

Measurement of rhizosphere respiration and organic matter decomposition using natural ^{13}C

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Abstract

Due to the limitations in methodology it has been a difficult task to measure rhizosphere respiration and original soil carbon decomposition under the influence of living roots. ^{14}C -labeling has been widely used for this purpose in spite of numerous problems associated with the labeling method. In this paper, a natural ^{13}C method was used to measure rhizosphere respiration and original soil carbon decomposition in a short-term growth chamber experiment. The main objective of the experiment was to validate a key assumption of this method: the $\delta^{13}\text{C}$ value of the roots represents the $\delta^{13}\text{C}$ value of the rhizosphere respiration CO_2 . Results from plants grown in inoculated carbon-free medium indicated that this assumption was valid. This natural ^{13}C method was demonstrated to be advantageous for studying rhizosphere respiration and the effects of living roots on original soil carbon decomposition.

Introduction

Carbon dioxide released by a system of living roots and soil has three origins: (1) root respiration; (2) microbial respiration utilizing carbon from live roots (rhizomicrobial respiration); and (3) microbial respiration using original soil carbon. Total rhizosphere respiration is defined as the sum of root respiration and rhizomicrobial respiration. Carbon used in total rhizosphere respiration is all derived from the living plants. Virtually all reported total rhizosphere respiration and original soil carbon decomposition have been quantified by either continuous ^{14}C -labeling (Barber and Martin, 1976; Merckx et al., 1987; Whipps and Lynch, 1983) or pulse-labeling (e.g. Cheng et al., 1993, 1994; Swinnen et al., 1994). However, both of these ^{14}C labeling methods have limitations. Continuous ^{14}C -labeling requires special facilities which are limited to a few places in the world. It often requires transplanting of seedlings, which may have considerable unlabelled food reserves and it may take some time for all plant parts to become evenly labeled. There are safety concerns due to the use of radioactive materials and are mostly applied to

experiments of short duration, several months at the maximum.

This paper reports a natural ^{13}C method for measuring total rhizosphere respiration and original soil carbon decomposition under the influence of living roots. Natural ^{13}C methods have been used in many studies of soil carbon dynamics (e.g. Balesdent et al., 1987; McPherson et al., 1993; Wedin et al., 1995). The principle of the ^{13}C natural tracer method is based on the differences in $^{13}\text{C} : ^{12}\text{C}$ ratios (often reported in $\delta^{13}\text{C}$ values) between plants with the C3 photosynthetic pathway, whose mean $\delta^{13}\text{C}$ is -27‰, and plants with the C4 pathway, whose mean $\delta^{13}\text{C}$ is -12‰ (Smith and Epstein, 1971). Similarly, there are subsequent differences in the $\delta^{13}\text{C}$ values between soil organic matter derived from the two types of plants. Soil organic matter derived from C4 plants (C4-derived soil) such as continuous corn fields has $\delta^{13}\text{C}$ values ranging from -12‰ to -14‰, whereas $\delta^{13}\text{C}$ values of soil organic matter derived from cold and temperate forests (C3-derived soil) range from -24‰ to -29‰. If one grows C3 plant in a C4-derived soil, the carbon entering the soil via roots will have a different $\delta^{13}\text{C}$ value than the $\delta^{13}\text{C}$ value of the soil. Based on Cerri et al. (1985), the following equation can be used to partition soil-derived

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C₄-carbon from plant-derived C₃-carbon:

$$C_3 = C_t \frac{\delta_t - \delta_4}{\delta_3 - \delta_4} \quad (1)$$

where $C_t = C_3 + C_4$, is the total carbon from below-ground CO₂, C_3 is the amount of carbon derived from C₃ plants, C_4 is the amount of carbon derived from C₄ soil, δ_t is the $\delta^{13}\text{C}$ value of the C_t carbon, δ_3 is the $\delta^{13}\text{C}$ value of the C₃ plant carbon, and δ_4 is the $\delta^{13}\text{C}$ value of the C₄ soil carbon.

The primary assumption of this method is that the total rhizosphere CO₂ has the same $\delta^{13}\text{C}$ value as that of roots, i.e. isotopic fractionation does not occur during rhizosphere respiration. One of the main objectives of this study was to validate this assumption by determining whether or not isotopic fractionation occurs during rhizosphere respiration. It has been shown that isotopic fractionation occurs in several plant-related processes and some microbial processes. Root tissues may be either depleted or enriched in ^{13}C (about 1‰–2‰) compared to shoots (Wedin et al., 1995). Lignin fractions of plant tissues are generally depleted in ^{13}C (about 3‰) compared to bulk tissues as a whole possibly due to isotopic fractionation during lignin synthesis (Benner et al., 1987; Wecin et al., 1995). Isotopic fractionation seems to occur during the decomposition of some organic materials (roots, root mucilage, and glucose) when these organic materials were mixed with pure sands, inoculated, and incubated under laboratory conditions (Mary et al., 1992). Therefore, the issue of isotopic fractionation is critical for all ^{13}C -related methods.

Materials and methods

Soil used in this experiment was collected from the 0–15 cm depth in the plowed continuous corn plots of the Wooster Long-term Tillage and Rotation Experiment site near Wooster, Ohio, USA. Detailed descriptions about the soil and the management practices were given by Dick (1983, 1984) and Dick et al. (1991). Briefly, the soil is a silt loam (fine, mixed, mesic Typic Fragidalf). The continuous corn plots have been maintained for 31 years.

A total of 15 plastic (polyvinyl chloride, or PVC) containers (32 mm ID, 180 mm in height) were made. Each container was closed at the bottom with a rubber stopper and has an air inlet and air outlet consisting of clear plastic tubing (Figure 1). Ten containers were filled with 80 grams of air-dried corn field soil mixed

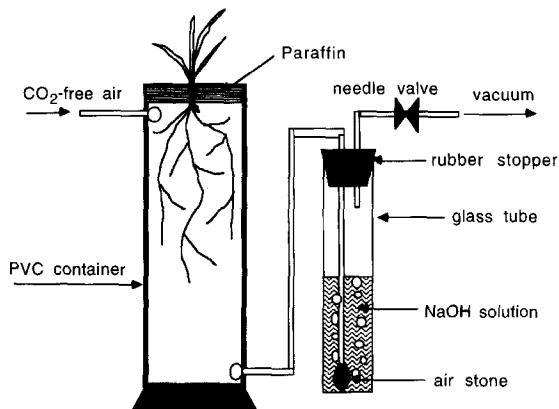


Figure 1. Design of the apparatus used in the experiment.

with 15 grams of vermiculite. Another 5 containers were filled with 100 grams of sand-vermiculite mixture plus 1 gram of soil as inoculant. To eliminate carbon in the sand and vermiculite, the sand and vermiculite were combusted at 500 °C for 4 hours before use. Five soil-filled containers and 5 sand-filled containers were planted with winter wheat (*Triticum aestivum*). Another five soil-filled containers were not planted, and used as non-vegetated controls. All containers were kept in growth chamber with a 14 hour light and 10 hour dark photo-period, 22 °C day-time temperature and 15 °C night temperature, and 40% relative humidity. The photosynthetic active photon flux density at the top of the canopy was approximately 800 $\mu\text{E m}^{-2} \text{s}^{-1}$. The water content of each container was checked daily by weighing and maintained at 80% water holding capacity by watering with deionized water (soil-filled containers) or Hoagland solution (for containers filled with sand plus vermiculite).

On the 13th day after emergence, the top of each container was sealed with low melting point Paraffin (m.p. 42–43 °C). 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 brought to 80% of its water holding capacity. During the 3-day period from the 14th to the 16th day after emergence, CO₂ evolved from the belowground components was trapped in 50 mL of 0.5 M NaOH solution by pulling CO₂-free air through the container (Figure 1). The trapping efficiency of this method was higher than 99.9% (Cheng and Coleman, 1989). Five replicates of CO₂-free air blanks and 3 replicates of normal air were also included in the CO₂ trapping. By the end of the 3-day trapping period, all containers were checked for possible air leaks

by pumping air into the container and submerging the whole container in water. None of the containers had air leaks. Total CO₂ trapped was determined by titrating 20 mL of the NaOH solution with 0.117 M HCl standard solution after adding 5 mL of 1 M BaCl₂ solution using phenolphthalein indicator. The remaining 30 mL of the trapping solution was mixed while 10 mL of 1 M BaCl₂ solution. The BaCO₃ particulates were washed with deionized water five times under CO₂-free conditions and dried at 105 °C in an oven. The δ¹³C value of the BaCO₃ obtained from each sample was determined with a Europa Scientific Tracermass Stable Isotope Analyser (Europa Scientific Ltd., Crewe, England) following combustion of the sample in a Carlo Erba CN Analyzer (Model NA1500, Carlo Erba Strumentazione, Rodano, Italy).

On the 17th day after emergence, all containers were destructively sampled. Roots were washed on a 0.1 mm screen. Shoots were cut at the base of each plant. Shoots, roots and soil samples were dried at 70 °C in an oven for at least 48 hours before weighing. Dry root, shoot and soil samples were pulverized in a ball mill before analysis for δ¹³C. The δ¹³C values of roots, shoots and soils were measured using the same equipment and procedure as for BaCO₃. Total belowground CO₂ from each container was separated into plant-derived carbon and soil-derived carbon using Equation (1).

Results and discussion

Belowground CO₂ from the sand+vermiculite treatment was mostly due to rhizosphere respiration since there were no other carbon sources in the container except for the small amount of soil inoculant (1 gram). For the sand+vermiculite treatment, the δ¹³C value of belowground CO₂ (total rhizosphere respiration) was -27.3‰, virtually the same as the δ¹³C value of root tissue (-27.1‰) (Table 1). This result indicated that isotopic fractionation did not occur during the rhizosphere respiration process for the young wheat plants. Therefore the assumption used in the above mentioned equation is valid. However, it is theoretically possible that isotopic fractionation did occur during both root respiration and rhizosphere microbial respiration if the directions of the fractionalization in the two processes were opposite to each other and totally canceled out. Literature on these aspects are scarce. More studies are needed to further investigate this possibility.

In a laboratory study, Mary et al. (1992) reported that isotopic fractionation occurred during the decomposition of maize roots, root mucilage, and glucose, and that the degree of fractionation varied during their 50-days incubation. The controversy between their results and the results of this study might have been caused by the differences in the designs and the conditions between the two studies. The isotopic fractionation was found between the original organic substrate and the CO₂ evolved from a microbial batch culture of the organic substrate (i.e., roots, mucilage or glucose) mixed with sands and mineral nutrients in their study. Whereas the microbial component of the total rhizosphere CO₂ was from the microbial metabolism of root-derived materials (mainly root exudates) in this study. The organic substrates were given as one batch at the beginning of their incubation. The supply of root exudates to the rhizosphere microorganisms was through continuous in this study. This difference in substrate supply might have created different types of metabolism and different microbial communities. It is commonly known that microorganisms change phases of growth in batch cultures, which may induce changes in metabolism and community structure. Another major difference between these two studies was the methods used to trap CO₂. Static alkaline absorption was used in the study of Mary et al. (1992). The static absorption technique could potentially cause isotope fractionation due to the kinetic isotope effect (Fritz et al., 1985). In this study CO₂ was trapped by using an open gas exchange system with an nearly 100% trapping efficiency, so that potential kinetic isotope fractionation was avoided.

The δ¹³C value of total soil carbon from the unplanted treatment was -20.5‰. The δ¹³C value of CO₂ evolved from the unplanted treatment was -12.2‰, very similar to the δ¹³C value of C4 plant tissues. These results indicated that there was still a substantial amount of old C3 carbon in this soil after 31 years of continuous corn (C4 plant) cultivation while virtually all short-term CO₂ carbon came from the newer carbon source of C4 plants (corn). The newer C4 carbon has replaced the old C3 carbon in the active fractions, but not in the inert humus fractions. These results were in agreement with existing literature which suggested that the half-life of this inert carbon might be in the order of thousands of years (Jenkinson and Rayner, 1977; Parton et al., 1987). Therefore it was not surprising that nearly all short-term CO₂ came from the newer C4 carbon. The δ¹³C value of respiratory CO₂ from a field of 7 years of continuous maize

Table 1. Separation of plant-derived C from soil-derived C in total belowground CO₂ using natural ¹³C tracer method in a growth chamber experiment. Numbers are the means of 5 replicates with standard errors in parentheses

Measurements	Treatments		
	Soil only	Soil+plant	Sand+plant
<i>δ¹³C-values (‰)</i>			
Roots	-	-27.4 (0.15)	-27.1 (0.34)
Belowground CO ₂	-12.2 (0.26)	-25.6 (0.24)	-27.3 (0.43)
Soil-C	-20.5 (0.14)	-20.3 (0.18)	-
<i>Belowground CO₂ (mg CO₂ container⁻¹ d⁻¹)</i>			
Total	2.44 (0.06)	13.88 (0.37)	10.59 (0.59)
Rhizosphere	-	12.35 (0.35)	10.59 (0.59)
Soil-derived	2.44 (0.06)	1.53 (0.25)	-

was quite similar to the $\delta^{13}\text{C}$ value of a typical C4 plant (Schönwitz et al., 1986). Vitorello et al. (1989) reported that the $\delta^{13}\text{C}$ value of total soil organic C in a 50 year old continuous sugar cane field converted from tropical forest was -20.2‰, which is close to the value of -20.5‰ for the soil used in this study. The $\delta^{13}\text{C}$ value of total soil organic C in the 0–20 cm soil layer in a 100 year old maize field originally converted from a tallgrass prairie was -20.2‰ (Balesdent et al., 1988).

During the three day trapping period (14–16 days after emergence), belowground CO₂ from soil only plus that from sand+plant was about equal to that from soil+plant treatment (Table 1), which might suggest that the plant component and the original soil carbon component in the total belowground CO₂ pool were additive, not interactive, indicating that no priming or conserving effect occurred. However, following analysis using the natural ¹³C method indicated that the presence of living roots suppressed the decomposition of original soil carbon.

Growing wheat (C3 plant) in the soil from a continuous corn field decreased the $\delta^{13}\text{C}$ values of total belowground CO₂ from -12.2‰ (soil only) to -25.6‰ (soil+plant), indicating that the majority of the CO₂ was from C3 carbon sources of either rhizosphere respiration of the wheat plants or the original old C3 carbon in the soil. The contribution of the old C3 soil carbon to this short-term CO₂ should be negligible because nearly all short-term CO₂ came from the newer C4 soil carbon in the unplanted treatment unless the presence of plant roots significantly stimulated the decomposition of the old soil organic matter, which

is highly unlikely since the presence of living roots depressed original soil carbon decomposition.

Assuming that all C3 carbon in the total belowground CO₂ came from the current wheat plants, we can calculate the total rhizosphere respiration rate and the original soil carbon decomposition rate in the soil+plant treatment using Equation (1). The rate of total rhizosphere respiration was 12.35 mg CO₂ container⁻¹ day⁻¹, which accounted for 89% of the total belowground respiration. The original soil carbon decomposition rate was 1.53 mg CO₂ container⁻¹ day⁻¹, or 11% of the total belowground respiration. This rate (1.53 mg CO₂ container⁻¹ day⁻¹) obtained under the influence of living roots was much lower than the rate (2.44 mg CO₂ container⁻¹ day⁻¹) without the influence of living roots in the unplanted treatment. The difference represented a 37.3% decrease in original soil carbon decomposition rate due to the presence of living roots. Therefore, the plant component and the original soil carbon component in the total belowground CO₂ pool were not additive, but interactive. Similar results have been obtained from ¹⁴C-labeling experiments. Decomposition of labeled plant material was markedly lowered in the presence of cultivated plant cover or in natural grasslands when compared to bare soil controls (Shields and Paul, 1973; Jenkinson, 1977). When ¹⁴C-labeled plant material was decomposed in soil planted with maize, ryegrass, wheat or barley, ¹⁴CO₂ release from the soil was reduced compared to bare soil controls during experiments under controlled conditions (Reid and Goss, 1982; 1983; Sparling et al., 1982). It was generally suggested that this negative effect of living roots on soil organic mat-

ter decomposition was probably due to the competition between the living roots and the rhizosphere microflora for substrates and due to the changed physical and chemical conditions by roots. In contrast, a stimulatory effect of living roots on soil organic matter decomposition was also reported based on experiments using ^{14}C -labeling methods (Cheng and Coleman, 1990; Helal and Sauerbeck, 1984; Sallih and Bottner, 1988). The break-down of soil aggregates and the stimulation of rhizosphere microflora were suggested to be the cause of this phenomenon. Obviously the effects of living roots on soil organic matter decomposition remains controversial.

This paper has demonstrated that the natural ^{13}C method can be used to study total rhizosphere respiration under true soil conditions and original soil organic carbon decomposition under the influence of living roots. The fundamental assumption of this method, i.e. the $\delta^{13}\text{C}$ value of the roots represents the $\delta^{13}\text{C}$ value of the rhizosphere resired CO_2 using plant-derived carbon, has been validated in a short-term growth chamber experiment. For this method to be applicable under broader conditions, further validation of the assumption is required.

This method has some shortcomings. It has low sensitivity and low resolution due to both the natural variation of $\delta^{13}\text{C}$ values in plant or soil samples and the relatively small difference in $\delta^{13}\text{C}$ value between C3 and C4 plants. Assuming that the standard deviation of replicated samples of plant tissues, soils, or CO_2 for ^{13}C analysis is 0.3‰ which is not uncommon, and assuming that the difference between the $\delta^{13}\text{C}$ value of a pure C4 source and the $\delta^{13}\text{C}$ value of a pure C3 source is 15‰ (i.e. 27‰ – 12‰), the coefficient of variation (CV) of the measurement will be 2%. This means that a change of one standard deviation of the $\delta^{13}\text{C}$ values represents a 2% change in the separation between C3-derived C and C4-derived C. Any statistically significant difference between means requires at least two standard deviations. Therefore the highest resolution or sensitivity of this natural ^{13}C method should be about 4% of the total soil CO_2 . Whereas the resolution of ^{14}C continuous labeling method is not constrained by these factors, and is rarely a concern in practice. The natural ^{13}C method is limited to C3 plants on C4-derived soils or vice versa. Sample analysis for natural ^{13}C is relatively slow and expensive. The natural ^{13}C method, however, has several advantages compared to ^{14}C -labeling of either the plants or the soil. First, both the plants and the soil are uniformly and naturally labeled, so that the problem of non-

uniformity in the ^{14}C -labeling approach is eliminated. Second, there are no radiation safety problems. Third, labeling procedure is not required for this method since the ^{13}C tracer happens naturally.

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