



Rhizosphere priming effect: Its functional relationships with microbial turnover, evapotranspiration, and C–N budgets

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ABSTRACT

Understanding soil organic matter (SOM) decomposition and its interaction with rhizosphere processes is a crucial topic in soil biology and ecology. Using a natural ^{13}C tracer method to separately measure SOM-derived CO_2 from root-derived CO_2 , this study aims to connect the level of rhizosphere-dependent SOM decomposition with the C and N balance of the whole plant–soil system, and to mechanistically link the rhizosphere priming effect to soil microbial turnover and evapotranspiration. Results indicated that the magnitude of the rhizosphere priming effect on SOM decomposition varied widely, from zero to more than 380% of the unplanted control, and was largely influenced by plant species and phenology. Balancing the extra soil C loss from the strong rhizosphere priming effect in the planted treatments with C inputs from rhizodeposits and root biomass, the whole plant–soil system remained with a net carbon gain at the end of the experiment. The increased soil microbial biomass turnover rate and the enhanced evapotranspiration rate in the planted treatments had clear positive relationships with the level of the rhizosphere priming effect. The rhizosphere enhancement of soil carbon mineralization in the planted treatments did not result in a proportional increase in net N mineralization, suggesting a possible decoupling of C cycling with N cycling in the rhizosphere.

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1. Introduction

Linking aboveground vegetation with the soil, plant roots constitute a major biological component of belowground systems and a main soil-forming agent (Jenny, 1941). Processes that are largely controlled or directly influenced by roots are often referred to as rhizosphere processes. These processes may include exudation, water uptake, nutrient mobilization, rhizosphere-associated soil organic matter (SOM) decomposition, and rhizosphere respiration. The substrates of rhizosphere respiration come from recently fixed C through photosynthesis, whereas SOM decomposition is primarily a function of soil heterotrophic activities utilizing solely original soil C. The two processes act simultaneously and are also linked through rhizosphere interactions (Cheng, 1999; Personeni et al., 2005). Large amounts of C and mineral nutrients recycle back to the soil through rhizosphere processes (Pregitzer et al., 1995). However, only limited effort has been made to link rhizosphere processes with soil processes such as organic matter decomposition and N transformation (Hanson et al., 2000). It is challenging to link rhizosphere processes with soil processes because of the inherent structural and functional complexity of rhizosphere systems.

Rhizosphere processes are crucial for the functioning of terrestrial ecosystems. At the global level, rhizosphere processes contribute as much as 50% of the total CO_2 release from terrestrial ecosystems (Schimel, 1995), regulate virtually all aspects of nutrient cycling (e.g., Smith and Read, 1997), and constitute the primary gateway for plant water uptake (Jackson et al., 2000). Many important soil functions, such as SOM decomposition and nutrient dynamics, are intimately coupled with plant roots and their associated rhizosphere processes (van Veen et al., 1991). Although the potential importance of rhizosphere processes in regulating soil functions is widely recognized, rates of SOM decomposition and N mineralization are commonly assessed by incubating soil samples in the absence of live roots with an implicit assumption that rhizosphere processes have little impact on the results (e.g., Bolker et al., 1998; Dalias et al., 2002). However, recent studies have overwhelmingly proved that this implicit assumption is often invalid. The rate of SOM decomposition in the presence of live roots can be inhibited by as much as 50% or accelerated by as much as 382% (Cheng and Kuzyakov, 2005), depending on the kind of plant–soil couplings and experimental conditions. The rhizosphere priming effect on SOM decomposition is often large in magnitude and significant in mediating plant–soil interactions. Both biotic and abiotic factors, e.g., N (Liljeroth et al., 1994; Cheng and Johnson, 1998), CO_2 (Cardon, 1996; Hungate et al., 1997), light (Kuzyakov and Cheng, 2001), plant species (Fu and Cheng, 2002; Cheng et al., 2003),

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and plant phenological stages (Cheng et al., 2003), have been found to influence the magnitude of rhizosphere effects on SOM decomposition. However, how the rhizosphere priming effect may influence C and N balance of the whole plant–soil system remains a subject of further studies.

Although the occurrence and the magnitude of the rhizosphere priming effect have been documented in several experiments under various experimental settings (Cheng and Kuzyakov, 2005), the mechanisms responsible for the rhizosphere priming effect have not been adequately investigated. In the book chapter, Cheng and Kuzyakov (2005) provided several hypotheses about the possible mechanisms for the rhizosphere priming effect. But direct testing of these hypotheses has been lacking. Mechanistically linking these rhizosphere functions with soil organic matter decomposition is needed.

The main objectives of this study are: (1) to investigate the balances of C and N in whole plant–soil systems after the documented occurrence of a significant rhizosphere priming effect on organic matter decomposition reported in an early paper (Cheng et al., 2003), (2) to seek mechanistic relationships between microbial biomass turnover rates and the amount of rhizosphere-primed soil organic matter decomposition, and between the rate of evapotranspiration and the magnitude of the rhizosphere priming effect. In order to investigate the potential influence of different plant species on the rhizosphere priming effect, two contrasting plant species, spring wheat (*Triticum aestivum* L.) and soybean (*Glycine max* L. Merr.), are chosen. Wheat is a monocot with a fibrous root system and relatively low tissue N content. In contrast, soybean is a dicot legume with a tap root system and relatively high tissue N content, and is capable of symbiotic N₂-fixation. The effects of plant phenological stages are also investigated by destructive sampling four times during the experiment.

2. Materials and methods

Detailed information about Section 2 was given by Cheng et al. (2003). In the experiment, two C₃-plant species, spring wheat and soybean, were grown in plastic containers (15 cm ID, 40 cm long) filled with 7.0 kg of air-dried, sieved (passing a 4 cm screen) soil in a greenhouse. The soil is a clay loam Mollisol from a Kansas tallgrass prairie field. The soil has 2.3% organic C, 0.2% N, and a pH of 7.6. Because the soil has been under C₄-dominated native vegetation for thousands of years, the $\delta^{13}\text{C}$ value of the total soil organic C was approximately -14.2‰ which is significantly different from the $\delta^{13}\text{C}$ value of the C₃ plants (ca -28‰). Because of this large difference in ^{13}C isotope abundance between the C₃-plant-derived C and the C₄ SOM-derived C, rhizosphere respiration (or root-derived CO₂) could be partitioned from SOM decomposition (or SOM-derived CO₂) using the following two-end-member separation equation (Cheng, 1996):

$$C_3 = C_t(\delta_t - \delta_4)/(\delta_3 - \delta_4) \quad (1)$$

where C_t ($C_t = C_3 + C_4$) is the total amount of C from belowground CO₂ as measured by CO₂ trapping, C_3 is the amount of CO₂-C derived from C₃ plants, C_4 is the amount of CO₂-C derived from C₄-soil (C_4 does not appear in Eq. (1)), δ_t is the $\delta^{13}\text{C}$ value of C_t , δ_3 is the $\delta^{13}\text{C}$ value of the C₃-plant C, and δ_4 is the $\delta^{13}\text{C}$ value of the C₄-soil-derived C. δ_t , δ_3 , and δ_4 were measured by mass spectrometry.

Sixteen seeds were planted into each container for the wheat treatment and four seeds per container were planted for the soybean treatment. After emergence, plants were thinned to one plant per container for soybeans, and 12 plants per container for wheat. Each container was one replicate for all measurements. There were four sequential destructive sampling dates. For wheat these were vegetative (35 days after planting or DAP), flowering (DAP = 68),

grain-filling (DAP = 89) and maturity (DAP = 110). For soybean these represented early-vegetative, late-vegetative, flowering, and grain-filling growth stages. A complete factorial randomized design was employed, with treatments replicated four times. An unplanted soil-only control was also included.

Water loss in each container was determined gravimetrically using a top-loading balance with a sensitivity of ± 1 g. Each container was watered to 80% of field water holding capacity with de-ionized water daily according to the amount of water loss to avoid a soil moisture difference between the planted treatments and the unplanted control. The weight changes due to plant growth were estimated using plant fresh weight measurements done on some extra containers periodically. Daily water loss from each container was recorded as the total evapotranspiration rate. In order to prevent possible occurrence of anaerobic conditions at the bottom portion of each container, all containers were aerated by using timer-controlled air pumps for 30 min during every 6 h period throughout the duration of the experiment. Maximum air temperature in the greenhouse ranged from 25 °C to 33 °C. Minimum air temperature ranged from 17 °C to 24 °C. Relative humidity ranged from 15% to 90%. Maximum solar energy received inside the greenhouse reached 1005 W m⁻² at the beginning of the experiment in March, and 1350 W m⁻² towards the end of the experiment in July. No supplemental lighting was used.

Immediately before each destructive sampling, rhizosphere respiration (or plant-derived CO₂ belowground) and SOM decomposition (or SOM-derived CO₂) were quantified using the natural ^{13}C tracer method described above (Cheng et al., 2003). Four containers from each treatment were sealed at the base of the plant with low melting point Paraffin (m.p. 42 °C). The total belowground CO₂ produced in each container was trapped for 48 h in a column containing 30 ml of 4 M NaOH solution mixed with acid-washed sand using a closed air circulating system. An aliquot of the NaOH solution in each column was analyzed for total inorganic C using a TOC Analyzer. Another aliquot of the trapping solution was mixed with excess SrCl₂ to yield pure SrCO₃, and then the $\delta^{13}\text{C}$ of the SrCO₃ was analyzed by mass spectrometry (Harris et al., 1997). Null CO₂ trapping units (blanks) without soil-roots were included to correct for contamination from carbonate in the NaOH stock solution and from sample handling.

After CO₂ trapping, plant shoots were cut at the soil surface and oven-dried at 65 °C for 48 h before weighing. Roots were hand-picked, washed, and oven-dried at 65 °C before weighing. Root-free soil samples were thoroughly mixed and sub-sampled. The first sub-sample of approximately 50 g was dried at 105 °C for 48 h for water, C and N content determination. Another sub-sample of approximately 500 g was put into a plastic bag and temporarily stored at 4 °C for soil microbial biomass determination. After grinding in a ball mill, the $\delta^{13}\text{C}$ values, C and N contents of oven-dried shoot, root, and soil samples were analyzed 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.

Carbon budgets of all three treatments (soybean, wheat, and unplanted) at the last sampling date were constructed using biomass and C content data for plant materials, cumulative soil CO₂ production across all sampling dates, and rhizodeposition. Cumulative CO₂ production during the whole experiment was estimated using linear interpolation between sampling dates, therefore, assessment of standard error was not permitted. Plant-derived C in the form of rhizodeposits at the end of the experiment was estimated using two approaches, C balance and isotope tracers. The C balance approach employed the following equation: $C_{\text{rh}} = (C_s + C_{\text{CO}_2}) - C_{\text{rcv}}$; where C_{rh} is the amount of C as rhizodeposits or plant-derived C remaining in the soil at the end of the experiment; C_s is the total amount of soil C remaining at the end of the

experiment; C_{CO_2} is the amount of soil C loss in the form of CO_2 as quantified using the natural tracer method; and C_{rcv} is the amount of total soil C recovered in the unplanted treatment which is the sum of remaining soil C and CO_2 -C. The isotope tracer approach used the two-end-member separation equation similar to Eq. (1). But in this case, C_t , C_3 and C_4 represented the amount of total remaining soil C, plant-derived rhizodeposit C, and original soil-derived C, respectively; and δ_t , δ_3 and δ_4 represented the $\delta^{13}C$ values of the total remaining soil C, root-derived plant C and original soil-derived C, respectively.

There were four components in the N budget for each of the three treatments at the last sampling: shoot N, root N, soil mineral N (KCl extractable nitrate and ammonium), and total mobilized N which is the sum of plant-N and soil mineral N. For soybean there was an additional component of N_2 -fixation as well. Plant-N was calculated using biomass and N content data. Soil mineral N was measured using KCl extraction method. N_2 -fixation in the soybean treatment was estimated using two approaches: N balance and ^{15}N natural abundance. In the N balance approach, the amount of N fixed in the soybean treatment was calculated by taking the difference between the total amount of N in the whole plant–soil system at the end of the experiment and the total amount of soil N at the start of the experiment. The ^{15}N natural abundance approach used the following two-end-member separation equation:

$$N_{fix} = N_{soy}(\delta_{ref} - \delta_{soy}) / (\delta_{ref} + \Delta) \quad (2)$$

where N_{fix} is the amount of N fixed by the soybean-Bradyrhizobium system; N_{soy} is the amount of N in soybean plant materials; δ_{ref} is the $\delta^{15}N$ value of the reference plant materials (wheat); δ_{soy} is the $\delta^{15}N$ value of the soybean plant materials; and Δ is the isotope fractionation factor associated with N_2 -fixation for soybeans which is 1.5‰ (Shearer and Kohl, 1986).

Soil microbial biomass C was measured using the fumigation–extraction method described by Vance et al. (1987) with some minor modifications. Fresh soil samples were fumigated with purified $CHCl_3$ for 48 h, and extracted with a 0.5 M K_2SO_4 solution. The concentration of total organic C in each extract was analyzed using a total organic C analyzer (Shimadzu 5050A). Microbial biomass C was calculated as the difference between fumigated and unfumigated samples, adjusted by a proportionality coefficient for C ($K_{ec} = 0.33$) (Vance et al., 1987). Microbial turnover time (MTT) was calculated using this equation:

$$MTT = (MB \times (1 - Y)/Y) / (R_s - MB \times R_m) \quad (3)$$

where MB is microbial biomass C ($mg\ C\ pot^{-1}$); Y is the so-called microbial yield factor (assuming $Y = 0.45$ as a reasonable estimate [Qian et al., 1997]), R_s is the microbial respiration rate as measured using SOM-derived CO_2 , R_m is the microbial maintenance respiration rate (assuming $R_m = 0.08\%$ of the $MB\ day^{-1}$ as a reasonable estimate [Anderson and Domsch, 1985]).

Statistix 7 software (Analytical Software, Tallahassee, Florida) was used for statistical analysis of the data. There were two main treatment factors (plant species and growth stage) and four replicates with each treatment-factor combination. The general linear model was used for analysis of variance (ANOVA). Fisher's LSD method ($P = 0.05$) was used for mean comparisons.

3. Results

Compared to the unplanted treatment, SOM decomposition rates increased significantly in the two planted treatments at the second sampling time and afterwards, indicating a high magnitude of rhizosphere priming effects on SOM decomposition (Table 1). Plant species and phenology strongly controlled the magnitude of

Table 1

Days after planting (DAP), SOM-derived CO_2 (SOM- CO_2 , $mg\ C\ pot^{-1}\ day^{-1}$), soil microbial biomass soil C (MB, $mg\ C\ pot^{-1}$), microbial biomass turnover time (MTT, days), and evapotranspiration (ET, $g\ H_2O\ pot^{-1}\ day^{-1}$) under the unplanted control, planted with wheat, and planted with soybean treatments

Treatments	DAP	SOM- CO_2	MB	MTT ^b	ET
Unplanted	35	32.9 (3.1)a ^a	2254 (140)	88	46 (3)a
	68	28.9 (2.2)a	2431 (62)	110	55 (4)a
	89	30.3(1.2)a	2173 (124)	93	53 (4)a
	110	32.3 (1.5)a	2365 (78)	95	43 (5)a
	Mean	31.1	2305	97	49
Wheat	35	35.2 (9.6)a	1976 (50)	72	135 (12)b
	68	111.7 (1.6)c	2063(82)	23	463 (25)c
	89	69.7 (3.6)b	2416 (49)	44	170 (20)b
	110	51.7 (1.7)b	2217 (108)	54	46 (6)a
	Mean	67.1	2168	48	203
Soybean	35	33.9 (1.8)a	1971 (98)	75	56 (6)a
	68	139.4 (5.9)c	1988 (107)	18	569 (41)c
	89	124.8 (4.6)c	2072 (111)	21	469 (38)c
	110	114.4 (6.9)c	2339 (132)	25	520 (45)c
	Mean	103.1	2093	35	403

Values are means of four replicates with standard errors in parenthesis.

^a Values with different letters are significantly different from each other ($P = 0.05$).

^b Microbial turnover time is calculated using this equation: $MTT = (MB \times (1 - Y)/Y) / (R_s - MB \times R_m)$, where MB is microbial biomass C; Y is the substrate utilization efficiency (assuming $Y = 0.45$ as a reasonable estimate, see Qian et al., 1997), R_s is the microbial respiration rate as measured using SOM-derived CO_2 , R_m is the microbial maintenance respiration rate (assuming $R_m = 0.08\%$ of the $MB\ day^{-1}$ as a reasonable estimate, see Anderson and Domsch, 1985).

the priming effect. The mean SOM decomposition rate in the soybean treatment increased by 231% over the unplanted treatment; while the rate only increased by 116% for the wheat treatment. The plant species effect was statistically significant ($P < 0.0001$). The level of the rhizosphere priming effect varied significantly during the experimental period for both planted treatments. No rhizosphere priming effect occurred at the first sampling time as indicated by the similar SOM decomposition rates among all three treatments. However, high levels of rhizosphere priming appeared at the second sampling time and through the end of the experiment for both planted treatments. The SOM decomposition rate of the soybean treatment increased several folds (3–4 times) above the unplanted treatment at the second sampling time and afterwards. The SOM decomposition rate of the wheat treatment more than tripled the rate of the unplanted treatment at the second sampling time, and more than doubled the rate of the unplanted at the third sampling time. These results indicated that plant phenology (sampling time) exerted significant ($P < 0.0001$) control on the level of rhizosphere priming effect.

There was far more C input in the forms of root biomass and rhizodeposition than the soil C loss in both planted treatments, even though the amount of extra soil C loss due to the rhizosphere priming effect was substantial (Fig. 1). Similar amounts of rhizodeposition were estimated using both the C budget method and the ^{13}C method, except that the standard error of the C budget method was much higher than the ^{13}C method. The agreement between the two methods assured reliability of the estimates. New C input in the form of rhizodeposits was much higher than root biomass C for both planted treatments. Overall, the rhizosphere priming effect only resulted in faster soil C cycling, no net C loss, instead, there was a C gain at the end of the experiment. The ultimate soil C status will depend on the degree of stabilization of the new C inputs.

Although the rhizosphere priming effect on SOM decomposition was highly significant for both planted treatments, soil microbial biomass C was not significantly affected by this priming effect (Table 1). The amounts of soil microbial biomass C were not significantly different between the unplanted control and the planted

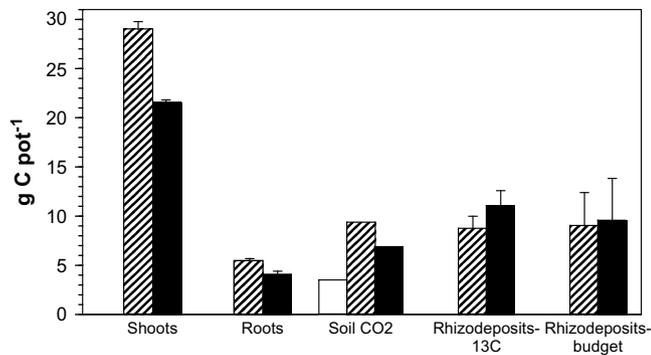


Fig. 1. Carbon budgets for the unplanted control (open bar), planted with soybean (gray bar), and planted with wheat (dark bar) treatments at the final sampling time (DAP = 110). Error bars = 1 standard error. Some error bars are too small to be seen. The amount of rhizodeposits was estimated using two methods: the ¹³C natural tracer method (Rhizodeposits-¹³C), and the carbon budget method (Rhizodeposits-budget).

treatments or between different sampling dates. This suggested that the rhizosphere priming effect mainly accelerated soil microbial biomass turnover rates. Soil microbial biomass turnover time in Table 1 was calculated using the soil respiration data and the microbial biomass data, assuming a microbial substrate utilization efficiency of 45% (Qian et al., 1997) and a soil microbial maintenance respiration rate of 0.08% of the biomass day⁻¹ (Anderson and Domsch, 1985). In general, larger rhizosphere priming effect in both planted treatments was associated with shorter soil microbial biomass turnover time. The amount of SOM-derived CO₂ output in all treatments and sampling times was negatively correlated with soil microbial biomass turnover time with a curve-linear relationship (Fig. 2). This relationship could be inferred from the fact that SOM-derived CO₂ increased several times in some of the planted treatments, but no significant changes in soil microbial biomass were found. Increased soil microbial biomass turnover rate was the plausible explanation for the rhizosphere priming effect. However, the quantitative relationship is clearly sensitive to the assumed value of microbial substrate utilization efficiency, but relatively insensitive to the value of the assumed microbial maintenance respiration rate (Fig. 2).

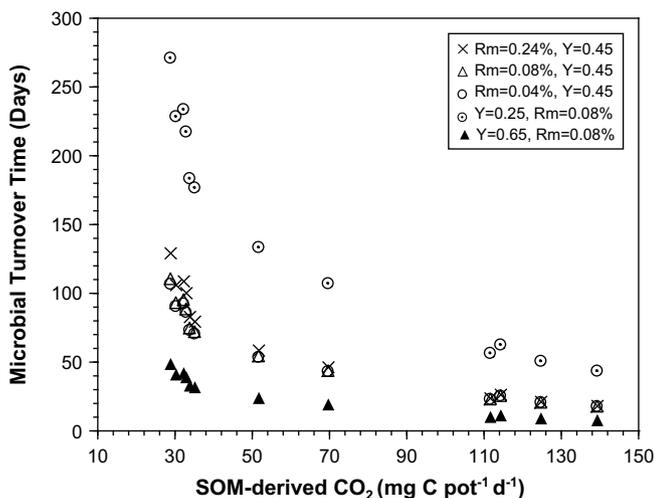


Fig. 2. A curve-linear relationship between soil microbial biomass turnover time in days and the rate of SOM-derived CO₂ efflux, when different values of microbial maintenance respiration rate (R_m) and substrate utilization efficiency (Y) were used to calculate biomass turnover time. The relationship is more sensitive to the changes of Y than R_m .

The amount of SOM-derived CO₂ output in all treatments and sampling times was also positively correlated with the amount of water loss through evapotranspiration, primarily from plant transpiration (Fig. 3). The highest degree of rhizosphere priming effect occurred at the second sampling time (DAP = 68) which corresponded to the highest evapotranspiration rate for both planted treatments. The higher amount of water loss also meant more drying–rewetting cycles in this experiment. This result indicated that the magnitude of the rhizosphere priming effect is controlled by plant photosynthesis or the degree and frequency of drying–rewetting cycles, or both, because plant transpiration rate is often positively correlated with plant photosynthetic activity.

In order to investigate the link between the rhizosphere priming effect (as mostly measured with organic C decomposition) and soil N dynamics, N budgets for all treatments were obtained at the last sampling time (Fig. 4). The percent recovery for soil N was nearly 100% (data not shown) of the initial amount, indicating that there was little N loss through either denitrification or leaching. Most mobilized N was found in shoot biomass and a small amount in root biomass at the end of the experiment for both planted treatments. Approximately 54% of the total N uptake in the soybean treatment came from biological N₂-fixation. The average amount of N₂-fixation estimated using the ¹⁵N natural abundance method was quite similar to the amount of N₂-fixation estimated using the N budget method, but the N budget method had a much higher standard error. The total amount of net soil N mineralized (the sum of plant uptake minus N₂-fixation, and the remaining soil NH₄⁺ and NO₃⁻) in the soybean treatment was 21% higher than in the unplanted control, indicating a significant stimulation of net N mineralization by rhizosphere priming. In contrast, net soil N mineralization in the wheat treatment only increased 6% compared to the unplanted control. Considering the significantly increased SOM decomposition (tripled for the soybean treatment, and doubled for the wheat treatment) in the planted treatments mentioned above, the rhizosphere priming effect on net soil N mineralization occurred to a lesser degree and was not proportional to that of soil organic C decomposition.

4. Discussion

Results from this experiment and several other studies (e.g., Fu and Cheng, 2002; Bader and Cheng, 2007; Dijkstra and Cheng, 2007) clearly indicate that the rhizosphere priming effect on SOM decomposition is a major biological factor controlling soil C

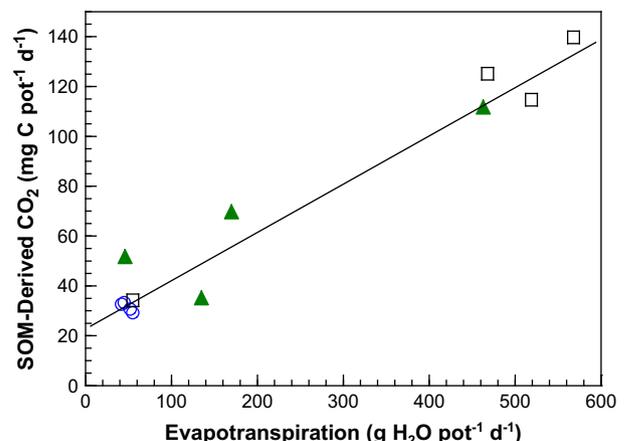


Fig. 3. A linear relationship between the rate of SOM-derived CO₂ efflux and the rate of evapotranspiration, using the data in Table 1. Open circle: unplanted treatment; filled triangle: wheat treatment; and open square: soybean treatment. The regression equation is: $Y = 0.19X + 24.8$ with $R^2 = 0.95$ and $P < 0.0001$.

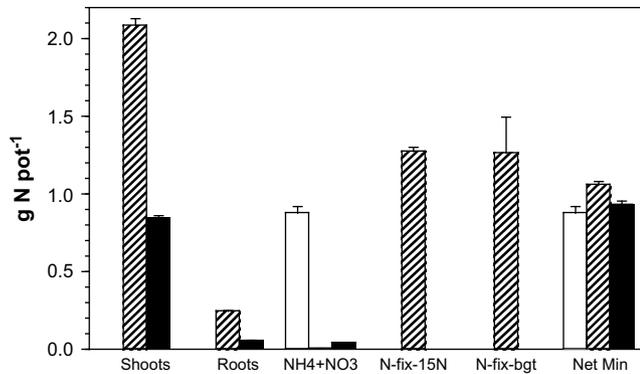


Fig. 4. N budgets for the unplanted control (Open bar), planted with soybean (gray bar), and planted with wheat (dark bar) treatments at the final sampling time (DAP = 110). Error bar = 1 standard error. Some error bars are too small to be seen. The amount of N₂-fixation in the soybean treatment was estimated using two methods: the ¹⁵N natural abundance method (N-Fix-¹⁵N), and the N budget method (N-Fix-bgt).

dynamics. The rhizosphere priming effect can range from 70% reduction to 380% higher than the unplanted control under various experimental configurations (Table 1, Cheng and Kuzyakov, 2005). Both biological and environmental agents have been shown to significantly influence the magnitude of the rhizosphere priming effect, even though exact mechanisms behind the rhizosphere priming effect remain largely unknown (Cheng and Kuzyakov, 2005). However, there is little doubt that the rhizosphere is constantly exerting its effects on SOM decomposition in all real terrestrial ecosystems. Therefore, any existing rates of SOM decomposition originated from either root exclusion experiments or unplanted soil incubations should be considered unrealistic.

The amount of extra soil C loss caused by the rhizosphere priming effect during the entire experiment rivals the amount of C in root biomass for both planted treatments (Fig. 1). However, when all C inputs and outputs are considered, the planted soil systems actually have net C gains, as C inputs from root standing biomass and rhizodeposition more than compensated for the total C loss during the growing season. But, directly applying the current results to a field situation is probably unwise because soil disturbance during preparation and greenhouse operation may have exacerbated the rhizosphere effect. The average C loss in the form of soil-derived CO₂ during the entire growing season represents 2–6% of the total initial soil C in this experiment. This range is comparable to the value of approximately 6% found in a field study using the ¹³C method (Rochette and Flanagan, 1997).

Some studies have suggested that the occurrence of priming effects is closely linked with the dynamics of soil microorganisms (Dalenberg and Jager, 1981; Alphei et al., 1996; Hamer and Marschner, 2005). Results from this current study directly establish the curve-linear relationship between the level of rhizosphere priming effect and soil microbial biomass turnover time (Fig. 2), and clearly demonstrate that accelerated SOM decomposition rates often result from enhanced microbial turnover rates instead of increased microbial biomass. This is shown by the relatively stable levels of total microbial biomass while SOM-derived CO₂ output varies by several folds in the planted treatments. Two critical assumptions are used when establishing the relationship between soil microbial biomass turnover time and the level of the priming effect on SOM decomposition: (1) a constant microbial substrate utilization efficiency, and (2) an invariant level of microbial maintenance respiration rate. If the two assumptions are invalid, the quantitative relationship between soil microbial biomass turnover time and the level of the priming effect on SOM decomposition will become fussy. But the qualitative relationship shall still hold. Further studies about this relationship and these parameter values are warranted.

In addition to the uncertainties associated with the two assumed parameters, the inevitable inclusion of plant-derived C in the total microbial biomass C for all planted treatments, which was used to calculate SOM-derived microbial biomass turnover time, may introduce another source of bias. The turnover times of SOM-derived microbial biomass C would be over-estimated (alternatively, turnover rates would be under-estimated) if plant-derived microbial biomass C contributed significantly to the total microbial biomass. This could be a serious bias particularly for the two later sampling dates when plant-derived C might have contributed to the total microbial biomass C. But it should be a minor bias for the two early sampling dates when most plant-derived C sources had not been incorporated into microbial biomass. Unfortunately, accurately and separately estimating SOM-derived and plant-derived microbial biomass turnover times remains difficult, because the measurements of root-derived CO₂ inevitably include both rhizosphere-microbial respiration and root respiration. Overall, this bias will tend to produce conservative soil microbial turnover rates, which should not seriously affect the qualitative conclusion about the relationship between rhizosphere priming effects and soil microbial biomass turnover rates.

Linking the rhizosphere priming effect with an increased soil microbial biomass turnover rate has important implications to the outcome of competition for mineral N between plants and soil microbes. Because of increased microbial turnover rates and SOM decomposition, plants are able to effectively compete with microbes for available N, even though a substantial amount of rhizodeposition has given the microbes more available C substrates for more N immobilization. The mechanism of this competition between plants and soil microbes for available N can be understood when one considers the difference between the turnover rate of plant roots and the turnover rate of microbial biomass and microbial extracellular enzyme production. Soil microbes in the rhizosphere grow and die (or turnover) at time scales of hours or days (Herman et al., 2006), while plant roots normally turnover at time scales of weeks, months or even years. Because of this difference in turnover rates between rhizosphere microbes and plant roots, N assimilated by rhizosphere microbes is quickly recycled back to the available N pool as microbial biomass turns over, forming bidirectional flows in between. In contrast, most N taken up by roots does not return back to the soil within a growing season, forming a unidirectional flow between the available N pool and roots. These flow structures allow plants to effectively compete with soil microbes for available N at longer time scales. Furthermore, accelerating microbial biomass turnover is often advantageous to the plants as long as the enhanced microbial activities release more extracellular enzymes that mobilize N from SOM. In this context, it is logical that the rhizosphere priming effect is closely linked with a much increased microbial turnover rate.

Results from this current experiment also indicate that the magnitude of the rhizosphere priming effect is affected either by plant photosynthesis or the degree and frequency of drying-rewetting cycles, or both (Fig. 3). Water loss through plant transpiration often results in relatively drier soil conditions in planted treatments than unplanted controls, despite frequent watering to compensate for the water difference between planted and the unplanted treatments. Therefore, drying-rewetting cycles have larger amplitudes in the planted treatment than the unplanted control (Sala et al., 1992). Some soil incubation studies have shown that short and frequent drying-rewetting cycles, as one may expect in the rhizosphere, tend to increase SOM decomposition (van Schreven, 1967; Lundquist et al., 1999). Our data indicate that drying and rewetting may explain the increased CO₂ efflux and N mineralization in the presence of the rhizosphere, since CO₂ efflux originating from SOM is positively correlated with total daily evapotranspiration rates (Fig. 3). However, evapotranspiration is

also positively correlated with leaf biomass and possibly root exudation. It is unclear at this stage whether drying–rewetting alone, drying–rewetting together with root exudation, or root exudation alone causes the rhizosphere effect on SOM decomposition, even though published reports have often attributed the rhizosphere effect to root exudation alone (e.g., Clarholm, 1985; Alpehi et al., 1996). As shown in a growth chamber experiment of shorter duration (55 days) with sunflower and soybean plants grown in soils taken from an organic farm, the level of rhizosphere priming is positively correlated with leaf biomass which can be a surrogate for the photosynthesis rate or transpiration rate (Dijkstra et al., 2006). However, the level of rhizosphere priming is not correlated with leaf biomass from either the wheat or the soybean treatment in the current experiment of longer duration (110 days) (data not shown). This is because the older leaves later in the growing season were not as active as the younger leaves early in the growing season. The close connection between photosynthetic activity aboveground and the degree of rhizosphere priming has been well illustrated in another growth chamber experiment (Kuzakov and Cheng, 2001) using spring wheat grown in the same C₄-soil as in this current experiment. However, the potential connection between the drying–rewetting cycles and the rhizosphere priming needs further investigation.

Proportional release of mineral N upon decomposition of SOM has often been assumed (e.g., Parton et al., 1987; Dalias et al., 2002). However, results from this current experiment suggest that the amount of net mineral N released from the accelerated SOM decomposition due to the rhizosphere priming effect is not nearly proportional to the C mineralization (Fig. 4). Only a small amount of extra net N mineralization is found in the planted treatments, even though C mineralization increases 2–3 folds due to the rhizosphere priming effect. The net amounts of rhizosphere-primed C decomposition are 5.88 and 3.38 g C pot⁻¹ for the soybean and the wheat treatments, respectively (Fig. 1); which would mean 0.51 and 0.29 g N pot⁻¹ of extra mineralized-N if the rhizosphere-primed SOM has the same C:N ratio (C:N = 11.5) as the original SOM at the beginning of the experiment. Instead, the rhizosphere priming effect is only responsible for 0.18 and 0.05 g N pot⁻¹ for the soybean and wheat treatments, respectively (Fig. 4). This suggests that N immobilization may account for approximately 64% and 81% of the extra gross mineralized-N from the rhizosphere priming effect for the soybean and the wheat treatments, respectively. In other words, the soybean plants are 36% efficient and the wheat plants are 19% efficient in the competition with soil microbes for the rhizosphere-primed gross mineralized-N, far from 100% efficient as often assumed. Unfortunately, the exact efficiencies cannot be calculated because gross N mineralization is not measured in this experiment. It is clear that this possible de-coupling of C and N cycling in the rhizosphere warrants further study.

Given that soybean rhizosphere priming effect on SOM decomposition and N mineralization is significantly stronger than wheat, it is not surprising that the soybean plants are also more efficient in acquiring soil N than wheat. However, considering the fact that soybean plants also fix significant amount of N₂ (<50% of the total plant-N) by harboring rhizobia in the nodules, the observed prudence of soybean plants in soil N acquisition is in direct contrast to the perception that N₂-fixing legumes may release some fixed-N to the soil during the growing season. Based on the N budget data (Fig. 4), extra release of plant-N into the soil is not detected at the end of the experiment for the soybean treatment as compared to the wheat treatment. There is little doubt that a portion of the fixed-N will be released back to the soil after soybean roots and leaves decompose, which will enrich the soil with more N.

The current results and results from published reports clearly indicate that plant species is an important determinant in the degree of rhizosphere priming (e.g., Cheng and Kuzakov, 2005;

Dijkstra and Cheng, 2007). However, the mechanistic link between plant traits and rhizosphere priming effects remains largely unknown. For the current study, soybean rhizosphere stimulates significantly more SOM decomposition and N mineralization than wheat rhizosphere. The relatively higher N concentration in the soybean tissues (C:N = 14.8) compared to wheat tissues (C:N = 28.7) is a possible cause of the difference in rhizosphere priming between the two plant species if the C:N ratio of their rhizodeposits is similar to the C:N ratio of their plant tissues. This is because N-containing labile compounds tend to produce higher degree of priming effects than carbon substrates alone (Dalenberg and Jager, 1989). Another possible cause of this difference in rhizosphere priming between the two plant species is their transpiration rates; the transpiration rates of soybean plants were substantially higher than the transpiration rates of wheat plants (Table 1). As shown in Fig. 3, there is a positive correlation between the level of rhizosphere priming and the amount of water loss through transpiration. Further studies are needed to ascertain what plant traits actually determine their rhizosphere priming effects on SOM decomposition and N mineralization.

In summary, the magnitude of the rhizosphere priming effect on SOM decomposition ranged from zero to more than 380% of the unplanted control in this experiment. Plant species and phenology exerted strong control on the level of the priming effect. Although the extra soil CO₂ efflux from the strong rhizosphere priming effect was a significant source of soil C loss, the whole plant–soil system remained with a net C gain at the end of the experiment, because the new C input in the forms of rhizodeposits and root biomass more than negated the loss of the extra soil CO₂ in the planted treatments. The increased soil microbial biomass turnover rate and the enhanced drying–rewetting cycles in the planted treatments were identified as the two important mechanisms that are closely linked to the rhizosphere priming effect. Contrary to the often-assumed proportionality between soil C and N processes, rhizosphere-primed C mineralization is largely de-coupled from net N mineralization.

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