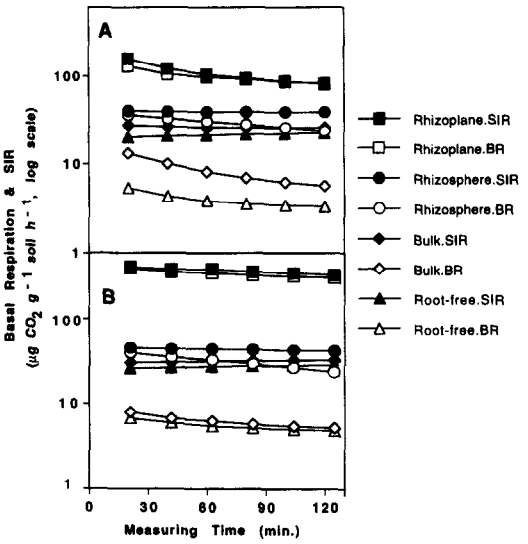

Fig. 2. Time-course of basal respiration (BR) and substrate-induced respiration (SIR) in the rhizosphere continuum of wheat plants grown in pots. (**A** the membrane column experiment; **B** the membrane cell experiment.)

systems. Any delay in handling time can lead to marked changes in the measured values.

The ratio of BR to SIR was used to give a quantitative measure of carbon availability (Parkinson and Coleman, 1991), or carbon availability index (CAI) based on the fact that the only difference between BR and SIR was the addition of available carbon in SIR. Microbial respiration was not limited by available carbon if the ratio, or CAI, was close to 1, and was limited by available carbon when CAI was less than 1.

The concept of CAI here is related to the concept of metabolic quotient for CO_2 (qCO_2) (Anderson and Domsch, 1985, 1993). If the microbial biomass component in qCO_2 is determined by the SIR method (e.g. Anderson and Domsch, 1978) and if the conditions in determining the BR and the SIR are set to be the same, qCO_2 can be linearly correlated with CAI because the SIR method is based on the linear regression with microbial biomass values obtained by using the fumigation–incubation method (Jenkinson and Powlson, 1976). But they are different in several aspects. CAI, an index of carbon availability without any unit, is different from qCO_2 which is basal respiration rate (e.g. mg $CO_2 - C\ h^{-1}$) per unit of microbial biomass (e.g. mgC). Secondly, most of the qCO_2 values reported in the literature were calculated using BR values measured after a pre-incubation or conditioning time ranging from 15 to 48 h; whereas the BR values in this study were determined without pre-incubation. CAI is mainly used to assess the carbon availability to microbial populations in a soil sample at the time of the measurement. Whereas

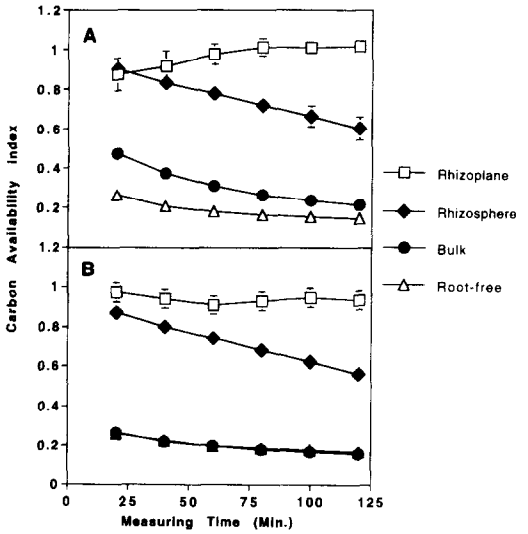

Fig. 3. Time-course of carbon availability indexes (BR/SIR) in the rhizosphere continuum of wheat plants grown in pots. (**A** the membrane column experiment; **B** the membrane cell experiment.) (Error bars are 2 standard errors; some error bars are invisible.)

qCO_2 has mostly been used as a specific activity property for a soil sample in order to elucidate the effects of different environmental factors.

The CAIs of the rhizoplane soils from the two pot experiments were close to 1 during the measuring period, indicating that carbon was not limiting microbial respiration (Fig. 3). The CAI of the rhizosphere soils from the pot experiments declined linearly during the 2 h with the initial values close to 1, indicating that carbon was not limiting microbial respiration initially, but became limiting during the time of the measurements. Readily-available carbon sources were rapidly utilized by microbes once rhizosphere soils were separated from roots, indicated by the rapidly declining trend of carbon availability indexes of rhizosphere soils in both pot experiments. The CAI values of the rhizoplane and the rhizosphere soils in the field maize experiment were close to 1 (Fig. 4), indicating that available carbon was not a limiting factor for microbial respiration in the rhizoplane and the rhizosphere soils in the field maize experiment. If the destructive sampling did not release significant amounts of available C, these results support the proposition (Helal and Sauerbeck, 1986) that the microbial activities in the immediate rhizosphere (i.e. rhizoplane and rhizosphere soil) are not limited by the available carbon source. However, they do not support the assumption (Newman and Watson, 1977; Darrah, 1991a,b) that the amount of available carbon is the sole controlling factor for microbial growth in the rhizosphere.

The destructive sampling used in this study could confound the interpretation of the results because it potentially could cause an increase of available C due to root injuries. In order to minimize root injuries, we took special precautions in our sampling procedures. We did not separate the root system into pieces. Instead, we kept the root system intact as much as possible, hence the amounts of available C released due to root injuries should be low. A separate study on this issue is needed to further clarify the degree of confounding by destructive sampling.

The field maize experiment was carried out as a point of reference to the pot experiments. Direct comparison between the two types of experiments was neither intended nor valid due to the differences in plant species, plant age and other factors.

The CAIs of the bulk soils and the root-free soils from the two pot experiments were much less than 1, and declined slowly during the test period, indicating that carbon was highly limiting.

Water-soluble C concentrations radically declined from the rhizoplane outwards in all three experiments (Fig. 5). The rhizoplane-to-rhizosphere (**RP:R**) ratio of water-soluble carbon was 6.11 in the membrane column experiment, 12.56 in the membrane cell experiment, and 8.60 times in the field maize experiment. The rhizosphere-to-bulk soil

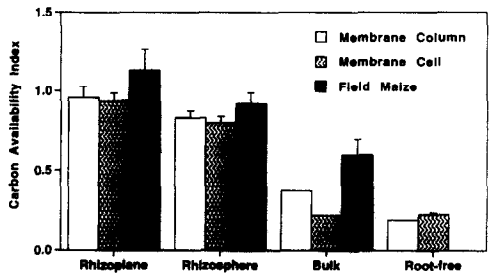

Fig. 4. Carbon availability indexes in the rhizosphere continuum. Measuring time commenced after 40 min. (Error bars are 1 standard error; some error bars are invisible.)

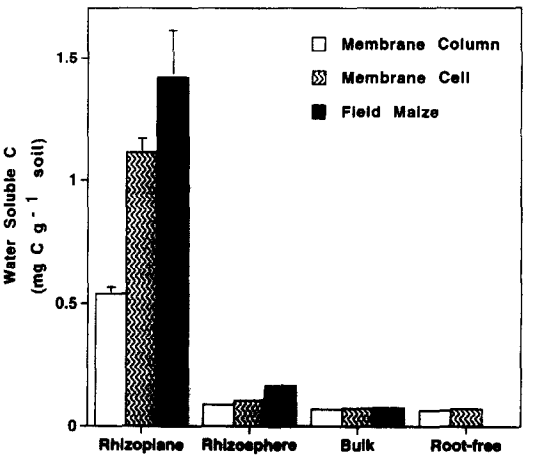

Fig. 5. Water soluble carbon in the rhizosphere continuum. (Error bars are 1 standard error; some error bars are invisible.)

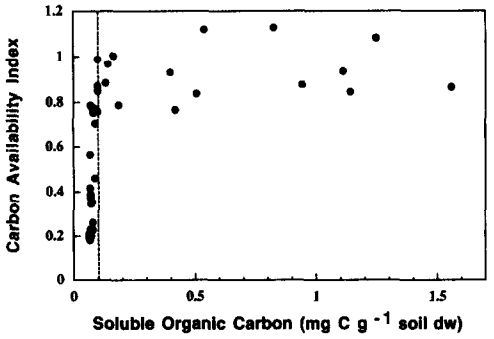

Fig. 6. Relationship between water soluble carbon concentrations and carbon availability indexes. The dotted line indicates the value of the saturation threshold (approximately 0.1 mg C g^{-1} of soil dw).

(**R:B**) ratio of water-soluble carbon was 1.26 in the membrane column experiment, 1.42 in the membrane cell experiment, and 2.20 in the field maize experiment. The bulk-to-root-free (**B:RF**) ratio of water-soluble carbon was 1.06 in the membrane column experiment, and 1.03 in the membrane cell experiment. In all three experiments there were sharper drops in the concentrations of water-soluble C from rhizoplane to rhizosphere than from rhizosphere to bulk soils. The most obvious case was in the membrane cell experiment where roots were confined within the membrane cells.

The relationship between CAI and water-soluble carbon concentrations was not linear (Fig. 6). There was a sharp turning point, or a threshold of saturation when water-soluble carbon concentration was at 0.1 mg C g^{-1} of soil. The values of CAI varied dramatically when water-soluble carbon concentrations ranged from 0 to 0.1 mg C g^{-1} of soil, and varied around 1 when water-soluble carbon concentrations ranged from 0.1 to 1.7 mg C g^{-1} of soil. This result indicated that microbial respiration was not limited by available carbon when water-soluble carbon concentrations were close to or higher than 0.1 mg C g^{-1} of soil in these experimental settings.

The values of water-soluble carbon in the rhizoplane soils were 0.538, 1.319, and 1.419 mg C g^{-1} of soil for the membrane column experiment, the membrane cell experiment, and the field maize experiment, respectively (Fig. 5). These values were much higher than 0.1 mg C g^{-1} of soil, the saturation threshold for microbial respiration. However, these values were much lower than the *in situ* value of 2.67 mg C g^{-1} of soil obtained in our previous wheat experiment (Cheng *et al.*, 1993) which was calculated based on an isotopic trapping procedure. These results were expected for the following two reasons. First, as the result of the destructive sampling in this study, the flow of root exudates into the surrounding soil was discontinued during the sample preparation and extraction, which could cause a rapid decrease in water-soluble carbon concentration due to the microbial uptake as has been shown above (Fig. 3). Second, the isotopic trapping procedure used in the previous study only measures the soluble carbon concentration in the rhizosphere of actively exuding roots or the immediate hot spots, not the average rhizosphere of all roots as measured in this study. The fact that the soluble C concentration values of the rhizoplane soils in this study were much lower than the values obtained *in situ* by using the isotopic trapping procedure in our previous wheat experiments (Cheng *et al.*, 1993) indirectly supports our belief that the amount of available C released due to root injuries was probably low.

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