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Zhu, Biao
Gutknecht, Jessica LM
Herman, Donald J
et al.

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Rhizosphere priming effects on soil carbon and nitrogen mineralization

Biao Zhu [a, b, *], Jessica L.M. Gutknecht [a, c], Donald J. Herman [d], Daniel C. Keck [a, e], Mary K. Firestone [d], Weixin Cheng [a]

[a] Department of Environmental Studies, University of California, Santa Cruz, CA 95064, USA
[b] Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
[c] Department of Soil, Water and Climate, University of Minnesota, St. Paul, MN 55108, USA
[d] Department of Environmental Science, Policy and Management, University of California, Berkeley, CA 94720, USA
[e] Environment, Health and Safety Program, University of California, Los Angeles, CA 90095, USA

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ABSTRACT

Living roots and their rhizodeposits affect microbial activity and soil carbon (C) and nitrogen (N) mineralization. This so-called rhizosphere priming effect (RPE) has been increasingly recognized recently. However, the magnitude of the RPE and its driving mechanisms remain elusive. Here we investigated the RPE of two plant species (soybean and sunflower) grown in two soil types (a farm or a prairie soil) and sampled at two phenological stages (vegetative and mature stages) over an 88-day period in a greenhouse experiment. We measured soil C mineralization using a continuous ^{13}C-labeling method, and quantified gross N mineralization with a ^{15}N-pool dilution technique. We found that living roots significantly enhanced soil C mineralization, by 27–245%. This positive RPE on soil C mineralization did not vary between the two soils or the two phenological stages, but was significantly greater in sunflower compared to soybean. The magnitude of the RPE was positively correlated with rhizosphere respiration rate across all treatments, suggesting the variation of RPE among treatments was likely caused by variations in root activity and rhizodeposit quantity. Moreover, living roots stimulated gross N mineralization rate by 36–62% in five treatments, while they had no significant impact in the other three treatments. We also quantified soil microbial biomass and extracellular enzyme activity when plants were at the vegetative stage. Generally, living roots increased microbial biomass carbon by 0–28%, β-glucosidase activity by 19–56%, and oxidative enzyme activity by 0–46%. These results are consistent with the positive rhizosphere effect on soil C (45–79%) and N (10–52%) mineralization measured at the same period. We also found significant positive relationships between β-glucosidase activity and soil C mineralization rates and between oxidative enzyme activity and gross N mineralization rates across treatments. These relationships provide clear evidence for the microbial activation hypothesis of RPE. Our results demonstrate that root–soil–microbial interactions can stimulate soil C and N mineralization through rhizosphere effects. The relationships between the RPE and rhizosphere respiration rate and soil enzyme activity can be used for explicit representations of RPE in soil organic matter models.

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1. Introduction

Soil organic carbon (SOC) functions as an important source and sink of atmospheric CO_2 (Amundson, 2001). Soil CO_2 efflux is approximately 10 times greater than anthropogenic CO_2 emissions from fossil fuel burning and land use change (Bond-Lamberty and Thomson, 2010). The two main components of soil CO_2 efflux are rhizosphere respiration by roots and microbes utilizing root-derived carbon substrates, and microbial decomposition of native SOC (Kuzyakov, 2006). Globally, SOC decomposition accounts for nearly half of total soil respiration (Hanson et al., 2000; Kuzyakov, 2006), and plays an important role in the global carbon cycle and its feedback to climate change (Davidson and Janssens, 2006; Heimann and Reichstein, 2008).

* Corresponding author. Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA. Tel.: +1 831 331 3627.
E-mail address: biaozhu@gmail.com (B. Zhu).

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Plants can stimulate or inhibit native SOC decomposition through rhizosphere processes (Dormaar, 1990; Kuzyakov, 2002; Paterson, 2003; Cheng and Kuzyakov, 2005; Cheng et al., 2014). Recent syntheses (Zhu and Cheng, 2011a; Cheng et al., 2014) noted that SOC decomposition rate in the presence of live roots can be suppressed by 50% or stimulated by up to 400% compared to unplanted control soils under similar temperature and moisture conditions. It is now becoming generally recognized that rhizosphere priming effects on SOC decomposition can play important roles in the global carbon cycle (Heimann and Reichstein, 2008; Kuzyakov, 2010; Cheng et al., 2014).

The actual mechanisms underlying rhizosphere priming effects still remain elusive (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). The positive rhizosphere effect on SOC decomposition (increased rates of decomposition) has been more frequently reported than the negative rhizosphere effect (Zhu and Cheng, 2011a; Cheng et al., 2014). One mechanism which has been proposed to explain the positive rhizosphere effect relates to root-released available substrates stimulating microbial growth in the rhizosphere thus leading to extracellular enzyme production and enhanced decomposition of native SOM (DeAngelis et al., 2008; Zhu and Cheng, 2011a; Phillips et al., 2011). However, the conditions under which this type of microbial activation occurs (Kuzyakov, 2002; Cheng and Kuzyakov, 2005) have not been clearly delineated.

In response to root-released carbon substrates in the rhizosphere, increases in microbial growth may stimulate microbial demand for nitrogen. This microbial N demand can be met by increasing enzyme synthesis (DeAngelis et al., 2008; Phillips et al., 2011) and gross N mineralization rate in the rhizosphere (Norton and Firestone, 1996; Herman et al., 2006; Koranda et al., 2011). The higher N mineralization rate may eventually lead to higher soil N availability for root uptake due to faster turnover of microbes compared to roots (Frank and Groffman, 2009; Kuzyakov and Xu, 2013). This microbial N mining hypothesis has been invoked as a mechanism to explain increased plant N uptake in elevated CO_2 studies (Zak et al., 1993; Cheng, 1999; Langley et al., 2009; Billings et al., 2010; Phillips et al., 2011), but only few studies (e.g. Herman et al., 2006; Dijkstra et al., 2009) have directly tested this hypothesis.

Here we investigated the rhizosphere priming effect on soil C and N mineralization in an 88-day greenhouse experiment. We measured soil C mineralization rate in the presence of live roots using a novel continuous ^{13}C-labeling method (Cheng and Dijkstra, 2007; Pausch et al., 2013), gross N mineralization rate in freshly sampled soils after root picking using a ^{15}N pool dilution method (Hart et al., 1994a; Herman et al., 2006), microbial biomass carbon by chloroform fumigation–extraction (Vance et al., 1987), and extracellular enzyme activities using fluorometric microplate assays (Saiya-Cork et al., 2002). The experiment included two plant species (a legume soybean (*Glycine max*), a non-legume sunflower (*Helianthus annuus*), and an unplanted control) grown in two soil types (a cultivated farm soil or a pristine prairie soil), and destructively sampled in two phenological stages (vegetative and mature stages). Our main objectives were to (1) investigate the control of rhizosphere priming effect on soil C mineralization by soil type, sampling time, and plant species, (2) test the microbial activation hypothesis for rhizosphere effect on soil C mineralization, and (3) explore the magnitude of rhizosphere effect on soil N mineralization.

2. Materials and methods

2.1. Experimental setup

We performed the experiment in a continuous ^{13}C-labeling greenhouse at University of California, Santa Cruz. During the experimental period, we maintained a constant CO_2 concentration (400 ± 5 ppm) and $\delta^{13}C$ value (-18.0 ± 0.5‰) inside the greenhouse by automatically adjusting the flow rate of CO_2-free air and pure CO_2 into the greenhouse. Details about this continuous ^{13}C-labeling method can be found in Cheng and Dijkstra (2007) and Pausch et al. (2013).

The experiment included two soil types (farm soil and prairie soil, Table 1), two plant species (soybean and sunflower) with an unplanted control, and two destructive samplings (53 and 88 days after planting). There were 8 or 11 replicates for each treatment combination ($2 \times 3 \times 2 = 12$) and 105 pots totally (Table 2).

We used two soil types in this experiment (Table 1). Surface (0–30 cm) soils were collected from a farm on the campus of University of California, Santa Cruz (farm soil) and from a tall-grass prairie at Konza Prairie Biological Station, Kansas (prairie soil). The farm was converted from coastal grassland in 1974 and has been planted with various C_3 crops and vegetables, while the prairie was dominated by C_4 grasses. The farm soil (Alfisol, sandy loam, pH 5.8) contained 14.0 mg C g soil^{-1} and 1.2 mg N g soil^{-1}, while the prairie soil (Mollisol, clay loam, pH 7.1) contained 17.1 mg organic C g soil^{-1} (plus 0.6 mg inorganic C g soil^{-1}) and 1.9 mg N g soil^{-1}. Our previous work using the prairie soil and similar plants (wheat and soybean) showed that soil inorganic carbon did not change significantly among control and planted treatments or during the experimental period (Cheng et al., 2003). Therefore, we are confident that carbonate did not contribute to the measured CO_2 flux from the prairie soil. The $\delta^{13}C$ value of soil organic carbon is −26.8‰ and −15.5‰ for the farm soil and the prairie soil, respectively.

The soils were sieved through a 4-mm screen and air-dried before use. A nylon bag filled with 1500 g washed sand was placed at the bottom of each bottom-capped polyvinyl chloride (PVC) pot (diameter 15 cm, height 40 cm, equipped with an inlet tube at the bottom for aeration and CO_2 trapping). We packed 7.32 kg farm soil or 6.60 kg prairie soil (dry weight equivalent) into each pot at a mean bulk density of 1.29 and 1.17 g cm^{-3}. After adjusting soil moisture to 60% water holding capacity (0.24 and 0.32 mL water g dry soil^{-1} for farm soil and prairie soil), we pre-incubated these 105 pots inside the greenhouse for two weeks. Then we planted five pre-soaked seeds of sunflower or soybean (inoculated with *Bradyrhizobium japonicum*) in 35 "sunflower" or 35 "soybean" pots, and kept 35 "control" pots unplanted. Seedlings germinated within one week and were thinned to one individual plant per pot.

During the experimental period, air temperature inside the greenhouse was maintained below 28 °C during the day (6 am to 6 pm) and above 18 °C during the night (18:00–06:00) by an air conditioner and a heater respectively, and relative air humidity was kept at 50% by a dehumidifier. Supplemental lighting was turned on during cloudy days (light intensity < 800 μmol m^{-2} s^{-1}). Soil moisture in each pot was maintained at 60% water holding capacity by frequent weighing and watering with deionized water.

Table 1
Properties of the two soil types.

Soil property	Farm	Prairie
Soil order	Alfisol	Mollisol
Soil texture	Sandy loam	Clay loam
Vegetation	C_3 crops	C_4 grasses
pH	5.8	7.1
Organic C (g kg^{-1})	14.0	17.1
Total N (g kg^{-1})	1.2	1.9
C:N	11.5	8.8
^{13}C of SOC (‰)	−26.8	−15.5

Table 2
Shoot and root biomass and $\delta^{13}C$ value, and total soil respiration (C_{total}) and its $\delta^{13}C$ value. Values represent mean ± standard errors. DAP stands for days after planting.

Soil type	Sampling time (DAP)	Plant species	Replicate	Shoot biomass (g pot^{-1})	Root biomass (g pot^{-1})	Shoot $\delta^{13}C$ (‰)	Root $\delta^{13}C$ (‰)	C_{total} $\delta^{13}C$ (‰)	C_{total} (mg C kg soil^{-1} day^{-1})
Farm	53	Control	8					−26.9 ± 0.1	9.5 ± 0.3
Farm	53	Soybean	8	4.40 ± 0.84	1.35 ± 0.22	−38.9 ± 0.2	−36.2 ± 0.4	−29.5 ± 0.4	16.9 ± 1.1
Farm	53	Sunflower	8	8.90 ± 1.15	3.61 ± 0.62	−40.3 ± 0.3	−38.6 ± 0.2	−34.7 ± 0.3	37.0 ± 3.0
Farm	88	Control	11					−25.1 ± 0.2	8.5 ± 0.4
Farm	88	Soybean	11	13.3 ± 1.01	2.50 ± 0.15	−39.2 ± 0.2	−36.7 ± 0.3	−28.2 ± 0.2	13.5 ± 0.5
Farm	88	Sunflower	11	63.0 ± 5.79	10.7 ± 0.64	−40.8 ± 0.2	−39.2 ± 0.4	−33.2 ± 0.2	30.4 ± 0.9
Prairie	53	Control	8					−17.3 ± 0.2	6.6 ± 0.8
Prairie	53	Soybean	8	2.81 ± 0.28	0.93 ± 0.12	−35.7 ± 0.3	−34.4 ± 0.4	−21.7 ± 0.8	12.5 ± 1.3
Prairie	53	Sunflower	8	2.24 ± 0.30	0.73 ± 0.10	−40.4 ± 0.2	−39.3 ± 0.2	−27.3 ± 0.8	19.8 ± 2.6
Prairie	88	Control	8					−18.0 ± 0.3	3.9 ± 0.3
Prairie	88	Soybean	8	4.62 ± 0.74	0.70 ± 0.06	−37.9 ± 0.5	−34.4 ± 0.6	−21.0 ± 0.6	7.8 ± 0.7
Prairie	88	Sunflower	8	12.1 ± 1.57	2.68 ± 0.46	−41.0 ± 0.4	−39.9 ± 0.3	−32.0 ± 0.6	33.8 ± 1.9
ANOVA *P*-values									
Soil type				<0.001	<0.001	<0.001	0.013	<0.001	<0.001
Sampling time				<0.001	<0.001	<0.001	0.108	0.984	0.356
Plant species				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Soil type × Sampling time				<0.001	<0.001	0.017	0.656	<0.001	<0.001
Soil type × Plant species				<0.001	<0.001	<0.001	<0.001	<0.001	0.253
Sampling time × Plant species				<0.001	<0.001	0.075	0.532	<0.001	0.001
Soil type × Sampling time × Plant species				<0.001	0.002	0.032	0.569	<0.001	<0.001

Anaerobic conditions were prevented by forcing ambient air through each pot for 60 min every night (20:00–21:00) using an aquarium pump. Once a week, positions of the pots on the greenhouse bench top were randomly relocated to ensure similar growing conditions for the plants.

2.2. Measurements and calculations

We measured total soil respiration from each pot using a closed-circulation CO_2 trapping system (Cheng et al., 2003) during the first (51–53 days after planting) and second (86–88 days after planting) sampling period. Briefly, we sealed each pot at the base of the plant with non-toxic silicone rubber (GI-1000, Silicones Inc., NC, USA) and removed CO_2 inside each pot by circulating the isolated air through a soda lime column for 1 h. Then CO_2 produced during the following 48-h period in each sealed pot was trapped in 400 mL 0.5 M NaOH solution by periodic air circulation for 30 min at 6-h interval. Blanks were included to correct for handling errors. An aliquot of each NaOH solution was analyzed for total inorganic C using a Shimadzu 5050A TOC analyzer. Another aliquot was precipitated as $SrCO_3$ and then analyzed for $\delta^{13}C$ using a PDZ Europa ANCA–GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Harris et al., 1997). The $\delta^{13}C$ values measured in $SrCO_3$ were corrected for contamination from carbonate in the NaOH stock solution and from sample handling (Cheng et al., 2003). We separated total soil respiration (C_{total}) into SOM decomposition (C_{soil}) and rhizosphere respiration (C_{root}) using a two-source mixing model:

$$C_{soil} = C_{total}(\delta^{13}C_{root} - \delta^{13}C_{total})/(\delta^{13}C_{root} - \delta^{13}C_{soil}) \quad (1)$$

$$C_{root} = C_{total} - C_{soil} \quad (2)$$

where $\delta^{13}C_{root}$ is the $\delta^{13}C$ value of rhizosphere respiration which was calculated based on the $\delta^{13}C$ value of shoots and the ^{13}C depletion of root-derived CO_2 relative to shoots (1.3‰ for soybean and 1.1‰ for sunflower, Zhu and Cheng, 2011b). This isotopic fractionation between root or shoot bulk tissue and root-respired CO_2 has been increasingly recognized (Werth and Kuzyakov, 2010; Ghashghaie and Badeck, 2014). Future research needs to measure and account for this fractionation in respiration partitioning at near natural abundance ^{13}C levels. $\delta^{13}C_{total}$ is the measured $\delta^{13}C$ value of total soil respiration, and $\delta^{13}C_{soil}$ is the mean $\delta^{13}C$ value of CO_2 from SOM decomposition measured in the unplanted treatment.

We quantified rhizosphere priming effect (C_{primed}) as the difference in C_{soil} between unplanted and planted treatment.

$$C_{primed} = C_{soil(planted)} - C_{soil(unplanted)} \quad (3)$$

Immediately after CO_2 trapping, we separated plants into shoots and roots, homogenized soils, and took a fresh soil sample (400 g) from each pot. Fine roots were removed from soil samples from planted pots by hand-picking. Then these soils were prepared for measuring gross N mineralization, microbial biomass, extracellular enzyme activity, soil moisture, and isotope abundance within three days.

Gross N mineralization rate was measured using the ^{15}N pool dilution method (Hart et al., 1994a). Fresh soil (50 g) was labeled with 2 mL of $(NH_4)_2SO_4$ solution (62.5 μg N mL^{-1}, 99 atom% ^{15}N). Each soil was divided in half and extracted in 2 M KCl at 15 min and 15 h after labeling. NH_4^+ in the extracts was quantified using a Lachat Autoanalyzer (QuikChem FIA+ Series 8000). $^{15}NH_4^+$ in the extracts was prepared by diffusion and determined on a PDZ Europa 20–20 isotope ratio mass spectrometer. Gross N mineralization rate was calculated using the equations in Hart et al. (1994a).

Microbial biomass C was measured using the chloroform fumigation–extraction method (Vance et al., 1987). One subsample (50 g) was extracted with 60 mL 0.5 M K_2SO_4 solution, another subsample (50 g) was fumigated by ethanol-free chloroform in the dark for 48 h and then extracted with 60 mL 0.5 M K_2SO_4 solution. The concentration of total organic C in each extract was analyzed using a Shimadzu analyzer (TOC 5050A). Microbial biomass C was calculated as the difference between fumigated and unfumigated samples, adjusted by a proportionality coefficient ($k_{EC} = 0.45$).

Potential activities of one hydrolytic and two oxidative enzymes were assayed using a fluorometric method modified from Saiya-

Cork et al. (2002). Briefly, β-glucosidase (BG) activity was determined using 200 μM 4-methylumbylliferone (MUB)-linked substrate, while phenol oxidase (PO) and peroxidase (PER) activities were assayed using 25 mM L-3,4-dihydroxyphenylalanine (L-DOPA) substrate. Soil homogenates were prepared by thoroughly mixing 1 g fresh soil in 125 mL buffer (50 mM Tris, pH = 7). After adding appropriate standards, homogenates, and substrates, the 96-well plates were incubated in dark at room temperature for 1 h (BG) or 24 h (PO and PER). Then sample fluorescence (for BG) was measured on a microplate reader (BioTek Synergy HT Multi-Mode) with 365 nm excitation and 450 nm emission filters, and sample absorbance (for PO and PER) was measured on the same microplate reader with a 460 nm filter. Enzyme activities were calculated as μmol g soil^{-1} h^{-1} (BG) and nmol g soil^{-1} h^{-1} (PO + PER).

Soil moisture was determined by oven-drying at 105 °C for 48 h. Subsamples of plants (shoots and roots) and soils (root-free) from each pot were dried, weighed, ground, and then analyzed for C%, N %, $\delta^{13}C$ and $\delta^{15}N$ using a Carlo Elba 1108 elemental analyzer interfaced to a ThermoFinningan Delta Plus XP isotope ratio mass spectrometer. Prairie soils contained some carbonate (~0.6 mg C g soil^{-1}) and were acid-fumigated to remove carbonates before elemental and isotope analysis.

2.3. Statistical analyses

For plant and soil variables that we measured across all treatment combinations (Figs. 1 and 2), we used three-way ANOVA to assess the effects of soil type, sampling time, plant species, and their two- and three-way interactions (Tables 2 and 3). As microbial biomass and extracellular enzymes were only measured after the first sampling (Fig. 3), we used two-way ANOVA to assess the effects of soil type, plant species, and their interaction (Table 4). One-way ANOVA (post-hoc LSD test) was also used to compare variables among unplanted control, soybean, and sunflower treatments at each soil type and sampling time combination (Figs. 1–3). We also calculated the percentage difference in soil variables between paired control and planted (soybean or sunflower) treatment as a relative measure of rhizosphere effect (Table 5). Simple linear regression was used to show the relationship between the variables in Fig. 4. We used PASW Statistics 18 to perform all statistical analyses and set the significance level at $P < 0.05$.

3. Results

3.1. Plant biomass and ^{13}C content

Plant growth appeared normal, with no signs of pests or pathogens. At the first sampling (53 days after planting), both plants were at vegetative stage. Sunflower produced significantly more shoot and root biomass than soybean in farm soil, but developed similar biomass as soybean in prairie soil (Table 2). Both plants grew better and produced more biomass in farm soil than in prairie soil (Table 2). These patterns became stronger at the second sampling (88 days after planting) when both plants were at mature stage. Sunflower produced 3–5 times more biomass than soybean, and both plants were 3–5 times bigger in farm soil than in prairie soil (Table 2).

Both plants were successfully labeled with ^{13}C-depleted CO_2. Shoots $\delta^{13}C$ value ranged between −39.2‰ and −35.7‰ for soybean and between −41.0‰ and −40.3‰ for sunflower, and roots $\delta^{13}C$ value ranged between −36.7‰ and −34.4‰ for soybean and between −39.9‰ and −38.6‰ for sunflower (Table 2). Moreover, sunflower was 1.5–4.9‰ more ^{13}C-depleted compared to soybean, and shoots were 1.0–3.5‰ more ^{13}C-depleted than roots (Table 2).

3.2. Soil C and N mineralization

Soil-derived CO_2 from unplanted pots showed $\delta^{13}C$ values between −26.9‰ and −25.1‰ in farm soil, and between −18.0‰ and −17.3‰ in prairie soil (Table 2). These values were slightly depleted or similar to the $\delta^{13}C$ values of soil organic carbon (−26.8‰ in farm soil and −15.5‰ in prairie soil). In contrast, soil-derived CO_2 from planted pots varied from −34.7‰ to −28.2‰ in farm soil, and from −32.0‰ to −21.0‰ in prairie soil (Table 2). These values reflected the mixture of two sources of CO_2 in total soil respiration (C_{total}) from planted pots: less ^{13}C-depleted soil-derived CO_2 (C_{soil}) and more ^{13}C-depleted root-derived CO_2 (C_{root}). Based on Eqs. (1) and (2), we partitioned C_{total} (Table 2) into SOM-derived C_{soil} (Fig. 1A) and root-derived C_{root} (Fig. 2B) using a two-source mixing model.

Soil-derived CO_2 ranged from 3.9 to 16.9 mg C kg soil^{-1} day^{-1} across all treatment combinations (Fig. 1A). Soil type, sampling time, and plant species all showed significant impact on C_{soil} ($P < 0.05$, Table 3). SOC in the farm soil appears to decompose faster than SOC in the prairie soil. From the first sampling to the second

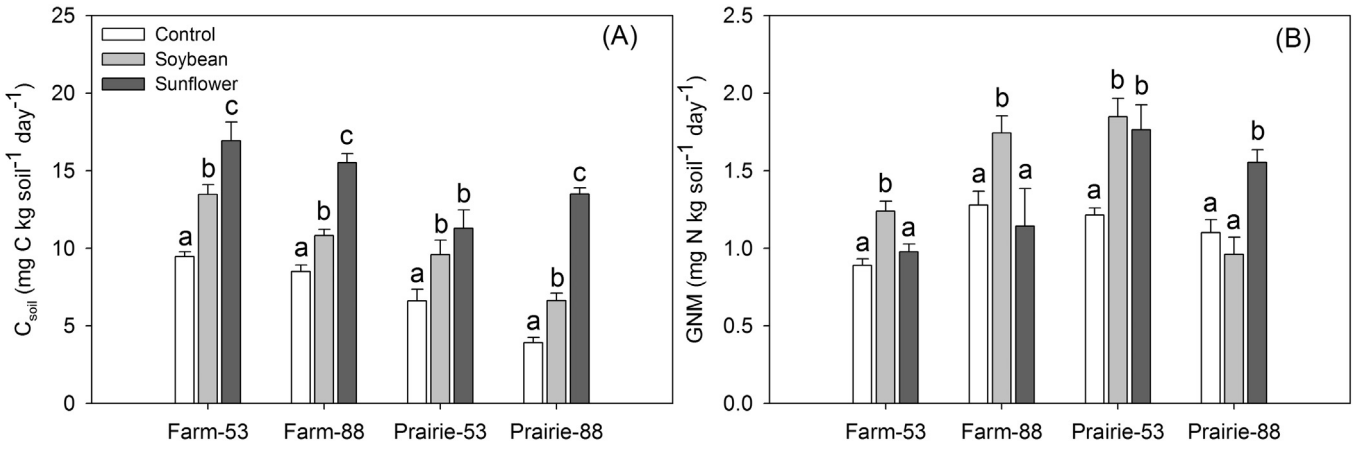

Fig. 1. Soil-derived CO_2 flux (C_{soil}, A) and gross N mineralization (GNM, B) averaged by soil type (farm versus prairie), sampling time (53 versus 88 days after planting), and plant species (control versus soybean versus sunflower). Different letters for each soil type at each sampling time denote significant differences among control and two plant species treatments (post-hoc LSD test, $P < 0.05$). Error bars indicate standard errors of the mean.

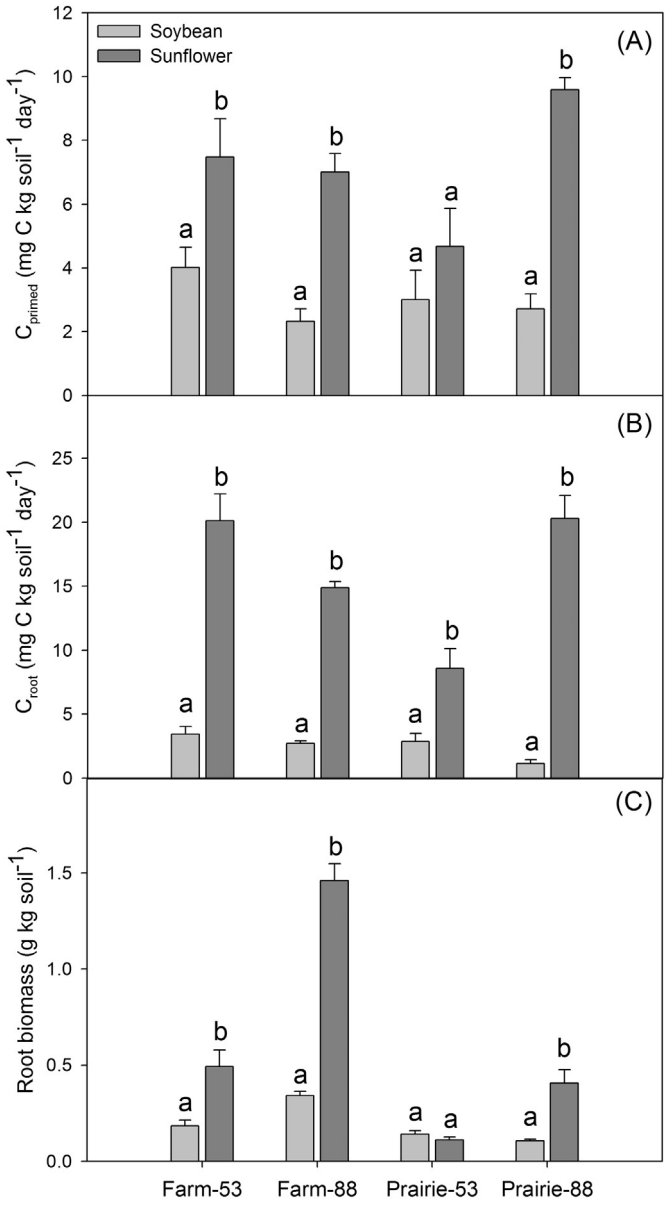

Fig. 2. Primed soil carbon (C_{primed}, A), root-derived CO_2 flux (C_{root}, B), and root biomass (C) averaged by soil type (farm versus prairie), sampling time (53 versus 88 days after planting), and plant species (soybean versus sunflower). Different letters for each soil type at each sampling time denote significant differences between soybean and sunflower treatments (post-hoc LSD test, $P < 0.05$). Error bars indicate standard errors of the mean.

sampling, C_{soil} decreased significantly in control and soybean pots, but remained at similar level or even increased in sunflower pots (Fig. 1A), indicating an interaction between sampling time and plant species on C_{soil} ($P < 0.05$, Table 3). Moreover, across all eight treatment combinations of two soil types, two sampling times, and two plant species, plants significantly enhanced SOC decomposition ($P < 0.05$, Fig. 1A, Table 3). These rhizosphere effects on SOC decomposition ranged between 27% and 245% compared to the unplanted control (Fig. 1A, Table 5).

Gross N mineralization (GNM) rate varied between 0.89 and 1.85 mg N kg soil^{-1} day^{-1} across all treatment combinations (Fig. 1B). Soil type and plant species, but not sampling time, exerted significant control of GNM ($P < 0.05$, Table 3). Compared to the unplanted control, soybean significantly enhanced GNM in farm soil at both sampling times and in prairie soil at the first sampling time ($P < 0.05$, Fig. 1B), but had no effect on GNM in prairie soil at the second sampling time ($P > 0.05$, Fig. 1B). In contrast, sunflower significantly enhanced GNM in prairie soil at both sampling times ($P < 0.05$, Fig. 1B), but had no effect on GNM in farm soil at both sampling times ($P < 0.05$, Fig. 1B). These contrasting results suggest significant interaction between soil type and plant species on GNM ($P < 0.05$, Table 3). Overall, plants significantly enhanced GNM compared to unplanted control by 36–52% in five treatment combinations ($P < 0.05$, Fig. 1B, Table 5), while had no significant impact in the other three treatment combinations ($P > 0.05$, Fig. 1B, Table 5).

3.3. Rhizosphere priming effect and rhizosphere respiration

Primed soil carbon (C_{primed}) was calculated as the difference in C_{soil} between unplanted and planted treatment (Eq. 3). Plant species, but neither soil type nor sampling time, significantly impacted C_{primed} ($P < 0.05$, Table 3). C_{primed} ranged between 2.32 and 4.01 mg C kg soil^{-1} day^{-1} among soybean treatments, and between 4.68 and 9.58 mg C kg soil^{-1} day^{-1} among sunflower treatments (Fig. 2A). Specifically, C_{primed} was significantly higher in sunflower treatment than in soybean treatment, in farm soil at both sampling times and in prairie soil at the second sampling time ($P < 0.05$, Fig. 2A), but not in prairie soil at the first sampling time ($P > 0.05$, Fig. 2A).

Root-derived CO_2 (C_{root}) ranged between 1.14 and 3.44 mg C kg soil^{-1} day^{-1} among soybean treatments, and between 8.57 and 20.29 mg C kg soil^{-1} day^{-1} among sunflower treatments (Fig. 2B). Soil type and plant species, but not sampling time, significantly impacted C_{root} ($P < 0.05$, Table 3). Compared to soybean, sunflower showed 3.0–17.8 times more C_{root} (Fig. 2B), but only 0.8–4.3 times more root biomass (Fig. 2C). These results suggest that mass-specific rhizosphere respiration rate was higher in sunflower than in soybean, although sunflower contained similar or less N in root tissue than soybean (0.96–2.38% versus 1.62–2.60%, data not shown).

The rhizosphere priming effect (C_{primed}) was significantly and positively correlated with rhizosphere respiration (C_{root}) across all treatment combinations ($R^2 = 0.660$, $P < 0.001$, Fig. 4D). However, C_{primed} was more poorly correlated with root biomass ($R^2 = 0.201$, $P < 0.001$, Fig. 4C) and did not change with increasing root density above a threshold of ~0.5 g kg soil^{-1}. These results suggest that variations of rhizosphere priming effect across treatments may be linked to variations of rhizosphere respiration rate, an index of root activity and rhizodeposit quantity. Indeed, when we include C_{root} as a covariate in the ANCOVA analysis, none of the main and interactive effects of soil type, sampling time, or plant species on C_{primed} was statistically significant ($P > 0.05$, Table 3).

3.4. Microbial biomass carbon and extracellular enzyme activities

We also measured microbial biomass carbon (MBC) and activities of three extracellular enzymes involved in soil C and N mineralization at the first sampling time. MBC was higher in prairie soil compared to farm soil ($P < 0.05$, Fig. 3A). Moreover, MBC in the farm soil was enhanced by sunflower (but not soybean) compared to the control treatment ($P < 0.05$, Fig. 3A), whereas MBC in the prairie soil was not affected by either plant ($P > 0.05$, Fig. 3A), suggesting a significant interaction of soil type and plant species on MBC ($P < 0.05$, Table 4). In addition, BG activity was 7–10 times higher in farm soil than in prairie soil (Fig. 3B). Compared to the unplanted control, sunflower enhanced BG activity in farm soil ($P < 0.05$, Fig. 3B) but not in prairie soil, and soybean did not affect BG activity in either soil ($P > 0.05$, Fig. 3B). In contrast to BG activity,

Table 3
ANOVA *P*-values for C_{soil} (soil-derived CO_2 flux), GNM (gross N mineralization), C_{primed} (the difference in soil-derived CO_2 fluxes between unplanted control and planted treatments), and C_{root} (root-derived CO_2 flux).

	C_{soil}	GNM	C_{primed}	C_{primed}[a]	C_{root}	C_{root}[b]
Soil type	<0.001	0.003	0.705	0.349	0.014	0.355
Sampling time	0.001	0.696	0.265	0.566	0.221	0.161
Plant species	<0.001	<0.001	<0.001	0.795	<0.001	<0.001
Soil type × Sampling time	0.501	<0.001	0.003	0.534	<0.001	<0.001
Soil type × Plant species	0.950	<0.001	0.856	0.380	0.226	0.253
Sampling time × Plant species	0.004	0.115	0.005	0.101	0.008	0.917
Soil type × Sampling time × Plant species	0.016	0.003	0.076	0.404	<0.001	<0.001

[a] ANOVA results with C_{root} as covariate ($P < 0.001$).
[b] ANOVA results with root biomass as covariate ($P = 0.003$).

the sum activity of PO and PER was higher in prairie soil than in farm soil ($P < 0.05$, Fig. 3C). Compared to the unplanted control, we observed higher activity of oxidative enzymes for sunflower (but not soybean) in farm soil and for both plants in prairie soil ($P < 0.05$, Fig. 3C). We also found a significant positive correlation between SOM decomposition rate (C_{soil}) and BG activity ($R^2 = 0.411$, $P < 0.001$, Fig. 4A), and between gross N mineralization rate (GNM) and the sum activity of PO and PER ($R^2 = 0.362$, $P < 0.001$, Fig. 4B).

4. Discussion

4.1. Rhizosphere priming effect on soil organic carbon decomposition

Consistent with recent studies (summarized by Cheng et al., 2014), this study showed that SOM decomposition was significantly enhanced in planted soil compared to unplanted control soil. The intensity of rhizosphere priming of soybean and sunflower ranged from 27% to 245% across treatment combinations, which was comparable to previous results on the same soil types, plant species, and plant phenology (e.g. Cheng et al., 2003; Dijkstra and Cheng, 2007b; Zhu and Cheng, 2011a). Moreover, woody species (tree seedlings) have also been shown to have a significant positive rhizosphere effect on SOM decomposition (e.g. Phillips and Fahey, 2006; Bader and Cheng, 2007; Dijkstra and Cheng, 2007a; Bengtson et al., 2012). In aggregate, these studies provide unequivocal evidence that plant–soil interactions can significantly control SOM decomposition through mechanisms commonly termed, rhizosphere effects. The importance of these interactions in controlling SOM decomposition is becoming increasingly recognized by the scientific community (Heimann and Reichstein, 2008; Kuzyakov, 2010; Bird et al., 2011; Gärdenäs et al., 2011; Phillips et al., 2011; Bengtson et al., 2012; Cheng et al., 2014).

Although the rhizosphere priming effect on SOM decomposition has been widely observed, the mechanisms underlying this effect remain elusive (Dormaar, 1990; Kuzyakov, 2002; Cheng and Kuzyakov, 2005). This study provides additional evidence supporting the microbial activation hypothesis (Cheng and Kuzyakov, 2005). Sampling during the vegetative growth stage showed that the presence of the rhizosphere led to 42–79% increase in SOM decomposition rate, 0–28% increase in microbial biomass carbon, 19–56% increase in β-glucosidase activity, and 0–46% increase in oxidative enzyme activity (Table 5). We also found significant correlation between β-glucosidase activity and SOM decomposition rate ($P < 0.001$, Fig. 4A), and between microbial biomass carbon and SOM decomposition rate ($P = 0.059$, data not shown). These results support the microbial activation hypothesis: roots release labile carbon substrates to the rhizosphere, which lead to microbial

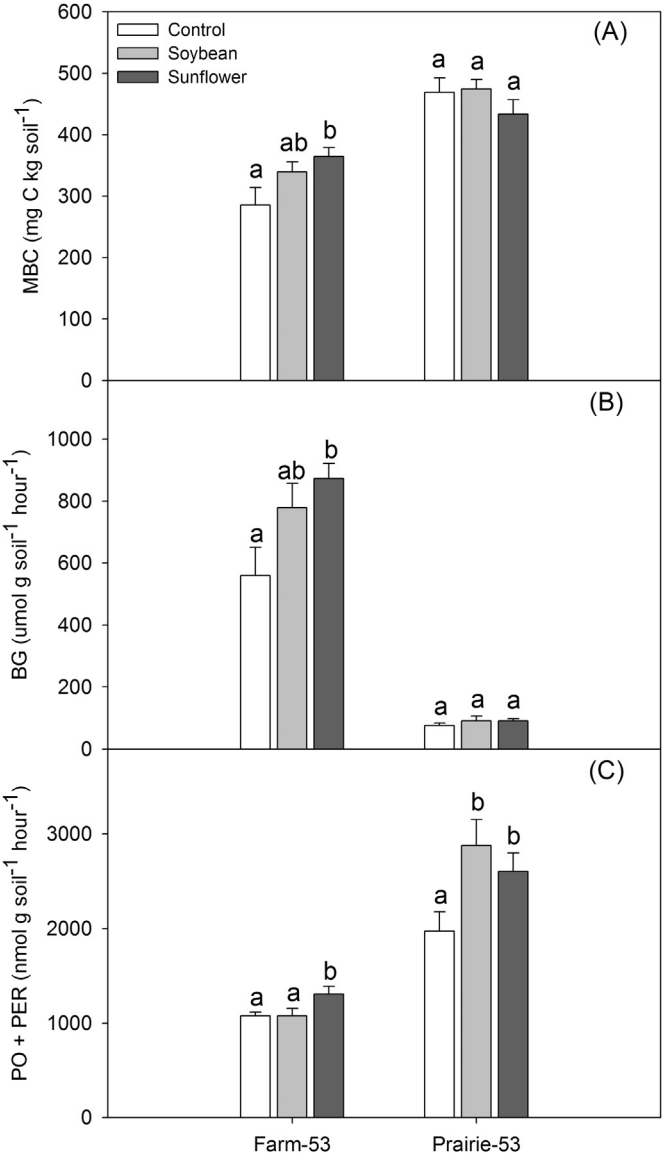

Fig. 3. Microbial biomass carbon (MBC, A), β-glucosidase activity (BG, B), and sum of phenol oxidase (PO) and peroxidase (PER) activity (PO + PER, C) averaged by soil type (farm versus prairie) and plant species (control versus soybean versus sunflower). These variables were measured at the first sampling (53 days after planting). Different letters for each soil type denote significant differences among control and two plant species treatments (post-hoc LSD test, $P < 0.05$). Error bars indicate standard errors of the mean.

growth, extracellular enzyme production, and SOM decomposition (Cheng and Kuzyakov, 2005; Kuzyakov, 2010). The significant positive correlation between β-glucosidase activity and SOM decomposition rate suggests that rhizodeposit-induced microbial enzyme production is an important mechanism for the rhizosphere priming

Table 4
ANOVA *P*-values for MBC (microbial biomass carbon), BG (β-glucosidase), and PO (phenol oxidase) + PER (peroxidase) activities measured after the first sampling (53 days after planting).

	MBC	BG	PO + PER
Soil type	<0.001	<0.001	<0.001
Plant species	0.350	0.008	0.024
Soil type × Plant species	0.030	0.017	0.045

Table 5
Rhizosphere effects on soil variables calculated as the percentage difference between unplanted control and planted treatment relative to the unplanted control. Bold values are significantly different than zero ($P < 0.05$). C_{soil}, soil-derived CO_2 flux; GNM, gross nitrogen mineralization; MBC, microbial biomass carbon; BG, β-glucosidase; PO, phenol oxidase; PER, peroxidase.

Treatment	C_{soil}	GNM	MBC	BG	PO + PER
Farm-53 – soybean	**42%**	**39%**	19%	39%	0%
Farm-53 – sunflower	**79%**	10%	**28%**	**56%**	**19%**
Farm-88 – soybean	**27%**	**36%**			
Farm-88 – sunflower	**82%**	−11%			
Prairie-53 – soybean	**45%**	**52%**	1%	19%	**46%**
Prairie-53 – sunflower	**71%**	**45%**	−8%	20%	**32%**
Prairie-88 – soybean	**69%**	−13%			
Prairie-88 – sunflower	**245%**	**41%**			

effect on SOM decomposition (Weintraub et al., 2007; DeAngelis et al., 2008; Phillips et al., 2011; Blagodatskaya et al., 2014).

In addition to testing the microbial activation hypothesis, this study also aimed to investigate the controls of the rhizosphere priming effect by soil type, sampling time, and plant species. Although the two soils had different texture, organic carbon content, and microbial biomass carbon (Table 1), we did not find significant difference in the rhizosphere priming effect between the two soils. This result is consistent with an earlier study on the same two soils (Dijkstra and Cheng, 2007b). Moreover, the rhizosphere priming effect did not differ significantly between the two sampling times. Although previous studies have showed significant change in the rhizosphere priming effect with plant phenology (Cheng et al., 2003), higher root biomass and lower rhizodeposition per unit of root biomass at the mature stage compared to the vegetative stage (Nguyen, 2003) may provide off-setting effects leading to similar intensities of the rhizosphere priming effect (Pausch et al., 2013).

In contrast to soil type and sampling time, plant species exerted significant control of the rhizosphere priming effect. Sunflower showed consistently higher intensity of rhizosphere priming than soybean, particularly at the mature stage in prairie soil. We expected that these two plant species (a legume and a non-legume) would show distinct rhizosphere priming for various reasons. One such reason could be that *B. japonicum* inside soybean nodules can fix N from the atmosphere and reduce the N demand in the rhizosphere, consequently lessening N mining from soil organic matter (Kuzyakov, 2002; Dijkstra et al., 2009; Phillips et al., 2011). However, when we included rhizosphere respiration rate as a covariate, the effect of plant species on rhizosphere priming effect disappeared (Table 3), suggesting that different intensity of rhizosphere priming between soybean and sunflower was likely due to

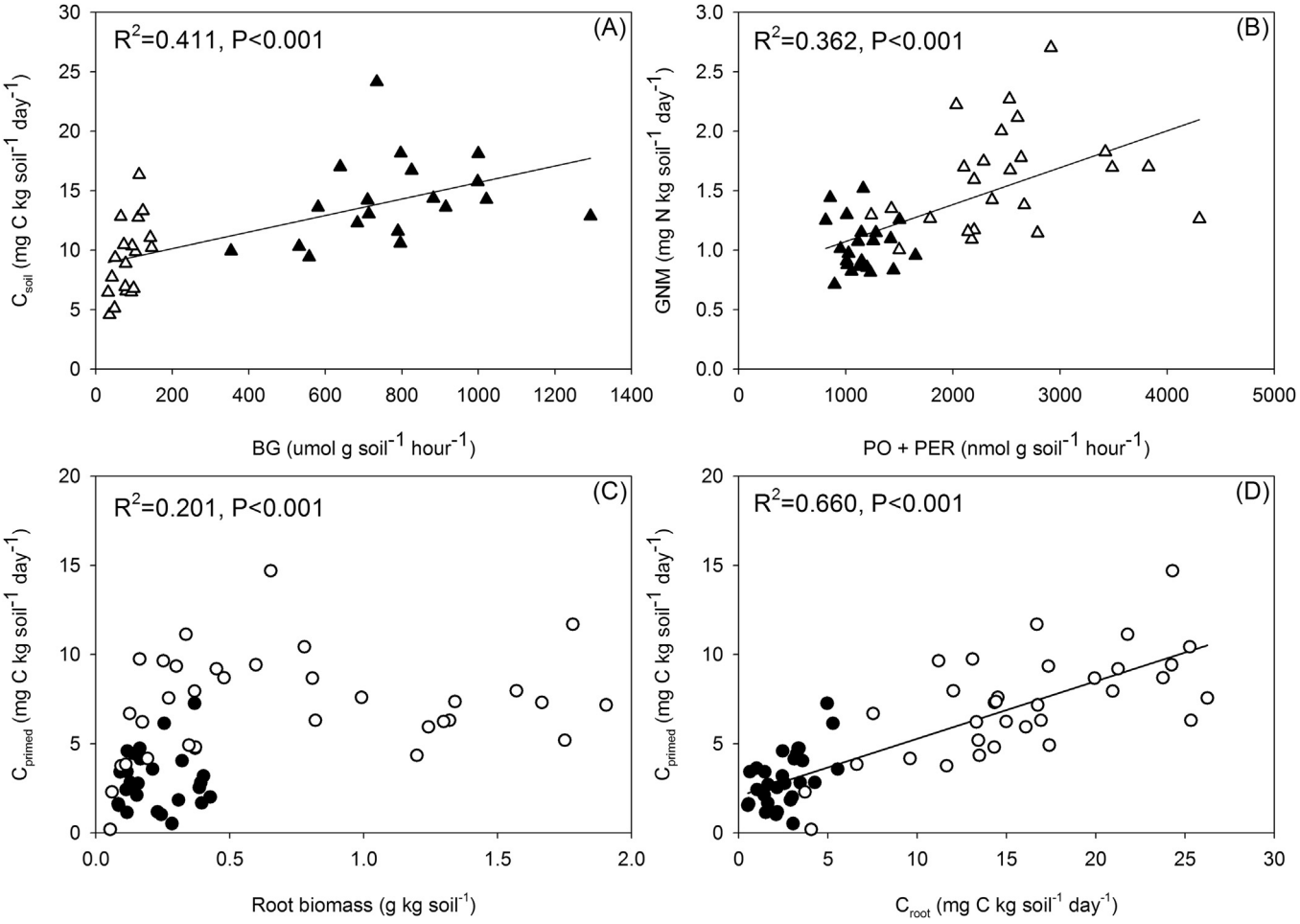

Fig. 4. Relationships (A) between β-glucosidase activity (BG) and soil-derived CO_2 flux (C_{soil}), (B) between the sum of phenol oxidase and peroxidase activity (PO + PER) and gross N mineralization rate (GNM), (C) between root biomass and primed soil carbon (C_{primed}), and (D) between root-derived CO_2 flux (C_{root}) and primed soil carbon. Data for (A) and (B) include two soil types (filled triangles for farm soil and empty triangles for prairie soil), control and two plant species, and the first sampling, while data for (C) and (D) include two soil types, two plant species (filled circles for soybean and empty circles for sunflower), and both samplings.

different root activity and rhizodeposit quantity. Although we did not directly measure rhizodeposit quantity, it is reasonable to assume that rhizodeposit quantity is positively correlated with rhizosphere respiration rate. Therefore, the significant positive correlation between rhizosphere respiration rate and the rhizosphere-primed SOM decomposition rate (Fig. 4D) suggests that the rhizosphere priming effect is determined in large part by rhizodeposit quantity; although rhizodeposit quality may also play a role (Zhu and Cheng, 2012; Drake et al., 2013). This idea is also supported by several recent studies. For example, Dijkstra and Cheng (2007a) reported a positive relationship between the rhizosphere priming effect and plant-derived carbon remaining in the soil (an index of rhizodeposition) across two tree species planted in three soil types. Bengtson et al. (2012) showed a positive correlation between the rhizosphere priming effect and root exudation rate of three tree species planted in a forest soil. Paterson and Sim (2013) observed a positive relationship between the priming effect and glucose addition rate within four soil types. If this relationship holds for different ecosystems across space and time, we may have identified an index that will enable incorporation of rhizosphere priming into future ecosystem models.

4.2. Rhizosphere priming effect on soil nitrogen mineralization

Rhizosphere priming of soil nitrogen mineralization has been inadequately studied (Frank and Groffman, 2009; Kuzyakov, 2010; Cheng et al., 2014). In this study, we quantified both gross N mineralization rate and SOC decomposition rate with and without the presence of the rhizosphere. Gross N mineralization rate was significantly higher (by 36–52%) in planted soil compared to unplanted control soil in five out of eight treatment combinations, and was similar between planted and unplanted soil in the other three treatment combinations (Table 5). These results suggested that rhizosphere-primed gross N mineralization rate is dependent on plant–soil–sampling combinations. Some other studies have also showed similar results. Dijkstra et al. (2009) found significantly higher rhizosphere-primed gross N mineralization rate in three out of six treatment combinations including two tree species planted in three soil types. Bengtson et al. (2012) observed higher gross N mineralization rate in soils with root access than in soils without root access for two out of three tree species. Taken together, these studies clearly indicate that gross N mineralization rate can be affected by plant–soil interactions in the rhizosphere.

Why is gross N mineralization rate higher in the rhizosphere and why is the N mineralization response so variable? A potential mechanism is related to microbial N mining (Kuzyakov, 2002; Cheng and Kuzyakov, 2005; Craine et al., 2007; Fontaine et al., 2011; Dijkstra et al., 2013). In order to assimilate root-derived labile carbon substrates, rhizosphere microbes need to take up inorganic nitrogen at a certain proportion. When soil mineral N is depleted due to root uptake, microbes can produce extracellular enzymes to break down macromolecular soil organic nitrogen into smaller molecular weight, more generally-usable organic N compounds (Schimel and Bennett, 2004). At the first sampling when we measured gross N mineralization rate and oxidative enzyme activity, the presence of rhizosphere depleted soil mineral N pool (data not shown), enhanced oxidative enzyme activity by 0–46%, and increased gross N mineralization rate by 10–52% (Table 5). Moreover, there was a significant positive relationship between gross N mineralization rate and oxidative enzyme activity (Fig. 4B). Although we did not measure other enzymes involved in soil N mineralization (e.g. urease, chitinase, protease), our data on gross N mineralization and oxidative enzyme activity are consistent with and supportive of the microbial N mining hypothesis in the nitrogen-deficient rhizosphere.

This rhizodeposition-induced higher enzyme activity and faster N cycling rate in the rhizosphere has also been reported in recent studies (e.g. Herman et al., 2006; DeAngelis et al., 2008; Dijkstra et al., 2009; Koranda et al., 2011; Phillips et al., 2011). Although soil N availability and plant N uptake are also controlled by plant–microbe competition for mineralized N, the increased gross N mineralization rate in the rhizosphere and the longer turnover time of roots compared to microbes indicate that long-term soil N availability should be higher in intact rhizosphere soils compared to root-free bulk soils (Schimel and Bennett, 2004; Frank and Groffman, 2009; Dijkstra et al., 2013; Kuzyakov and Xu, 2013). Although many studies have reported positive rhizosphere effects on potential net N mineralization rate based on short-term lab incubations of root-free soils sampled from regions at different distance from live roots (e.g. Phillips and Fahey, 2006; Brzostek et al., 2013), a detailed N-budgeting analysis in intact plant communities (e.g. Cheng, 2009; Zhu and Cheng, 2012) may provide a more comprehensive assessment of soil N availability and its response to the presence of roots.

Gross N mineralization rate was not correlated with SOM decomposition rate across all treatments (data not shown). This result is not consistent with previous studies in root-free soils (Hart et al., 1994b) or rhizosphere soils (Bengtson et al., 2012). One potential reason may be associated with the methods. Gross N mineralization rate was measured on freshly sampled soils after root-picking and homogenization (Hart et al., 1994a), while the measured SOM decomposition rate resulted from intact soils when live roots were still present (Cheng et al., 2003). The disturbance in soil preparation may affect gross N mineralization rate to a different extent across treatments (Murphy et al., 2003). Another possible reason may be due to different microbial community composition and biomass C:N ratio across treatments. Although microbial biomass nitrogen was not measured, PLFA data (unpublished data) indicated shifts in microbial community composition (fungi versus bacteria) and likely biomass C:N ratio across treatments. Further study on soil C and N mineralization rates in intact rhizosphere soils using less disturbing techniques may offer more insights into soil C and N dynamics. For example, one could add $^{15}NH_4^+$ solution to intact soils (with living plants present) homogenously (e.g. multiple injections using needles), and harvest soils 24 h later to estimate gross N mineralization rate during the 24-h period (e.g. Dijkstra et al., 2009). Moreover, one could also trap CO_2 flux during this same 24-h period using the method in this study, and measure rhizosphere priming of C and N mineralization during the exact same time. However, this method would be limited to small soil volume (small containers with small plants) because of the high cost of $^{15}NH_4^+$ solution and the difficulty of homogeneously adding $^{15}NH_4^+$ solution to soil without disturbing the living root–soil system.

4.3. Implications for soil C and N mineralization

Our results have three major implications for soil C and N mineralization. First, SOM decomposition can be enhanced by root–soil interactions in the rhizosphere compared to bulk soil. The intensity of rhizosphere priming effect on SOM decomposition in this study ranged from 27% to 245%. This result suggests that root–soil interactions should be included with soil temperature and soil moisture as significant controlling factors of SOM decomposition rate (Cheng et al., 2014). Second, we found some evidence for the microbial activation hypothesis of rhizosphere priming effect (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). The enhanced SOM decomposition rate in the rhizosphere was associated with higher enzyme activity and higher microbial biomass (to a lesser extent). The rhizosphere priming effect was positively correlated

with rhizosphere respiration rate, which can be considered as an index of root activity and rhizodeposit quantity. These results combined with other recent findings (Dijkstra and Cheng, 2007a; Bengtson et al., 2012; Paterson and Sim, 2013) may provide a promising way to explicitly incorporate rhizosphere priming effect into mechanistic SOM models (Cheng et al., 2014). Lastly, gross N mineralization rate was enhanced in the rhizosphere (by up to 52%) compared to bulk soil. This result is in line with previous studies (Norton and Firestone, 1996; Herman et al., 2006; Koranda et al., 2011; Phillips et al., 2011), although this study compared planted soil versus unplanted soil while other studies compared soil adhering to live roots versus soil at some distance from roots. Accurate measurement of soil N availability should account for rhizosphere N processes (Frank and Groffman, 2009; Dijkstra et al., 2013; Kuzyakov and Xu, 2013). Overall, our results suggest that conceptual and numerical models of SOM decomposition and organic nitrogen mineralization in soil should incorporate the impacts of root–soil–microbe interactions in order to encompass the major controllers of these processes.

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