

# Rhizosphere Effects on Decomposition: Controls of Plant Species, Phenology, and Fertilization

Weixin Cheng,\* Dale W. Johnson, and Shenglei Fu

## ABSTRACT

Plant species and soil fertility presumably control rhizosphere effects on soil organic matter (SOM) decomposition, but qualitative and quantitative descriptions of such controls are still sparse. In this study, rhizosphere effects of soybean [*Glycine max* (L.) Merr.] and spring wheat (*Triticum aestivum* L.) on SOM decomposition were investigated at four phenological stages under three levels of fertilization in a greenhouse experiment using natural  $^{13}\text{C}$  tracers. The magnitude of the rhizosphere effect ranged from 0% to as high as 383% above the decomposition rate in the no-plant control, indicating that the rhizosphere priming can substantially intensify decomposition. The rhizosphere priming effect was responsible for a major portion of the total soil C efflux. Cumulative soil C loss caused by rhizosphere effects during the whole growing season equated to the amount of root biomass C for the soybean treatment, and 71% of root biomass C for the wheat treatment. Different plant species produced significantly different rhizosphere priming effects. The overall rhizosphere priming effect of soybean plants was significantly higher than for wheat plants. Plant phenology significantly influenced the rhizosphere priming effect. Little rhizosphere effect occurred in both wheat and soybean treatments initially. The priming effect of the wheat rhizosphere reached 287% above the no-plant control at the flowering stage and declined significantly afterward. The priming effect of the soybean rhizosphere peaked at 383% above the no-plant control during the late vegetative stage and remained at high levels onward. Contrary to many published reports, NPK fertilization did not significantly modify the rhizosphere priming effect.

CONSIDERING ALL THE POOLS and fluxes of C within ecosystems, C cycling belowground is increasingly being recognized as one of the most significant components of the global C cycle (Jackson et al., 1997), yet the least understood (Zak and Pregitzer, 1998). Belowground  $\text{CO}_2$  efflux can be partitioned into two distinct processes: (i) rhizosphere respiration or root-derived  $\text{CO}_2$ , including root respiration and microbial respiration utilizing materials released from live roots and, (ii) microbial decomposition of SOM, or soil-derived  $\text{CO}_2$ . Separating these two processes is necessary for assessing how environmental changes may alter the balance of  $\text{CO}_2$  flux from belowground ecosystems because the controls and responses of the two processes are likely to respond distinctly to variable environments. While the two processes act separately, they may also be linked through rhizosphere interactions (Andrews et al., 1999), which may exert a stimulative (priming effect)

or a suppressive influence on SOM decomposition (Van Veen et al., 1991; Cheng, 1999). As a measure of main energy use for the acquisition of belowground resources (e.g., nutrients and water), rhizosphere respiration may range from 30 to 80% of total belowground  $\text{CO}_2$  efflux (Rochette and Flanagan, 1997; Hanson et al., 2000) in various ecosystems. Root-associated C fluxes represent a major portion of the input to and the output from the belowground C pool (Schimel, 1995). However, the regulating mechanisms and the magnitude of the rhizosphere's contribution to such fluxes have not been adequately addressed, particularly with respect to the ecological linkages that functionally connect rhizosphere processes with soil fertility and SOM decomposition. Although studies have indicated that input of labile substrates in the rhizosphere may significantly enhance SOM decomposition as a result of the priming effect (Helal and Sauerbeck, 1986; Liljeroth et al., 1994), rates of SOM decomposition are commonly assessed by laboratory incubations of soil samples with an assumption that rhizosphere processes have little impact on the results. This assumption has rarely been rigorously tested.

Roots have been found to have both stimulatory and inhibitory effects on SOM decomposition. Laboratory experiments have shown that when  $^{14}\text{C}$ -labeled plant material was decomposed in soils planted with maize (*Zea mays* L.), spring wheat, or barley (*Hordeum vulgare* L.),  $^{14}\text{CO}_2$  release from the soil was reduced compared with bare soil (Reid and Goss, 1982, 1983; Sparling et al., 1982). In contrast, a stimulatory effect has been reported in other laboratory experiments using  $^{14}\text{C}$ -labeled litter (Helal and Sauerbeck, 1986; Cheng and Coleman, 1990). Furthermore, other research has shown that SOM decomposition is dependent on the length of exposure to living roots. In a 2-yr study, roots suppressed the decomposition of newly incorporated  $^{14}\text{C}$ -labeled plant material during the first 200 d but stimulated the mineralization of the  $^{14}\text{C}$ -labeled materials in the soil during the latter stage when compared with bare soil (Sallih and Bottner, 1988). Recently we have found that different combinations of plant species and soils resulted in different rhizosphere effects on SOM decomposition (Fu and Cheng, 2002). Several studies have also suggested that soil mineral nutrition is an important modifier of rhizosphere effects (Merckx et al., 1987; Liljeroth et al., 1994; Ehrenfeld et al., 1997); the direction of rhizosphere effects may change depending on the level of the soil mineral nutrients. However, most studies have focused on the occurrence of rhizosphere effects, with little attention being paid to the multiple factors that may influence it.

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**Abbreviations:** DAP, days after planting; DI- $\text{H}_2\text{O}$ , deionized water; PVC, polyvinyl chloride; SOM, soil organic matter.

Studies of rhizosphere processes have been restricted by the limitations of existing methods. Separating soil C from current plant-derived C has been accomplished by using isotope techniques. Total rhizosphere respiration is often defined as the sum of root respiration and rhizomicrobial respiration of substrates derived from roots. Total rhizosphere respiration and original soil C decomposition have been quantified by either continuous  $^{14}\text{C}$  labeling (Barber and Martin, 1976; Whipps and Lynch, 1983; Liljeroth et al., 1994) or pulse labeling (Cheng et al., 1993). However, both of these  $^{14}\text{C}$ -labeling methods have limitations. Continuous  $^{14}\text{C}$ -labeling requires special facilities and often requires transplanting of seedlings which may have considerable unlabeled food reserves, therefore requiring some time for all plant parts to become evenly labeled. Because pulse labeling does not uniformly label all plant C, total plant-derived C cannot be separated from soil-derived C in a pulse-labeling experiment. Because of the safety issue when radioactive materials are used, most  $^{14}\text{C}$ -labeling experiments have been of short duration. A  $^{13}\text{C}$  natural tracer method for measuring total rhizosphere respiration and soil organic C decomposition has recently been developed (Cheng, 1996; Qian et al., 1997; Rochette and Flanagan, 1997). This natural tracer method eliminates some of the major limitations of earlier labeling methods, and makes possible for systematic investigations of rhizosphere effects on SOM decomposition.

The main objective of this study was to investigate simultaneously three potentially important factors: plant species, plant phenology, and fertilization, that may significantly control the magnitude and the direction of the rhizosphere effect on SOM decomposition. Research questions were: (i) What are key interactions between different factors that control the direction and magnitude of rhizosphere effects? (ii) What is the relative significance of each controlling factor when all factors are studied together in one experiment? (iii) How does the rhizosphere effect change as controlling factors change through time?

## MATERIALS AND METHODS

The  $^{13}\text{C}$  natural tracer method was used to separately measure soil-derived C and plant-derived C in the greenhouse experiment by growing  $\text{C}_3$  plants (spring wheat and soybean) in soils developed under  $\text{C}_4$ -dominated vegetation (Kansas prairie) ( $\text{C}_4$  soils). All plants were grown in polyvinyl chloride (PVC) plastic containers (15 cm ID, 40 cm in height, and 5.3 L of effective volume) (Fig. 1). The experimental treatments were (i) three levels of fertilization, (ii) two plant species, (iii) four times of sequential destructive sampling, and (iv) a no-plant control.

### $^{13}\text{C}$ Natural Tracer Method

The principle of the  $^{13}\text{C}$  natural tracer method is based on the difference in  $^{13}\text{C}:^{12}\text{C}$  ratio, reported in  $\delta^{13}\text{C}$  values, between plants with  $\text{C}_3$  vs.  $\text{C}_4$  photosynthesis pathways.  $\text{C}_3$  plants such as wheat or soybeans are depleted in  $^{13}\text{C}$  and have a mean  $\delta^{13}\text{C}$  of  $-27\text{‰}$ , while  $\text{C}_4$  plants such as maize have a mean  $\delta^{13}\text{C}$  of  $-12\text{‰}$  (Smith and Epstein, 1971). The  $\delta^{13}\text{C}$  values of belowground C roughly reflect the original source of plant C.

If  $\text{C}_3$  plants are grown in a  $\text{C}_4$ -derived soil, the C entering the soil via roots of the  $\text{C}_3$  plants has a different  $\delta^{13}\text{C}$  value than the  $\delta^{13}\text{C}$  value of the soil. The following equation can be used to partition soil-derived  $\text{C}_4$ -C from plant-derived  $\text{C}_3$ -C (Cheng, 1996):

$$C_3 = Ct(\delta_t - \delta_4)/(\delta_3 - \delta_4), \quad [1]$$

where  $Ct$  ( $Ct = C_3 + C_4$ ) is the total amount of C from belowground  $\text{CO}_2$ ,  $C_3$  is the amount of  $\text{CO}_2$ -C derived from  $\text{C}_3$  plants,  $C_4$  is the amount of  $\text{CO}_2$ -C derived from  $\text{C}_4$  soil ( $C_4$  does not appear in Eq. [1]),  $\delta_t$  is the  $\delta^{13}\text{C}$  value of  $Ct$ ,  $\delta_3$  is the  $\delta^{13}\text{C}$  value of the  $\text{C}_3$  plant C, and  $\delta_4$  is the  $\delta^{13}\text{C}$  value of the  $\text{C}_4$ -soil C.

Results from several studies have indicated that this method worked as expected (Cheng and Johnson, 1998; Cheng et al., 2000; Fu and Cheng, 2002). No isotopic fractionation was found between total root C and rhizosphere  $\text{CO}_2$ -C since the  $\delta^{13}\text{C}$  values of both C sources obtained from a C-free inoculated sand treatment were not statistically different from each other (Cheng, 1996). Analysis of  $^{13}\text{C}$  abundance of all samples was performed using a PDZ Europa (Cheshire, UK) Hydra 20-20 continuous flow isotope ratio mass spectrometer coupled with an automated C and N combustion analyzer at University of California-Davis Stable Isotope Facility.

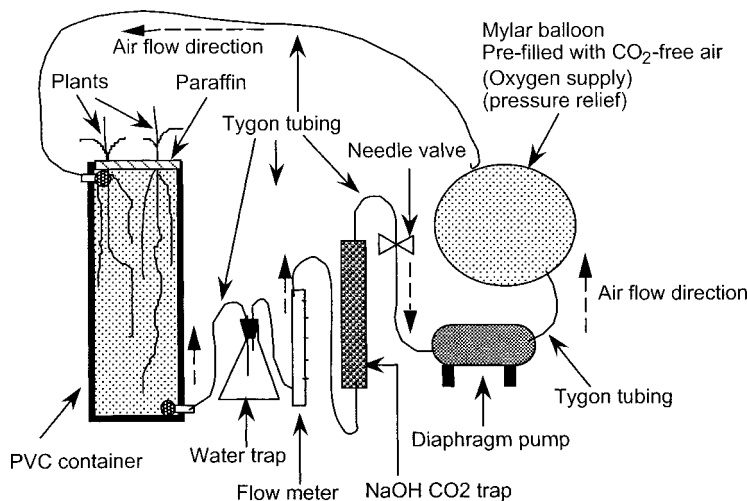


Fig. 1. A plant growth apparatus and a closed-circulation  $\text{CO}_2$  trapping system.

### Soil Used

Surface layer soil ( $\approx 0$ –30 cm) was obtained from a tallgrass prairie field at the Konza Prairie Long-Term Ecological Research site. Vegetation at this site has been dominated by  $C_4$  grasses. The  $\delta^{13}C$  value of the total soil organic C is  $-14.2\%$ . The soil is a clay loam, and classified as a Mollisol. The soil contains 2.3% organic C and 0.2% N, and has a pH of 7.6. The soil was sieved ( $< 2$  mm), homogenized, and air-dried before use.

### Experimental Design

Spring wheat and soybeans were grown in  $C_4$  soils with three levels of fertilization (F0, F1, F2) and four sequential destructive sampling dates. The four sequential destructive sampling dates represented vegetative (35 d after planting or DAP), flowering (DAP = 68), grain-filling (DAP = 89), and maturity (DAP = 110) growth stages for wheat, and early-vegetative, late-vegetative, flowering, and grain-filling growth stages for soybeans, respectively. A complete factorial randomized design was employed, with treatments replicated four times. A no-plant control was also included. Fertilization treatments were done by applying  $NH_4NO_3$  for N, and  $KH_2PO_4$  for P and K in a solution at the time of initial watering to air-dried soils. Deionized water ( $DI-H_2O$ ) was used for fertilization level one (F0, zero NPK added). For the second fertilization level (F1), 500 mg of N, 50 mg of P, and 63 mg of K were added per container (equivalent to 288 kg N, 29 kg P, and 36 kg K  $ha^{-1}$ ). For the third fertilization level (F2), 1000 mg of N, 100 mg of P, and 126 mg of K were added per container (equivalent to 566 kg N, 57 kg P, and 71 kg K  $ha^{-1}$ ).

### Plant Growth

A plant growth apparatus and a closed-circulation  $CO_2$  trapping system are illustrated in Fig. 1. Polyvinyl chloride containers (15 cm i.d., 40 cm long) were used to grow plants. Each container was the basic unit for all treatments and measurements, and was instrumented for measuring soil respiration. A closed-circulation system was used for trapping total  $CO_2$  from the soil-root system. The potential problem of minor air leakage was minimized because of its closed-circulation design and the use of a Mylar balloon for air pressure buffering.

Each container was packed with 7000 g of air-dried soil to a predetermined bulk density ( $1.2$  kg  $L^{-1}$ ), which is equivalent to 6250 g of oven-dried (at  $105^\circ C$ ) soil in each container. Sixteen seeds were planted into each container for the wheat treatment, and four seeds per container for the soybean treatment. For even distribution of applied fertilizers, fertilization treatments were applied at the first watering of the air-dried soil in each container. Nutrient solutions at two concentrations were prepared according to the fertilization treatments specified in the experimental design section and the amount of water to be applied to each container. Air-dried soils in each container were watered using either  $DI-H_2O$  (F0, zero fertilization), or nutrient solutions (F1 and F2) to 80% field water holding capacity. All containers were weighed on a top-loading balance after watering. All seeded and watered containers were randomly distributed in an area in a greenhouse. After emergence, plants were thinned to one plant per container for soybeans, and 12 plants per container for wheat. Water loss in each container was determined gravimetrically using a top-loading balance with a sensitivity of  $\pm 1$  g. Each container was watered with  $DI-H_2O$  daily according to the amount of water loss to avoid a soil moisture difference between the planted treatments and the no-plant treatments. To prevent possible occurrence of anaerobic conditions, all containers

were aerated for 30 min. every 6 h throughout the duration of the experiment by connecting an aquarium air pump to each container. A digital timer was used to control the aeration system. The experiment started on 5 Mar. 1999 and lasted until 3 July 1999 for a total period of 120 d. Maximum air temperature in the greenhouse ranged from 25 to  $33^\circ C$ . Minimum air temperature ranged from 17 to  $24^\circ C$ . Relative humidity ranged from 15 to 90%.

### Measurements

The method of Cheng (1996) was used to measure SOM decomposition and total rhizosphere respiration. Briefly, before each destructive harvesting, four containers from each treatment were sealed at the base of the plant with low melting point Paraffin (m.p.  $42^\circ C$ ) to separate the aboveground atmosphere from the belowground atmosphere. The integrity of the seal was verified by submerging the PVC container in water and checking for gas leaks. The Paraffin seal did not damage the plant seedlings, and was strong enough to withstand routine fluctuation of air pressure. The  $CO_2$  was trapped for 48 h in a column containing 30 mL of 4 M NaOH solution mixed with acid-washed sand (Fig. 1). The  $CO_2$  trapping efficiency of this system was nearly 100% as checked by an infrared gas analyzer (Model LI-6262, LI-COR, Lincoln, NE). Thus, preferential sorption of  $^{13}CO_2$  vs.  $^{12}CO_2$  was eliminated. After  $CO_2$  trapping, the contents (sand and NaOH) of each column were transferred into a plastic bottle with a sealed cap by rinsing with  $DI-H_2O$ . The bottle was capped and shaken vigorously. After settling overnight, an aliquot of the NaOH solution in each plastic bottle was analyzed for total inorganic C using a TOC Analyzer. Another aliquot of the trapping solution was mixed with excess  $SrCl_2$ , and the  $\delta^{13}C$  of the precipitate ( $SrCO_3$ ) was analyzed by mass spectrometry (Harris et al., 1997). The amounts of soil-derived  $CO_2$  and plant-derived  $CO_2$  were separated using the  $^{13}C$  method described above. Soil-derived  $CO_2$  was used as a measure of original SOM decomposition. Plant-derived belowground  $CO_2$  was used as the measure of total rhizosphere respiration. Analytical and experimental controls were employed during the  $^{13}C$  analysis. Null  $CO_2$  trapping units (blanks) without soil-roots were included to correct for contamination from carbonate in the NaOH stock solution and from sample handling. The average amount of inorganic C trapped by four replicate null units was used as a measure of the amount of contamination in all samples. The highest amount of contaminant C among all samples was  $< 5\%$  of the total C. The effect of the contaminant C on the  $\delta^{13}C$  value of each sample was corrected using the following equation:

$$\delta_j = (C_t \delta_t - C_c \delta_c) / (C_t - C_c),$$

where  $\delta_j$  is the  $\delta^{13}C$  value from a sample after correction,  $\delta_t$  is the  $\delta^{13}C$  value of a sample before correction,  $\delta_c$  is the  $\delta^{13}C$  value of the contaminant C ( $-8\%$ ; the value of the  $CO_3^{2-}$  in NaOH stock and from the air);  $C_t$  is the total amount of C in the sample solution including contaminant C;  $C_c$  is the amount of C in the blank control as contamination.

After each destructive sampling, plant shoots were cut at the soil surface and oven-dried at  $65^\circ C$  for 48 h before weighing. Roots were hand-picked, washed, and oven-dried at  $65^\circ C$  before weighing. After grinding, the  $\delta^{13}C$  value of each shoot or root sample was analyzed for isotopic composition. Total soil inorganic C content was analyzed using an infrared gas analyzer. After grinding and mixing in a SPEX ball mill, 500 mg of oven-dried soil sample was put into a 125-mL flask. The flask was then connected to a  $CO_2$ -free carrier gas system at a constant flow rate of  $500$  mL  $min^{-1}$ . Carbonates in each

**Table 1. Soybean and wheat biomass under three levels of fertilization and at four growing stages. Values are means of four replicates with standard errors in parenthesis.**

Plant	Fertilization	Days after planting			
		35	68	89	110
		g per pot			
		Shoot Biomass			
Soybean	Zero	0.38(0.05)	19.55(1.22)	47.16(3.52)	67.37(1.88)
Soybean	Low-NPK	0.53(0.06)	16.75(1.95)	47.78(1.35)	67.54(1.84)
Soybean	High-NPK	0.30(0.12)	9.58(0.81)	38.05(2.52)	66.68(1.11)
Wheat	Zero	9.54(0.83)	30.86(0.82)	46.09(1.67)	50.06(0.67)
Wheat	Low-NPK	14.20(1.12)	42.69(3.17)	65.21(2.90)	57.05(4.35)
Wheat	High-NPK	18.08(0.58)	50.60(1.45)	71.22(1.41)	71.60(4.37)
		Root Biomass			
Soybean	Zero	0.16(0.03)	3.82(0.15)	7.53(0.81)	10.80(0.51)
Soybean	Low-NPK	0.28(0.03)	3.88(0.55)	7.49(0.58)	12.23(1.61)
Soybean	High-NPK	0.08(0.05)	2.66(0.32)	6.90(0.47)	13.78(0.60)
Wheat	Zero	2.68(0.60)	7.94(0.62)	6.04(1.42)	7.94(1.68)
Wheat	Low-NPK	2.65(0.51)	10.94(1.23)	6.63(0.38)	9.09(2.29)
Wheat	High-NPK	4.11(0.85)	11.66(0.86)	7.68(0.54)	9.92(2.84)

soil sample were purged for 30 min by injecting 20 mL of acid solution (1 M H<sub>2</sub>SO<sub>4</sub> with 5% FeSO<sub>4</sub> as antioxidant) under magnetic stirring. The CO<sub>2</sub> concentration of the outflow gas was recorded digitally at 1-s intervals during the entire purging period. The amount of total inorganic C in each sample was calculated using the total area under the curve and known CaCO<sub>3</sub> standards.

### Statistical Analysis

All statistical analyses of data were performed using Statistix 7 software (Analytical Software, Tallahassee, FL). This experiment consisted of three factors (plant species, levels of fertilization, and growth stages) with four replicates. Growth stages were not considered repeated measures because they were represented by sequential harvests of random replicates. Analysis of variance (ANOVA) of all data sets was performed using the general linear model with all possible interacting terms. Mean comparisons were performed using Fisher's LSD method.

## RESULTS

### Plant Biomass

Plant growth appeared normal, with no obvious signs of pests or pathogens. Wheat plants produced significantly more shoot and root biomass than soybean plants at the first and the second sampling times (Tables 1, 2). Wheat and soybean plants had similar biomass at the last sampling time. Fertilization significantly increased shoot biomass of wheat, but had limited influence on shoot biomass of soybean. Mean comparisons indicated that the high fertilization treatment (F2) significantly reduced soybean shoot biomass as compared with the no-fertilization control ( $P < 0.05$ ) at both the late vegetative and flowering growth stages. There was a trend of increasing root biomass of wheat in response to fertilization, but this was only statistically significant at the flowering stage. Fertilization significantly ( $P < 0.05$ ) increased soybean root biomass at the last sampling date, even though there was a weak trend of decreasing soybean root biomass at the high fertilization level for the other three sampling dates.

### Total Belowground CO<sub>2</sub> Efflux

Total belowground CO<sub>2</sub> effluxes were relatively stable throughout the experiment for the no-plant control (Fig. 2). Belowground CO<sub>2</sub> effluxes were high at the two initial sampling times and declined linearly to a lower level at the last sampling time for wheat treatment. For the soybean treatment, total belowground CO<sub>2</sub> effluxes showed a different pattern from the wheat treatment, were low at the first sampling date, increased to a much higher level at the second sampling date, and remained high till the end of the experiment.

Fertilization significantly and consistently increased total belowground CO<sub>2</sub> effluxes for the wheat treatment. Fertilization significantly affected total belowground CO<sub>2</sub> effluxes for the soybean treatment but in an inconsistent way, and had no effect on total belowground CO<sub>2</sub> effluxes for the no-plant treatment. The CO<sub>2</sub> efflux rate of the wheat treatment, averaged across the three fertilization levels, was about seven times that of the no-plant control during the initial two sampling periods, indicating that a major portion of the total belowground CO<sub>2</sub> efflux came from plant roots and rhizosphere microbial activities. The magnitude of the rhizosphere contribution to the total belowground CO<sub>2</sub> efflux was even higher for soybean treatment at the second, third, and fourth sampling (nearly eight times that of no-plant control).

**Table 2. Analysis of variance table for shoot biomass, root biomass, and total plant biomass with three main factors (Plant: soybean, wheat; Sampling: 4 times; Fertilization: zero, low, high).**

Source	df	Shoots		Roots		Total plant biomass	
		F	P	F	P	F	P
Plant (A)	1	209	0.000	11.4	0.001	169	0.000
Sampling (B)	3	899	0.000	71.5	0.000	748	0.000
Fertilization (C)	2	24	0.000	2.7	0.071	21	0.000
A × B	3	72	0.000	24.7	0.000	75	0.000
A × C	2	66	0.000	1.6	0.204	50	0.000
B × C	6	3	0.025	0.5	0.808	2	0.070
A × B × C	6	4	0.002	0.7	0.651	3	0.011

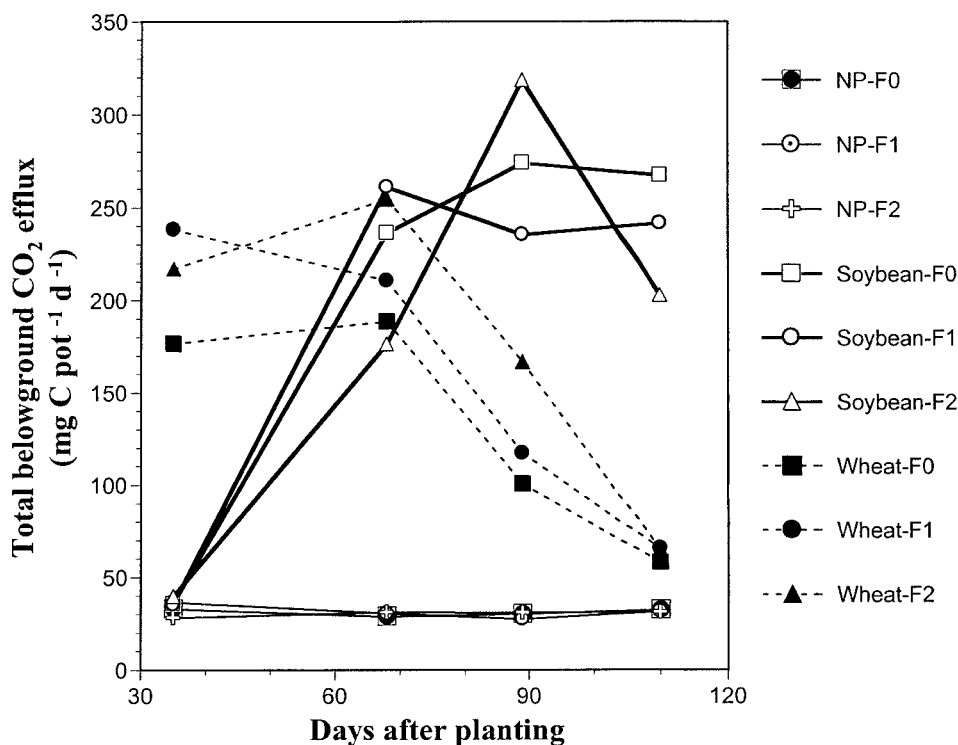


Fig. 2. Total belowground CO<sub>2</sub> effluxes from no-plant (NP), soybean, and wheat treatments without NPK fertilization (F0), with a low level of NPK fertilization (F1), and with a high level of NPK fertilization (F2). Error bars are two standard errors, and some error bars are too small to be seen.

### Natural <sup>13</sup>C Analysis

The average  $\delta^{13}\text{C}$  value was  $-27.59\text{‰}$  for roots of wheat or soybean. No significant difference in  $\delta^{13}\text{C}$  values was detected among C<sub>3</sub> plant materials. The  $\delta^{13}\text{C}$  value of  $-27.59\text{‰}$  was used to represent the  $\delta^{13}\text{C}$  value of plant-derived C in this experiment, because there was no detectable isotopic discrimination associated with rhizosphere processes (Cheng, 1996).

As expected, the  $\delta^{13}\text{C}$  value of CO<sub>2</sub> evolved from the no-plant control remained in the range of  $-13$  to  $-14\text{‰}$  during the whole experiment, indicating that the origin of the C was primarily from C<sub>4</sub> sources (Fig. 3). The  $\delta^{13}\text{C}$  value of CO<sub>2</sub> evolved from the wheat treatment was low (mostly from the C<sub>3</sub> source) at the first sampling, increased almost linearly as sampling stages progressed to the end of the experiment, indicating that the portion of wheat-derived C in the total belowground CO<sub>2</sub> decreased. On the contrary, the  $\delta^{13}\text{C}$  value of CO<sub>2</sub> evolved from the soybean treatment was high (near the value from the C<sub>4</sub> soil only) at the first sampling and decreased linearly during the course of the experiment, indicating that the portion of soybean-derived C in the total belowground CO<sub>2</sub> increased as soybean plants grew and approached maturity. Analysis of variance indicated that both the plant factor (no-plant, wheat, or soybean) and the time of sampling significantly influenced the  $\delta^{13}\text{C}$  values of belowground CO<sub>2</sub>, but the overall effect of fertilization was not statistically significant (Table 3).

### Total Soil Inorganic Carbon

At the last sampling, the total inorganic C content was 0.56, 0.59, and 0.57 mg C g<sup>-1</sup> of soil for the no-

plant control, wheat, and soybean treatments without fertilization, respectively. Similar concentrations of total soil inorganic C were found in both the no-plant control and the planted treatments. Therefore, root growth and associated rhizosphere microbial activities in the planted treatments did not significantly alter soil inorganic C concentration, as compared with the no-plant control (Fig. 4).

### Soil Organic Matter Decomposition and Rhizosphere Priming Effects

Using the natural <sup>13</sup>C tracer method (Eq. [1]), soil-derived CO<sub>2</sub> (decomposition of original SOM) was separated from root-derived CO<sub>2</sub>. Because soil inorganic C content was not significantly altered by the presence of the rhizosphere (Fig. 4), soil-derived CO<sub>2</sub> is a good measure of SOM decomposition in all treatments. Initially, similar SOM decomposition rates were found from all treatments (Fig. 5), indicating that no significant rhizosphere effect on SOM decomposition occurred at the first sampling time. However, the SOM decomposition rate from the wheat treatment across all levels of fertilization was approximately four times of that from the no-plant control at the second sampling time, indicating a strong rhizosphere priming effect on SOM decomposition. The magnitude of the rhizosphere priming effect for the wheat treatment declined significantly after the second sampling time. Also at the second sampling time, the SOM decomposition rate from soybeans was 383, 368, and 218% higher than the no-plant control treatment at fertilization levels F0, F1, and F2, respectively. This indicated a large rhizosphere priming effect

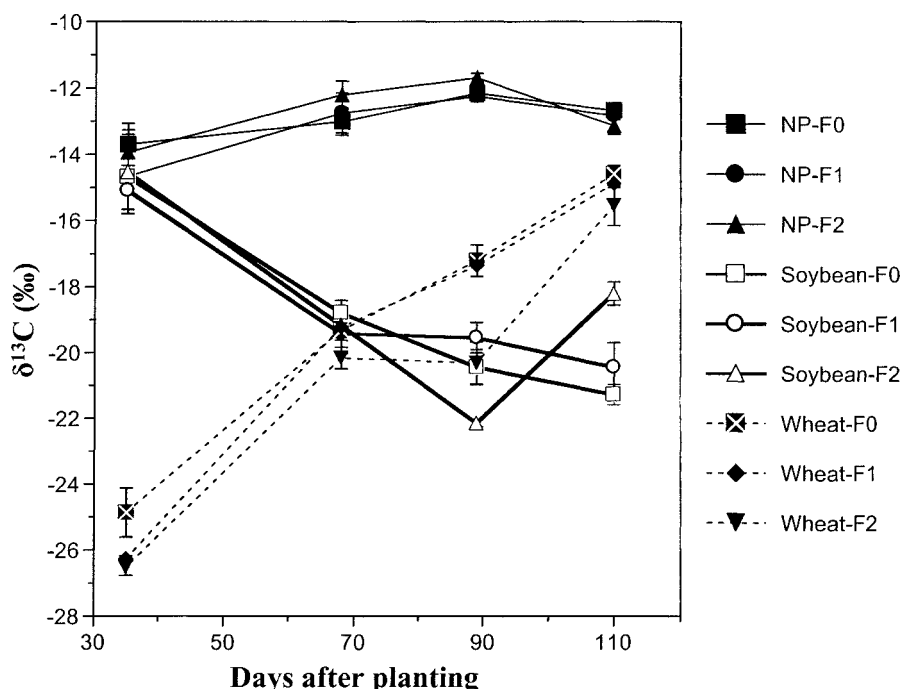


Fig. 3. The  $\delta^{13}\text{C}$  values of  $\text{CO}_2$  evolved from the no-plant control (thin solid lines), wheat (dotted lines), and soybean (thicker solid lines) treatments with three levels of fertilization (Zero NPK, square symbol; low NPK, circle symbol; high NPK, triangle symbol) and four sequential samplings. Error bars are two standard errors, and some error bars are too small to be seen.

on SOM decomposition in all fertilization treatments. The differences in SOM decomposition rates between plant treatments and growth stages were highly significant (Table 3). However, fertilization generally did not significantly affect SOM decomposition rates with only one exception. The SOM decomposition rate of the soybean treatment with the high fertilization rate at the second sampling was significantly ( $P < 0.05$ ) lower than zero fertilization. This might be a result of smaller soybean plants under the high fertilization treatment (Table 1).

Plant phenology (sampling times) and different species were the main factors significantly controlling the amount of rhizosphere-primed soil C loss ( $P < 0.0001$ ) (Table 4). The next important controlling term was the interaction between plant species and phenology ( $P < 0.0001$ ). Although the three-factor interaction term was statistically significant, 98% of the variation in the rhizosphere-primed soil C loss was contributed by plant phenology (55.8%), plant species (35.3%), and the interaction (6.7%). Fertilization as a main factor did not

Table 3. Analysis of variance table for  $\delta^{13}\text{C}$  values of total  $\text{CO}_2$  efflux and soil-derived  $\text{CO}_2\text{-C}$  with three main factors (Plant: no-plant, soybean, wheat; Sampling: 4 times; Fertilization: zero, low, high).

Sources	df	$\delta^{13}\text{C}$ ( $\text{CO}_2$ )		Soil-derived $\text{CO}_2\text{-C}$	
		F	P	F	P
Plant (A)	2	669	0.000	658	0.000
Sampling (B)	3	33	0.000	276	0.000
Fertilization (C)	2	2	0.130	2	0.148
A × B	6	163	0.000	102	0.000
A × C	4	6	0.001	2	0.164
B × C	6	4	0.001	2	0.127
A × B × C	12	2	0.010	5	0.000

significantly affect the amount of rhizosphere-primed soil C loss.

Cumulative soil-derived  $\text{CO}_2$  release from the soybean treatment during the whole growing season was 275, 262, and 256% compared with the no-plant treatment for zero, low, and high fertilization levels, respectively (Fig. 6). The cumulative rhizosphere priming effect for the wheat treatment was also very significant: 201, 186, and 201% of that from no-plant for zero, low, and high fertilization levels, respectively. The amount

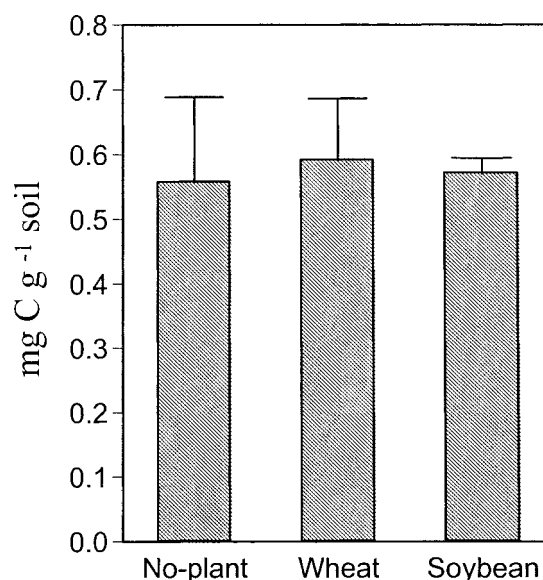


Fig. 4. Total soil inorganic C content ( $\text{mg C g}^{-1}$  soil) from the no-plant control, wheat, and soybean treatments without fertilization (Zero NPK) at the end of the experiment. Each error bar represents one standard error.

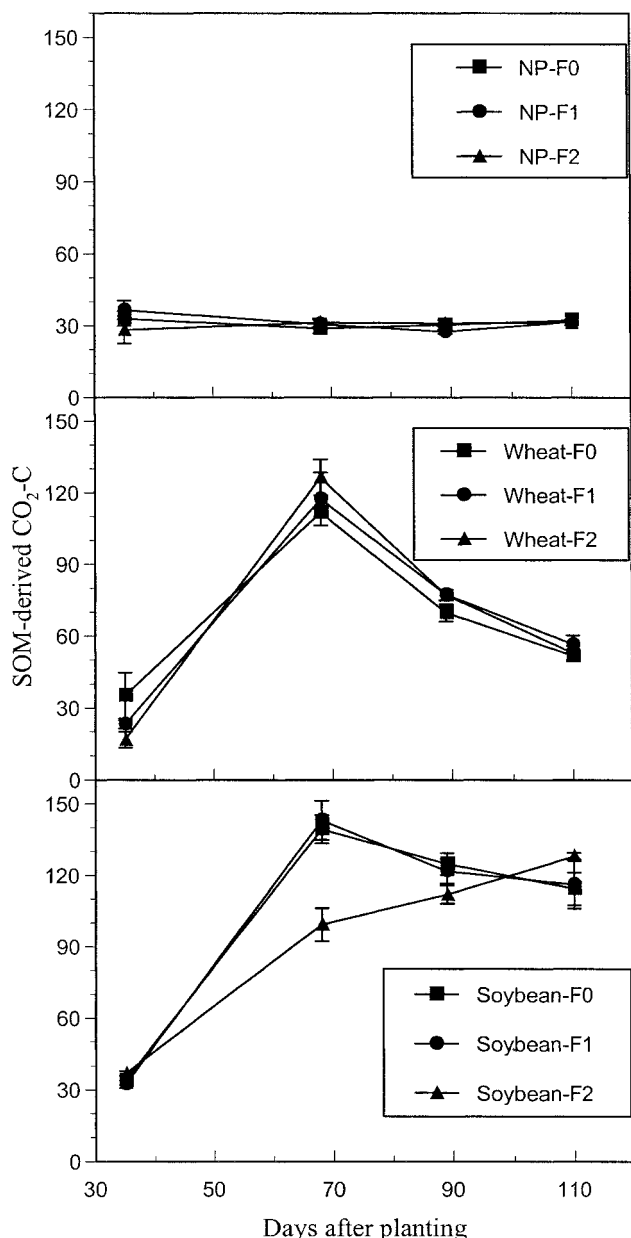


Fig. 5. Soil-derived CO<sub>2</sub> (mg C per pot d<sup>-1</sup>) from no-plant (top), wheat (middle), and soybean (bottom) treatments with three levels of fertilization (Zero NPK, square symbol; low NPK, circle symbol; high NPK, triangle symbol) and four sequential samplings. Error bars are two standard errors, and some error bars are too small to be seen.

of extra soil C loss due to rhizosphere priming effects during the whole growing season represented approximately the same amount of C in standing root biomass

Table 4. Analysis of variance table for rhizosphere-primed soil C with three main factors (Plant: soybean, wheat; Sampling: 4 times; Fertilization: zero, low, high). Rhizosphere-primed soil C was calculated by subtracting the mean value of soil-derived CO<sub>2</sub>-C of the no-plant control from the planted treatments.

Source	df	SS	MS	MS (%total)	F	P
Plant (A)	1	24 881	24 881	35.3	209.89	0.0000
Sampling (B)	3	117 942	39 314	55.8	331.65	0.0000
Fertilization (C)	2	415	207	0.3	1.75	0.1811
A × B	3	14 179	4 726	6.7	39.87	0.0000
A × C	2	496	248	0.4	2.09	0.1309
B × C	6	1 475	246	0.3	2.07	0.0669
A × B × C	6	4 513	752	1.1	6.34	0.0000
Residual	72	8 535	119	0.2		

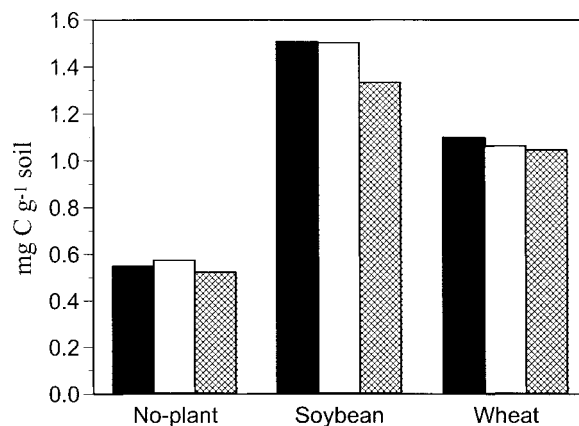


Fig. 6. Cumulative soil-derived CO<sub>2</sub>-C during the whole growing season calculated by using linear extrapolation. Filled bars, zero fertilization; open bars, low fertilization; and crossed bars, high fertilization. Setting the value for the no-plant treatment as 100%, the value for the soybean treatment is 275, 262, and 256% for zero, low, and high fertilization levels, respectively; and for the wheat treatment is 201, 186, and 201% for the three fertilization levels, respectively.

for the soybean treatment and approximately 71% of root biomass C for the wheat treatment. The extra soil C loss from the rhizosphere priming effect accounted for a major portion of the net C input belowground for both plant species.

## DISCUSSION

The rhizosphere effect on SOM decomposition ranged from zero to 383% higher than the no-plant control, depending on plant species and sampling time (Fig. 5). This demonstrates that the rhizosphere effect on SOM decomposition is an important component of C loss from ecosystems and cannot be ignored.

The C<sub>4</sub> soil used in this experiment had a pH value of 7.6 and contained on average 0.57 mg of inorganic C g<sup>-1</sup> of soil (Fig. 4). No statistically significant rhizosphere effect on soil inorganic C content was found at the end of the experiment. This result indicated that the enhanced soil-derived CO<sub>2</sub> effluxes from planted treatments mostly came from rhizosphere-stimulated SOM decomposition instead of soil carbonates. The cumulative amount of soil C loss in the soybean treatment averaged across all fertilization levels was 1.44 mg C g<sup>-1</sup> soil, which is 0.90 mg C g<sup>-1</sup> soil higher than the control (0.54 mg C g<sup>-1</sup> soil). This extra C loss caused by the soybean rhizosphere effect was even greater than the total soil inorganic C. The cumulative soil C loss in

the wheat treatment averaged across the three fertilization levels was  $1.07 \text{ mg C g}^{-1} \text{ soil}$ , or  $0.52 \text{ mg C g}^{-1} \text{ soil}$  higher than the no-plant control, or nearly twice as much as the total inorganic C.

Because of the rhizosphere effect, cumulative original soil C loss averaged across all fertilization levels during the entire growing season was 164% (or  $0.90 \text{ mg C g}^{-1} \text{ soil}$ ) and 96% (or  $0.52 \text{ mg C g}^{-1} \text{ soil}$ ) more than that in the no-plant control for soybean and wheat treatments respectively (Fig. 6). The amount of soil C loss due to soybean rhizosphere effect in another experiment (Fu and Cheng, 2002) using the same  $C_4$  soil was 70% higher than the no-plant control, much lower than the level found in our current study. Differences in environmental conditions and soybean varieties between the two experiments might have caused these apparently dissimilar levels of rhizosphere effects. The greenhouse used for this study generally had higher air temperatures, higher light intensities (full sunlight vs. lime shaded), and much higher variation in temperature and humidity. Different soybean varieties were chosen because of different locations where the two experiments were conducted. The real causes of these dissimilar levels of rhizosphere effects on SOM decomposition warrant further studies.

The amount of extra soil C loss due to rhizosphere effects during the entire growing season is approximately the same amount of C in standing root biomass for soybean, and 71% of root biomass C for wheat. If all C inputs and outputs are considered, the planted soil systems should have net C gains, as C inputs from root standing biomass, aboveground litter, and rhizodeposition more than compensated for the total C loss during the growing season. However, directly applying our current results to a field situation is probably unwise because soil disturbance during preparation and greenhouse operation may have exacerbated the rhizosphere effect. Averaging across the three levels of fertilization, the amount of C loss in the form of soil-derived  $\text{CO}_2$  during the entire growing season represents 2.4, 4.6, and 6.3% of the total initial soil C for the no-plant control, wheat, and soybean treatments, respectively. These values are in a reasonable range compared with the value of 6% found in the field study using the  $^{13}\text{C}$  method (Rochette et al., 1999). Planting density may also have influenced the level of rhizosphere effects in the greenhouse experiment. The level of rhizosphere effects on the decomposition rate of  $^{14}\text{C}$ -labeled litter ranges from 71% reduction (Sparling et al., 1982) to as high as 350% increase (Helal and Sauerbeck, 1987) above the no-plant control. Litter decomposition is often different from decomposition of mineral-associated SOM because of differences in substrate quality, decay dynamics, and environmental conditions. Therefore, the results obtained from experiments using labeled litter should be interpreted as rhizosphere effects on litter decomposition instead of on SOM decomposition. Negative rhizosphere effect on  $^{14}\text{C}$ -labeled litter decomposition were reported earlier (Reid and Goss, 1982, 1983; Sparling et al., 1982). They also reported that a significant amount of  $^{14}\text{C}$ -labeled C was taken up by plant roots,

which might have contributed to the negative effect on the decomposition rate. However, this root uptake hypothesis was largely dismissed (Sallih and Bottner, 1988; Cheng and Coleman, 1990). The high level of rhizosphere priming effects on the decomposition rate of  $^{14}\text{C}$ -labeled litter reported by Helal and Sauerbeck (1987) was estimated by budgeting  $^{14}\text{C}$ -labeled materials remaining in the planted soil zones, in the soil zones away from roots, or in the unplanted control rather than by direct measurement of soil C loss as  $^{12}\text{CO}_2$ . Because of the inherent high variability of SOM, the budgeting approach was widely acknowledged to have low sensitivity and low accuracy. Because our present study employed a natural  $^{13}\text{C}$  tracer method, the rhizosphere effect on SOM decomposition was measured directly in the form of soil-derived  $\text{CO}_2$ . The above-mentioned limitations associated with experiments using either  $^{14}\text{C}$ -labeled litter or C budgeting were avoided. The level of sensitivity of the natural  $^{13}\text{C}$  tracer method in this study was higher than that reported early by Cheng (1996). Standard errors of  $\delta^{13}\text{C}$  values ranged from 0.07 to 1.13‰, with a mean of 0.33‰. The difference in  $\delta^{13}\text{C}$  values between the two sources (plant-derived and soil-derived) was 13.6‰. The accuracy of the natural  $^{13}\text{C}$  tracer method used in this study ranged from 74 to 98% with a mean of 92%, using a 95% confidence level ( $t_{0.025} = 3.182$ ,  $n = 4 - 1$ ). For the level of rhizosphere effects found in this study, the accuracy of the natural  $^{13}\text{C}$  method was adequate beyond any reasonable doubt.

Using continuous  $^{14}\text{C}$  labeling technique in a relatively short experiment ( $\approx 50$  d) with wheat as the test plant, Liljeroth et al. (1994) reported a rhizosphere priming effect of  $\approx 200\%$ . Helal and Sauerbeck (1984, 1986) reported much higher rhizosphere priming values of 336 and 432%. However, these higher values were indirectly derived from soil C budgets with large measurement errors caused by high variability in soil C data. Nevertheless, the consistently lower amounts of soil C remaining in the soil sections with roots or closer to the rooting zone than sections away from the rooting zone indicate priming effects by roots in their studies. These continuous labeling studies require the use of a special labeling facility which only permits short experimental duration and limited plant growth space. Because of these limitations, the duration of the two experiments mentioned above was relatively short, roughly corresponding to the first or the second sampling of this present study. However, as shown here, plant phenology (or sampling times) was the most important variable in controlling the amount of rhizosphere-primed soil C loss. The magnitude of rhizosphere effects changed significantly through time. Moreover, the amount of rhizodeposition and root activities differs at different plant growth stages (Cheng et al., 1990; Martin and Merckx, 1992), resulting in different rhizosphere effects at different phenological stages.

The rhizosphere effect of soybeans is significantly different from wheat. This finding is further confirmed by another study using four plant species (Fu and Cheng, 2002). The stronger rhizosphere effect of soybeans may have been caused by the higher substrate quality of

soybean rhizodeposition because the N concentrations of soybean tissues are generally higher than tissues of nonleguminous plants. The N-rich compounds usually produce stronger priming effects than compounds of low or no N (Dalenberg and Jager, 1989). This clearly demonstrates that plant species is an important factor controlling SOM decomposition through rhizosphere processes. The effects of plant species on the rhizosphere effects on SOM decomposition have rarely been considered.

In general, fertilization did not significantly affect SOM decomposition in this experiment, with or without a rhizosphere. This is a surprise because several studies have suggested that soil mineral nutrition is an important modifier of rhizosphere effects (Merckx et al., 1987; Jackson et al., 1989; Schimel et al., 1989; Liljeroth et al., 1994; Ehrenfeld et al., 1997). Fertilization reduced rhizosphere effect on SOM decomposition as shown by some studies using soils from agricultural fields and  $^{14}\text{C}$ -labeling (Merckx et al., 1987; Lekkerkerk et al., 1990; Liljeroth et al., 1994). This negative impact of fertilization on the rhizosphere effect has been put forward as a hypothesis of preferential substrate utilization (Cheng, 1999). This hypothesis states that, when mineral nutrient supply is abundant, soil microorganisms prefer labile root-derived C to soil-derived C, resulting in a decreased rhizosphere effect on SOM decomposition. If mineral nutrients are in short supply, soil microorganisms prefer nutrient-rich SOM to root-derived C, resulting in increased SOM decomposition. However, some other studies (Jackson et al., 1989; Schimel et al., 1989; Ehrenfeld et al., 1997) seem to suggest that the presence of the rhizosphere reduces SOM decomposition in nutrient-poor soils, possibly because of the competition for mineral nutrients between roots and rhizosphere microbes. The extra root-derived C input in the rhizosphere may decrease SOM decomposition because the increased root uptake of mineral nutrients in the rhizosphere may retard microbial growth under mineral nutrient-poor conditions. The result of our experiment supports neither of these hypotheses because fertilization did not significantly modify the rhizosphere effects on SOM decomposition. This apparent controversy may be caused by the different soils, plant species, environmental controls, and experimental methods used in the various studies.

## CONCLUSIONS

The presence of live roots significantly controls the rate of original SOM decomposition through the rhizosphere effect. Rhizosphere priming may result in SOM decomposition rates four times higher than soil incubation alone. This finding challenges the ecological validity of any results from SOM decomposition studies using the approach of root exclusion or physical separation of soil from roots. Different plant species produce rhizosphere priming of different magnitude and seasonal pattern. This indicates that plant species is an important factor controlling SOM decomposition through rhizosphere effects. These results pose some serious ques-

tions about models of decomposition that take only climatic variables and litter quality into account (Bolker et al., 1998). Temporal scale is an important part of the rhizosphere priming effect. Plant phenology and temporal variation have significant implications for the level of rhizosphere priming. Although soil mineral nutrition has been frequently proposed as an important modifier of rhizosphere priming effects, results from the fertilization experiment did not support this mechanism.

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