Soil Biology and Biochemistry 116 (2018) 369–377

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Rice rhizodeposits affect organic matter priming in paddy soil: The role of N fertilization and plant growth for enzyme activities, CO_2 and CH_4 emissions

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ARTICLE INFO

Keywords:
Rice paddy
N fertilization
CH_4 production
Enzyme activity
Rhizosphere priming effect
C and N cycles

ABSTRACT

Carbon dioxide (CO_2) and methane (CH_4) production in paddy soils play a crucial role in the global carbon (C) cycle and greenhouse gas emissions. A rhizosphere priming effect (RPE) may change these emissions, but the relationships between RPE, CH_4 emission, and the effect of N fertilization are unknown. We investigated the RPE on CO_2 and CH_4 emissions and their dependence from N fertilization in a $^{13}CO_2$ continuous labelling experiment by partitioning total CO_2 and CH_4 derived from roots and soil organic matter (SOM). Because of plant-derived CO_2, rice plants strongly increased total CO_2 emission compared to that from unplanted soil. SOM-derived CO_2 and CH_4 increased in the presence of roots but decreased after N fertilization. The RPE for CO_2 at an early growth stage ($\leq$40 days) was negative: -1.3 and -1.9 mg C day^{-1} kg^{-1} soil without and with N fertilization, respectively. However, 52 days after transplanting, RPE for CO_2 got to positive. The RPE for CH_4 increased gradually up to 1.6 and 0.5 mg C day^{-1} kg^{-1} soil at the end of the experiment without and with N fertilization, respectively. Moreover, the RPE for CH_4 got half of the RPE for CO_2 after 64 days showing the relevance of CH_4 emissions for greenhouse gases balance and C cycling in paddy ecosystems. The RPE for CO_2 and CH_4 emissions increased with microbial biomass content and activities of xylanase and *N*-acetylglucosaminidase. Supporting the results to RPE, the enzyme activities decreased with N fertilization, suggesting that reduced N limitation decreased microbial potential to mine N from SOM. In conclusion, for the first time we showed that root-microbial interactions stimulated SOM mineralization in rice paddies through rhizosphere priming effects not only for CO_2 but also for CH_4, but the RPE decreased with N fertilization.

1. Introduction

Soil organic matter (SOM) functions as an important source and sink of atmospheric carbon dioxide (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). Soil CO_2 mainly derives from rhizosphere respiration (including root respiration), microbial decomposition of rhizodeposits from living roots, and microbial decomposition of SOM (Kuzyakov, 2006). It is well accepted that root-mediated processes regulate SOM dynamics, but their relationships with edaphic physical and microbial factors are less clear.

Plants can regulate SOM decomposition via rhizosphere processes (Cheng et al., 2014; Dijkstra et al., 2013; Kuzyakov, 2010). Living roots release available substrates, which are used as the primary energy source for microorganisms, stimulate microbial growth in the rhizosphere, thus leading to extracellular enzyme production, and enhance (400%) or suppress (50%) soil organic carbon (SOC) decomposition compared with unplanted soil (Kuzyakov, 2010; Shahzad et al., 2015; Zhu and Cheng, 2011). The amounts of rhizodeposition and root activities depend on plant growth, which in turn affects physical and chemical conditions, such as water content, oxygen (O_2) concentration, pH, and redox potential (Eh), in the rhizosphere depending on phenological stage (Cheng et al., 2003; Yuan et al., 2014). These soil changes induced by roots can also significantly affect the magnitude of SOM decomposition (Kumar et al., 2016; Mwafulirwa et al., 2016).

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http://dx.doi.org/10.1016/j.soilbio.2017.11.001
Received 6 June 2017; Received in revised form 27 September 2017; Accepted 2 November 2017

Furthermore, plants can alter rhizosphere microbial activities by competing with microorganisms for nutrients such as nitrogen (N), which leads to nutrient limitation in the rhizosphere and stimulates microorganisms to mine SOM to meet their nutrient requirements (Hodge et al., 2000; Kuzyakov and Xu, 2013).

Global ecosystems are experiencing increased inputs of anthropogenically derived N fertilizer, which increase N loading by 30–50% compared with that from natural sources (Canfield et al., 2010; Zang et al., 2016). Increasing N fertilizer inputs affect the above-/belowground distribution of plant C and the fate of plant-derived C in agricultural soils (Kuzyakov et al., 2002; Zang et al., 2016). Plants differ in their capacity to acquire N during growth stages because the rhizosphere microbial composition changes owing to the effects of different root exudates (Kuzyakov and Xu, 2013). The N availability in plant-soil systems, especially the rhizosphere, affects microbial activity and SOM decomposition. In soils with low nutrient availability, microorganisms meet their nutrient demands by increasing enzyme synthesis to mine nutrients from SOM (DeAngelis et al., 2008; Phillips et al., 2011). This accelerates SOM decomposition, resulting in a positive priming effect (PE). Alternatively, in nutrient-rich soils, microorganisms will switch from decomposing SOM (older C) to utilize newly deposited C and mineral N, resulting in a negative PE (Cheng et al., 2014; Dijkstra et al., 2013). Understanding how additional N inputs affect plant-soil ecosystems is becoming increasingly important within the context of C and N budgets and cycling. This is especially the case in paddy soils, as the number of studies on the PE under anaerobic conditions is very limited (i.e., Conrad et al., 2012; Yuan et al., 2014), and the effects on methane (CH_4) emissions are disregarded in nearly all studies.

Flooded rice fields are important wetland ecosystems contributing to significant CH_4 emissions (Cai et al., 2010; Yuan et al., 2014). In contrast to many investigations of the rhizosphere effects on SOM decomposition in upland soils, much less attention has been paid to wetland soils and CH_4 emission. Partitioning CH_4 production to its sources, i.e., plant-derived C and SOC, is crucial for improving process-based modeling of CH_4 emission from rice fields, which plays an important role in predicting CH_4 flux and global climate change (Fumoto et al., 2008). However, prediction and partitioning of CH_4 emissions from rice soils is challenging owing to high variability in water regime, availability of organics for microorganisms, SOM content, and organic and mineral fertilizer applications, especially N fertilization (Cai et al., 2010; Khalil et al., 2008). Liu and Greaver (2010) suggested that N fertilizer increased soil CH_4 emission by 97% and reduced CH_4 uptake (oxidation in soil) by 34%. Bodelier (2011) reported that N fertilization stimulated CH_4 production, while inhibiting CH_4 oxidation in soil. Previous studies have also reported that N fertilization stimulates methanotrophic bacteria and increases CH_4 uptake in soil (Prasanna et al., 2002; Shrestha et al., 2010). However, there is little information that quantifies the synergistic effects of living roots and N fertilizers on CH_4 emission in rice paddies, and we hypothesized that root C and SOM contribution to CH_4 emission changes greatly with rice growth and N fertilization.

Here, we investigated the effects of rice rhizodeposits and N fertilization on RPE and its ecological implications in a paddy field ecosystem by applying continuous ^{13}C labelling with and without N addition. ^{13}C continuous labelling enabled partitioning of total CO_2 and CH_4 efflux for root- and SOM-derived C, allowing estimation of the RPE in a rice field ecosystem and its implications for changing C and nutrient cycling. The activities of three enzymes (β-1,4-glucosidase [BG], β-xylosidase [XYL], and β-1,4-*N*-acetylglucosaminidase [NAG]) were determined to link CO_2 and CH_4 emissions to microbial activities and N transformations. We hypothesized that (i) rice roots accelerate SOM decomposition because their exudates promote microbial and enzyme activities, (ii) N fertilization reduces RPE for both CO_2 and CH_4 emissions via decreasing microbial activity and decreasing competition between roots and microorganisms for N, as well as additional electron acceptors reducing organic matter conversion to CH_4, and (iii) the RPE for CH_4 emission increases with rice growth as O_2 limitation increases during flooding.

2. Materials and methods

2.1. Soil

Typical Stagnic Anthrosol soil developed from granite was collected from a rice field (113° 19′ 52″ E, 28° 33′ 04″ N, 80 m a.s.l.) located at the Changsha Research Station for Agricultural and Environmental Monitoring, Subtropical Region of China. The climate of the study site is subtropical with a mean annual temperature of 17.5 °C and yearly rainfall of 1300 mm. Moist soil samples were collected from the plough layer (0–20 cm) and sieved through < 4 mm mesh to remove visible plant residues. The soil texture was 7.5% clay, 68.4% silt, and 24.1% sand; contained 15.6 g kg^{-1} organic C, 1.6 g kg^{-1} total N, and 0.5 g kg^{-1} total phosphorus; and had a pH of 5.8 (2:5 soil/water ratio).

2.2. Experimental setup

The experiment included a control and three treatments in pots: (1) unplanted soil with no N fertilization; (2) unplanted soil with 100 mg N kg^{-1}; (3) soil planted with rice, with no N fertilization; and (4) soil planted with rice, with 100 mg N kg^{-1}. Because isotopic fractionation between root tissue and rhizosphere respired CO_2, CH_4 in particular, has been increasingly recognized, additional pots filled with silica sand were included (Wang et al., 2016). The sand pots, inoculated with 1% (w/w) of paddy soil before planting, included the treatments of rice planted with and without 100 mg N kg^{-1} fertilization. The silica sand-filled pots were watered with basal nutrients solution but free of organic C, which was same as the paddy soil nutrient element content. For N fertilization, urea was applied at 160 kg N ha^{-1} and homogenized with soil before planting. Samples were collected at 40, 52 and 64 days after planting, with four replicates for each treatment.

We used the experimental protocol described previously (Ge et al., 2012, 2017), with some modifications. Briefly, on May 25, 2016, for each replicate, two 20-day-old rice seedlings (*Oryza sativa* L. 'Two-line hybrid rice Zhongzao 39', average dry matter weight 0.10 g per plant) were transplanted to a pot that was filled with 1.0 kg soil. Rice plants underwent continuous $^{13}CO_2$ labelling from 22 June (28 days after planting) to 28 July (64 days after planting) during their most vigorous growth. During the labelling period, plants were transferred to an automatically controlled gas-tight growth chamber system (110 × 250 × 180 cm). Growth chambers were placed in a rice field with sufficient sunlight for plant growth. Pot surfaces were covered by black plastic sheets to prevent algal photosynthesis and to allow only the rice shoots to be exposed to $^{13}CO_2$. The paddy soil pots were irrigated with deionized water, with a 2–3 cm water layer maintained above the soil surface, throughout the experiment.

The $^{13}CO_2$ (20 atom % ^{13}C) concentration in the growth chamber was maintained between 360 and 380 μl·L^{-1} and monitored using a CO_2 analyser (Shsen-QZD, Qingdao, China). When the CO_2 concentration in the chamber fell below 360 μl·L^{-1}, $^{13}CO_2$ generated by reacting $NaH^{13}CO_3$ (20 atom % ^{13}C, Cambridge Isotope Laboratories, Inc.) with H_2SO_4 (0.5 M) was introduced into the chamber. Conversely, when the CO_2 concentration in the chamber was higher than 380 μl·L^{-1}, a switch diverted gas flow to pass through CO_2 traps comprised of NaOH solution. One temperature and humidity sensor (SNT-96S, Qingdao, China) was installed inside the chamber and another was placed in the surrounding rice field. Air was continuously circulated in the growth chamber, and an air-conditioning system was used to control the temperature inside the chamber to within 1 °C of the ambient temperature in the rice field. Control pots did not undergo ^{13}C labelling and were placed outdoors 10–15 m away from labelled plants.

2.3. Rhizosphere respiration sampling

At each sampling point (40, 52, and 64 days after planting), each pot was sealed with a respiration collection chamber (Fig. S1; China Patent No. ZL201510242495.3) containing two parts (Fig. S1). The lower part of the chamber was fixed on the top of the pot, with any gaps between the rice stems and the opening in the middle of the chamber sealed with silicone paste. This part was used for sampling CO_2 and CH_4. The upper part of the chamber was fixed onto Part 1 to collect the CH_4 emitted from rice stems and leaves. The respiration collection chamber was flushed with CO_2-free air for 5 min until the outlet airflow had < 10 ppm CO_2 and was then sealed for 60 min by stoppering the tube ends to accumulate soil CO_2 and CH_4 efflux in the chambers of parts 1 and 2 (Mwafulirwa et al., 2016). Immediately thereafter, approximately 35 mL gas was sampled from the chambers using a gas syringe connected to the outlet tubing. One part of the sampled air was injected into a gas chromatograph (Agilent 7890A, Agilent Technologies, Alto Palo, California, USA) equipped with a thermal conductivity detector for measuring CO_2 and a flame ionization detector for measuring CH_4. The remaining gas was used to analyse the stable C isotope composition of CO_2 and CH_4 with an isotope ratio mass spectrometer coupled with a GasBench (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Total CO_2 respired was calculated per sampling point using the CO_2 concentration. The CO_2 in the lower chamber (Part 1) was collected and regarded as the total rhizosphere-respired CO_2, and the CH_4 in both the lower chamber (Part 1) and upper chamber (Part 2) was collected and considered the total rhizosphere-respired CH_4. Total soil CO_2 and CH_4 efflux were separated into SOM-derived (C_{SOM}) and plant-derived C (C_{plant}) fractions using a two-source mixing model (Kuzyakov and Bol, 2006; Zhu and Cheng, 2012):

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

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

where C_{total} was the total CO_2 efflux of the planted treatment (mg C day^{-1} kg^{-1} soil) and $\delta^{13}C_{total}$ was the corresponding $\delta^{13}C$ value (‰). $\delta^{13}C_{SOM}$ was the mean $\delta^{13}C$ value (‰) of CO_2 from SOM mineralization measured in the unplanted control pots. C_{plant} was the plant-derived CO_2 in the planted pots (mg C day^{-1} kg^{-1} soil), and the $\delta^{13}C_{plant}$ value was the $\delta^{13}C$ value (‰) of CO_2 emitted from the sand pots (Wang et al., 2016). The fraction of CH_4 derived from SOM was calculated with an analogous equation.

The priming effect of rice rhizodeposits on SOM decomposition and CO_2 and CH_4 emissions was defined as the amount of CO_2 and CH_4 released from the surface soil, not including CO_2 and CH_4 dissolved in the soil solution. The RPE for CO_2 and CH_4 were calculated as the difference of C_{SOM} between planted and unplanted soils, as shown below in Eq. (3) and Eq. (4).

$$RPE = C_{SOM,\ Planted} - C_{SOM,\ Unplanted} \quad (3)$$

$$RPE = C_{SOM\ Planted+N} - C_{SOM\ Unplanted+N} \quad (4)$$

where $C_{SOM,\ Planted}$ and $C_{SOM,\ Unplanted}$ were the CO_2 or CH_4-C derived from SOM in the unfertilized pots with and without rice plants, respectively, and $C_{SOM,\ Planted+N}$ and $C_{SOM,\ Unplanted+N}$ were the CO_2 or CH_4-C derived from SOM in the fertilized pots with or without rice plants, respectively.

2.4. Soil microbial biomass and enzyme assays

Directly after gas sampling, soil Eh was measured using a portable Eh meter (PRN-41, DKK-TOA Corporation, Tokyo, Japan), and the total weight of each pot was determined before the pots were harvested. Shoots were cut at the base and dried at 70 °C for 48 h. Soil cores were carefully removed from the pot. Shoots and roots were separated and analysed for $\delta^{13}C$ values. A representative homogenized soil sample (400–500 g) was taken from each pot to determine soil moisture, soil mineral N, microbial biomass C (MBC), microbial biomass N (MBN), and extracellular enzyme activities. Soil microbial biomass C and N were calculated by dividing the difference between extracted C and N from fumigated and non-fumigated soil samples with a K_{EC} and K_{EN} factor of 0.45 and 0.54, respectively (Wu et al., 1990). The N contents from nonfumigated soil samples were considered mineral N.

Extracellular enzymes activities were measured using the method described by Marx et al. (2001). Fluorogenic methylumbelliferone-based artificial substrates were used to estimate the activities of BG, XYL, and NAG (Sinsabaugh and Follstad Shah, 2012). Briefly, a soil suspension was made by dissolving 1 g fresh soil sample in 50 mL autoclaved water using low-energy sonication (50 Js^{-1}) for 120 s. An aliquot of 50 μL was dispensed in a 96-well black microplate while stirring the soil suspension to ensure uniformity. Thereafter, 50 μL of 2-(*N*-morpholino)ethanesulfonic acid buffer (pH 6.5) was added to the well. Finally, 100 μL serial concentrations of substrate solutions (20, 40, 60, 80, 100, 200, and 400 μmol substrate g^{-1} soil) were added to the wells. The microplate was rippled and measured fluorometrically using excitation at 360 nm and emission at 450 nm, with an automated fluorometric plate-reader (Victor3 1420–050 Multi-label Counter, PerkinElmer, Waltham, Massachusetts, USA) at 0, 30, 60, and 120 m after substrate addition. To estimate enzyme activity (V), we used the Michaelis-Menten equation for enzyme kinetics:

$$V = (Vmax * [S]) / (Km + [S]) \quad (5)$$

where Vmax is the maximal rate of enzyme activity, Km (Michaelis constant) is the substrate concentration at which Vmax is half, and [S] is the substrate concentration.

2.5. Statistical analysis

One-way ANOVA with Duncan's test (in SPSS 17, SPSS Inc., Chicago, Illinois, USA) was used to compare plant biomass, CO_2 and CH_4 emission, priming effect for CO_2 and CH_4, soil mineral N, microbial biomass and soil enzyme activities between treatments. A two-way ANOVA was conducted to assess the effects of N addition and rice growth stage on rhizosphere priming effect for CO_2 and CH_4 (Table S1). Redundancy analysis was performed with CANOCO 5.0 for Windows (Microcomputer Power, Ithaca, New York, USA) to identify the relationships between RPE for CO_2 and CH_4 and soil physiochemical parameters, microbial biomass, and extracellular enzymes. Variance decomposition was performed with CANOCO 5.0 to identify the contribution of N fertilization and rice growth to the variations of the RPE for CO_2 and CH_4.

3. Results

3.1. Plant biomass

Shoot biomass per pot was higher in the N-fertilized plant than in unfertilized plants ($p < 0.05$), and increased with rice growth ($p < 0.05$) (Fig. 1). And the root biomass per pot was also higher in N-fertilized plants, although the difference was not statistically significant ($p > 0.05$). However, the root to shoot ratio was higher in unfertilized plants than N-fertilized plants ($p < 0.05$).

3.2. Fluxes and sources of CO_2 and CH_4

In the unplanted pots, CO_2 efflux ranged from 0.3 to 2.1 mg C day^{-1} kg^{-1} soil, and CH_4 effluxes ranged from 0.02 to 0.13 mg C day^{-1} kg^{-1}. Total CO_2 and CH_4 effluxes were higher in planted pots. N fertilization increased the total CO_2 efflux but inhibited the CH_4 efflux. The highest CO_2 fluxes were 19.5 and 11.6 mg C day^{-1} kg^{-1} soil in N-

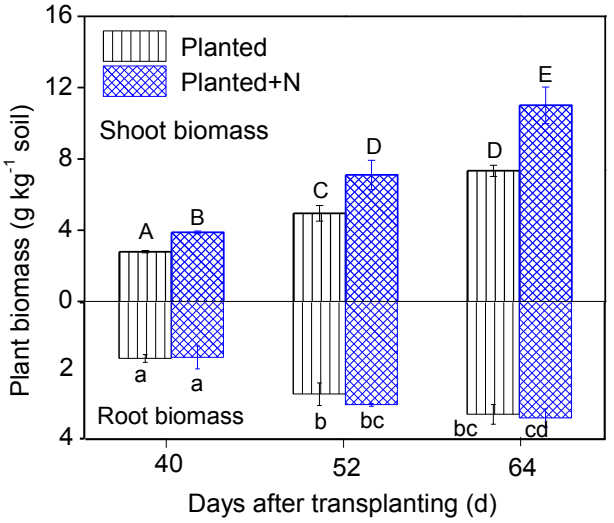

Fig. 1. Shoot and root biomass (bars) of rice from the unfertilized (Planted) and N-fertilized (Planted + N) pots over the 64-day period. Letters indicate significant differences at $p < 0.05$ based on Duncan's multiple range test. Values shown are means (n = 4) ± one standard error.

fertilized and unfertilized pots, respectively, and the highest CH_4 fluxes were 0.8 and 2.8 mg C day^{-1} kg^{-1} soil in N-fertilized and unfertilized pots, respectively (Fig. 2).

The contributions of root- and SOM-derived sources to total CO_2 and CH_4 efflux were calculated based on a linear two-source isotopic mixing model (Fig. 2). Plant-derived CO_2 efflux from fertilized soil was lower than that from unfertilized soil at 40 days after transplanting ($p > 0.05$). However, plant-derived CO_2 from N-fertilized soil was approximately 50% higher than that from unfertilized soil at the latter two sampling points (Fig. 2). CH_4 derived from root-released C significantly increased during rice growth, but N fertilization inhibited CH_4 efflux (Fig. 2). Both SOM-derived CO_2 and CH_4 were lower in N-fertilized and planted soil compared with unfertilized planted soil.

The RPE for CO_2 was −1.3 and −1.9 mg C day^{-1} kg^{-1} soil, respectively, in unfertilized and N-fertilized pots at 40 days, while the RPE for CO_2 was positive at the two latter sampling points (Fig. 2C). The RPE for CH_4 was gradually increased to 1.6 and 0.5 mg C day^{-1} kg^{-1} in unfertilized and N-fertilized pots, respectively ($p < 0.01$) (Fig. 2F). N fertilizer application reduced the RPE for CO_2 and CH_4, which were negatively correlated with soil mineral N ($R^2 = 0.34$, $p < 0.01$) (Fig. 4D). N fertilization and rice growth stage had a significant effect on RPE ($p < 0.05$, Table S1), contributing 10.5% and 54.6%, respectively, to RPE for CO_2, and 41.6% and 44.5% to RPE for CH_4 (Fig. S2).

3.3. Soil mineral N and microbial biomass

The mineral N content in N-fertilized unplanted soil was approximately 50% higher than that of unfertilized soil, and there were no changes of mineral N content across the three sampling times (Fig. 3A). Because of plant uptake, the mineral N content in planted soil was 19.1 (N-fertilized) or 4.6 (unfertilized) times lower compared to the unplanted control at 40 days after transplanting. The mineral N content in planted pots at 40 days was 25.8 g kg^{-1} with N fertilization, which was 8.9-fold greater than that in unfertilized pots. However, the mineral N content in the N fertilized planted soil sharply decreased to 3.5 g kg^{-1} at 64 days, when it was almost equal to that of the unfertilized planted pots.

Soil microbial biomass gradually increased with rice growth. N fertilization decreased MBC by 50% at 40 days after transplanting but did not affect MBC at 52 and 64 days (Fig. 3B). MBN decreased in the planted soil. N fertilization decreased MBN by 30–70% at the first two sampling points (40 and 52 days) in both planted and unplanted soils (Fig. 3C). The MBC to MBN ratio was higher in the treatment of planted and N-fertilization than in other treatments ($p < 0.05$) (Fig. 3D). Both MBC and MBN were positively correlated with RPE for both CO_2 and CH_4 ($p < 0.05$; Fig. 5).

3.4. Soil extracellular enzyme activities

The activities of three enzymes involved in soil C and N mineralization, BG, XYL and NAG, were stimulated by growing plants and reduced by N fertilization (Fig. S3). There was a negative relationship between BG activity and RPE for CO_2 ($R^2 = 0.45$, $p < 0.01$) and a positive correlation between XYL activity and RPE for CO_2 ($R^2 = 0.29$, $p < 0.01$). No relationships were observed between BG and XYL activities and RPE for CH_4. The NAG activity was positively correlated with RPEs for both CO_2 and CH_4 ($R^2 = 0.46$, $p < 0.01$; $R^2 = 0.69$, $p < 0.01$, respectively) (Fig. 4).

4. Discussion

4.1. Effects of living rice roots on RPE

RPE has been estimated in numerous studies of upland soils (e.g., Cheng et al., 2014; Kuzyakov and Bol, 2006) but have rarely been considered for flooded paddy soils (Conrad et al., 2012; Yuan et al., 2014). Here, we provided measurements of RPE in paddy soil with rice plants based on SOM-derived CO_2 and CH_4. Previous studies have found that SOM decomposition is affected by plants and their phenology (Cheng et al., 2003; Zhu and Cheng, 2012). In this study, rice plants accelerated SOM decomposition to CO_2 and CH_4, with the exception of SOM mineralized to CO_2 at the earliest sampling time (≤40 days). However, because of very small plant biomass, with was grown before day 28 (before the continuous ^{13}C labeling started), we slightly underestimate the contribution of root C to the CO_2 and CH_4 production by sampling at day 40. However, because root-derived CO_2 (Kuzyakov and Domanski, 2002; Werth and Kuzyakov, 2008) and CH_4 (Dorodnikov et al., 2011) are both produced from very recent assimilates, the contribution of unlabeled C is of very minor importance. Rice growth contributed to variations of the RPE, accounting for 54.6% of CO_2 and 44.5% of CH_4 emission from SOM (Fig. S2). These results indicate that during rice development, root exudates stimulate the growth and activity of rhizosphere microorganisms by providing a source of easily available C, inducing SOM decomposition (Fig. 6).

A negative RPE for CO_2 emission was observed in both N-fertilized and unfertilized planted soils on day 40, but the RPE was positive at the latter two samplings (Fig. 2). This reflects higher rhizodeposition per unit of root biomass at the vegetative stage (40 days) (Nguyen, 2003). Rice roots provided a greater C source for soil microorganisms at the first sampling, inducing microorganisms to switch from using humified organic matter as their main energy source to using easily available rhizodeposits (Ge et al., 2012; Kuzyakov and Xu, 2013). As a result, SOM decomposition decreased.

A positive RPE for CO_2 emission could be explained by the microbial activation hypothesis, wherein rhizodeposits stimulated microbial growth and extracellular enzyme activity and hence promoted SOM decomposition (Phillips et al., 2011; Tian et al., 2013; Yevdokimov et al., 2006; Zhu and Cheng, 2011). However, an excess of easily available C was typically depleted within a few days via microbial uptake, utilization, and decomposition (Kuzyakov and Xu, 2013). Lower rhizodeposits per unit of root biomass have been observed at latter growth stages compared to earlier stages in rice (Cheng et al., 2003). This change might cause microorganisms previously growing on the excess substrate to starve, inducing them to mine SOM for C and nutrients (Blagodatskaya et al., 2011; Kuzyakov and Xu, 2013).

A positive RPE for CH_4 emission was observed across the entire rice growth (Fig. 2F). This was consistent with previous studies

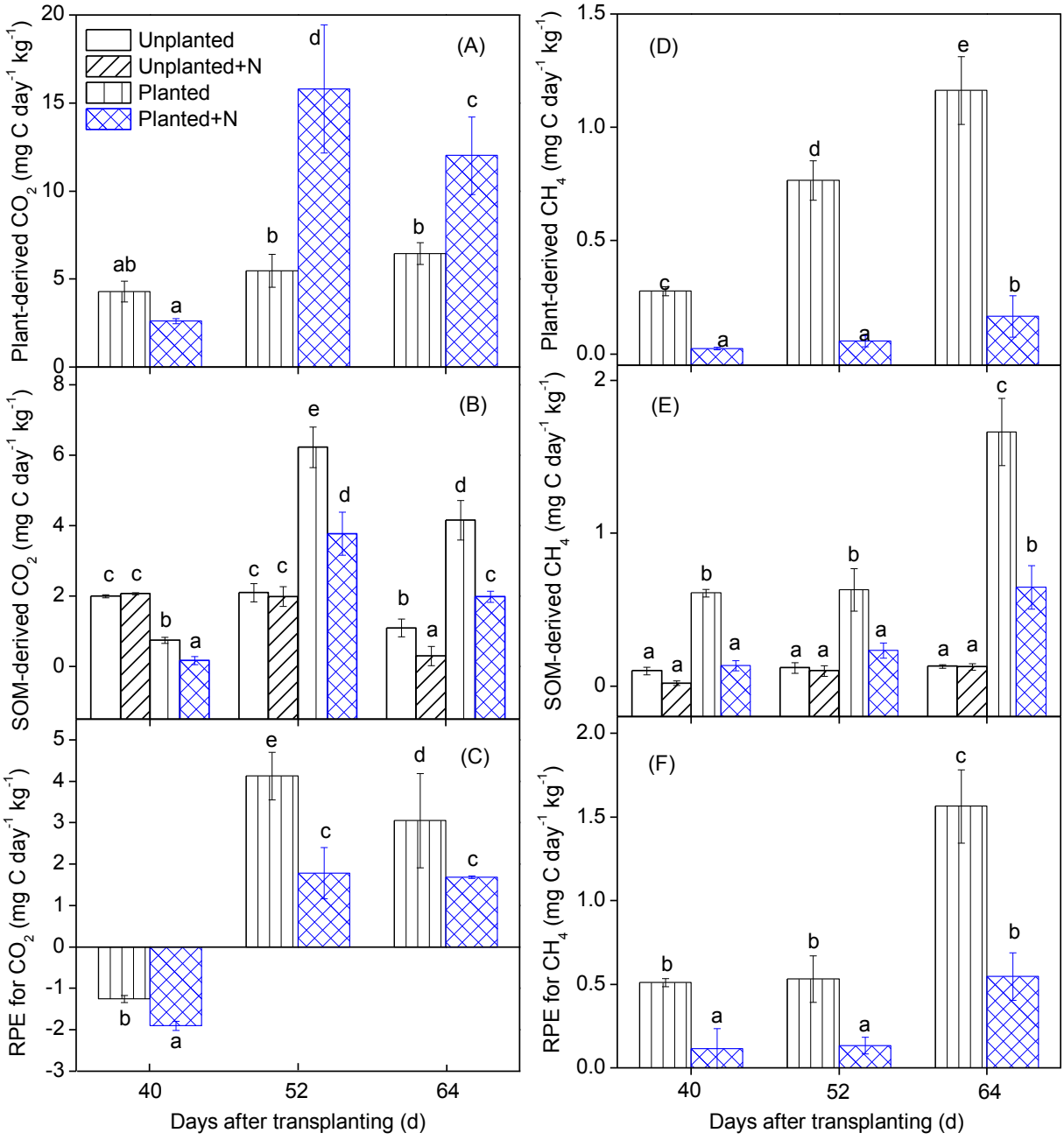

Fig. 2. Total CO_2 and CH_4 effluxes were separated for two sources: plant-derived C (A and D) and SOM-derived C (B and E) using the $\delta^{13}C$ after continuous labeling. The rhizosphere priming effect (RPE) for CO_2 (C) and CH_4 (F) is shown in planted unfertilized (Planted) and planted N-fertilized (Planted + N) pots over the 64-day rice growth period. Letters indicate significant differences at $p < 0.05$ based on Duncan's multiple range test. Values show means (n = 4) ± one standard error.

demonstrating that root exudates were a key source of substrate for microorganisms under anaerobic conditions (Cai et al., 2010; Dorodnikov et al., 2011; Yuan et al., 2014). Rice rhizodeposits (acting as electron donors) created reduced environments favourable for methanogenic production (Reddy and DeLaune, 2008) and provided organic precursors for methanogens, both of which might increase the abundance and activities of methanogenic archaea, and also stimulated additional CH_4 production from SOM (Cheng et al., 2003; Yuan et al., 2014). This hypothesis was supported by the positive correlation of rice root biomass with RPE for CH_4 and the negative relationship with soil redox potential and RPE for CH_4 (Fig. 5).

4.2. Effects of N fertilization on RPE for CO_2

N is the most important limiting nutrient for plant and soil microorganisms, and N fertilization might strongly increase plant growth, microbial activity, and SOM decomposition (Bobbink et al., 2010). The mechanisms of N fertilization affecting RPE might be primarily through regulating the microbial biomass stoichiometric ratio and extracellular enzyme activity (Kumar et al., 2016; Zhu et al., 2014). Soil microbial biomass could be a sensitive indicator of nutrient availability changes (Guillaume et al., 2016; Heuck et al., 2015). Meta-analysis suggested that mineral N addition decreased microbial biomass by 15–20%, thereby decreasing soil CO_2 emissions (Liu and Greaver, 2010). In the present study, although N fertilization did not show a significant effect on SOM decomposition in unplanted soil (Fig. 2B), it strongly reduced the SOM decomposition under rice plants (Fig. 1). Microbial decomposition did not seem limited by N availability in unplanted soils, as evidenced by MBC/MBN ratios of approximately 16 (Fig. 3). The lower SOM-derived CO_2 emission at 40 days was likely due

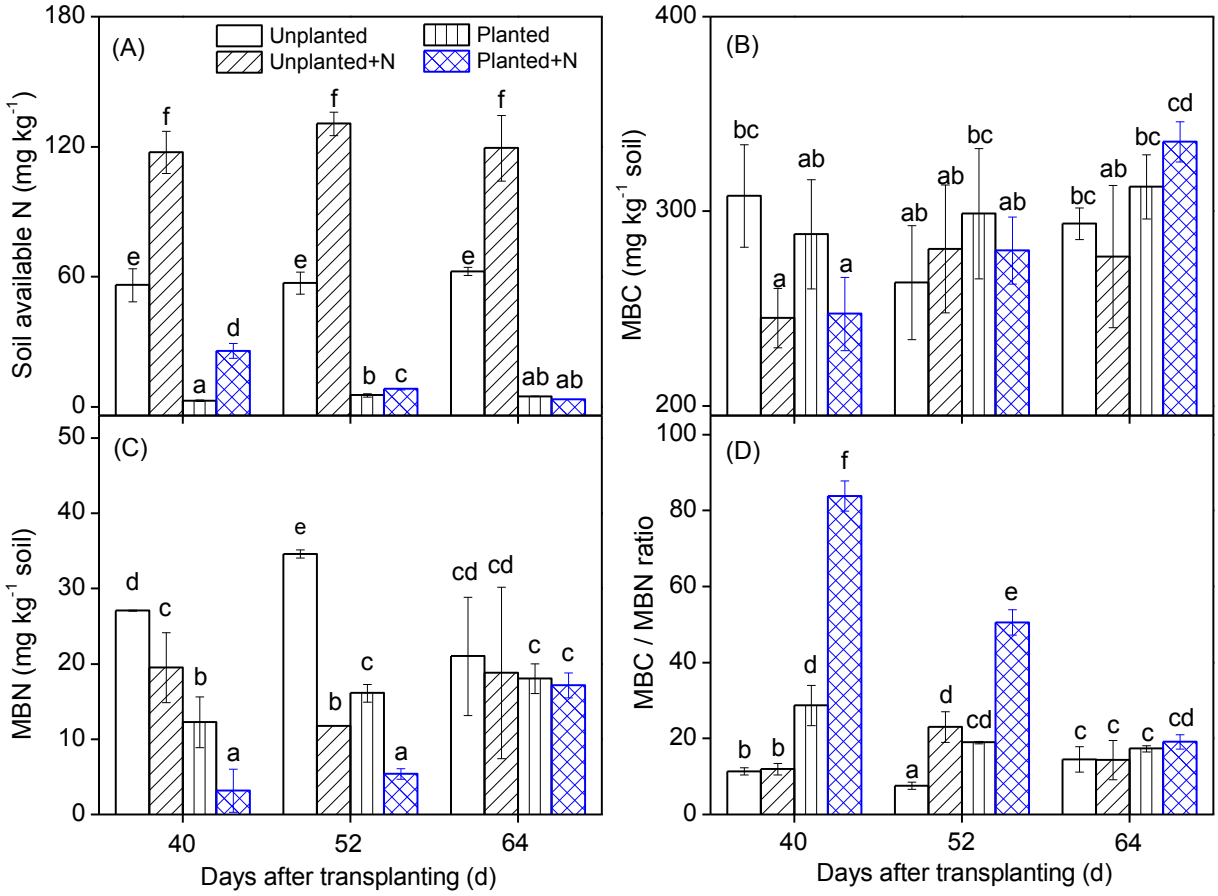

Fig. 3. Soil mineral N (A), microbial biomass C (MBC; B), microbial biomass N (MBN; C) and the MBC to MBN ratio (MBC/MBN ratio) in unplanted (Unplanted), unplanted with N fertilization (Unplanted + N), planted unfertilized (Planted), and planted N-fertilized (Planted + N) soils over the 64-day rice growth period. Letters indicate significant differences at $p < 0.05$ based on Duncan's multiple range test. Values shown are means (n = 4) ± one standard error.

to the N fertilizer application, which significantly increased rice root biomass and rhizodeposits that presumably resulted in preferential microbial utilization of plant-derived C (negative RPE) (Kuzyakov and Xu, 2013; Lu et al., 2002), as indicated by the consistent MBC regardless of fertilization (Fig. 3). However, a positive RPE for CO_2 emission was observed at the latter two sampling points, which could be attributed to the substantial decrease of mineral N in planted soil. Plant N uptake and a strong increase in competition between plants and microorganisms for N induces N limitation in the rhizosphere (Hodge et al., 2000; Kuzyakov and Xu, 2013). In the planted soil, the higher MBC/MBN ratios also suggested that microbes were N limited. Rhizosphere microorganisms need to take up mineral N to assimilate plant-derived substrates, thereby maintaining stoichiometric ratios of microbial biomass (Dijkstra et al., 2013; Kirkby et al., 2013). As a result, microbes accelerated the mineralization of SOM to obtain nutrients, leading to a positive RPE.

Extracellular enzyme activities reflect the functions of decomposer communities, which are limited by metabolic requirements and nutrient availability (Caldwell, 2005). In the planted soil, the greater microbial activity, stimulated by labile rhizodeposits, was characterized by increased Vmax for BG and XYL in comparison with unplanted soil. Furthermore, Vmax for these enzymes responded negatively to N fertilization (Fig. S2); such a response suggested that the N addition increased rhizodeposits and provided more available C sources for microbes that subsequently reduced carbohydrate hydrolase production (Chen et al., 2014; Sinsabaugh and Follstad Shah, 2012). The positive relationships between BG and XYL activity and RPE for CO_2 (Fig. 4) could indicate a strong correspondence between microbial growth and extracellular enzyme production (Dorodnikov et al., 2009). The amount of mineral N declined significantly with rice growth in planted, fertilized soil (Fig. 2); rhizodeposits might provide a substrate with a high C/N ratio (Fontaine et al., 2011), which caused N limitation for soil microbes that accordingly begun producing N-degrading enzymes to obtain N from SOM (Chen et al., 2014; Fontaine et al., 2011). Our data supported the 'microbial stoichiometry' theory: the increasing potential activity of NAG (Fig. 4C), a common enzyme involved in degrading organic N compounds such as chitin (Sinsabaugh and Follstad Shah, 2012), was consistent with the decrease in mineral N across the three sampling points (Fig. 2). Moreover, the close correlation between the Vmax of NAG and RPE (Fig. 4C) indicated that more N hydrolase was produced under N limitation and more SOM was mineralized to release mineral N (Fig. 6).

4.3. Effects of N fertilization on RPE for CH_4

CH_4 production is the terminal step of anaerobic decomposition, and substrates for CH_4 production are derived from SOM, root C, acetate (from roots and microorganisms), and CO_2 (Conrad et al., 2012; Ye et al., 2015). Presumably rice rhizodeposits provided available C for methanogens, their abundance and activities increase and consequently, they utilize more active SOM produced more SOM-derived CH_4 (positive RPE) (Dorodnikov et al., 2011). However, N fertilization increased root biomass and root exudate secretion (Fig. 1) and effectively reduced the CO_2 emission from SOM (Fig. 2). This results might attribute to the decreased CO_2 concentration causing substrate limitation for hydrogenotrophic methanogenesis (Yuan et al., 2014), resulting in a lower positive RPE for CH_4 compared with that of unfertilized soil. This was evident in the simultaneous decrease of SOM-derived CO_2 and

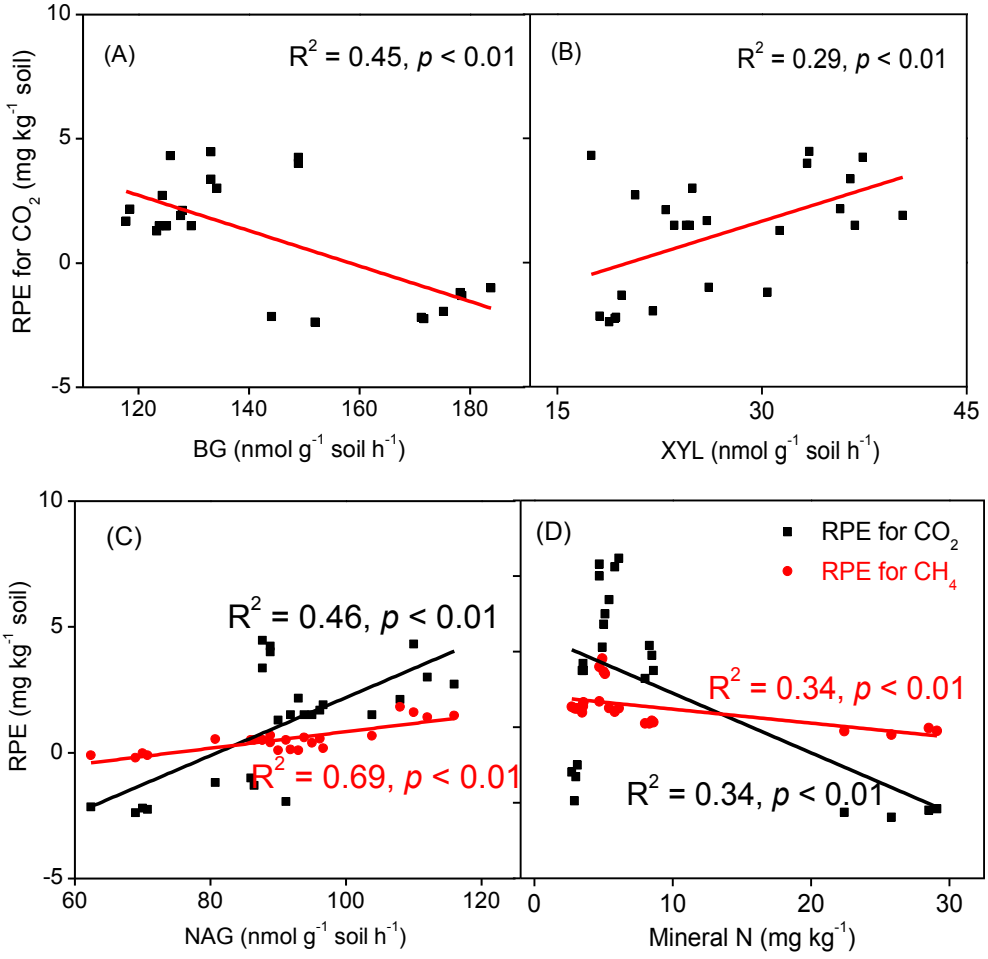

Fig. 4. Relationships between β-1, 4-glucosidase activity (BG) (A), β-xylosidase (XYL) activity (B), and the rhizosphere priming effect (RPE) for CO_2; relationships between β-1,4-*N*-acetylglucosaminidase activity (NAG) (C), soil mineral N (D) and RPE for CO_2 and CH_4.

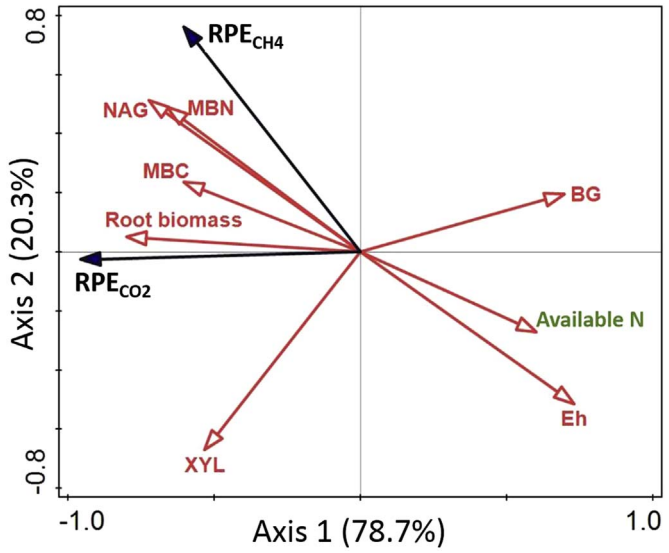

Fig. 5. Redundancy analysis plot showing the relationships of rice root biomass, potential redox (Eh), soil mineral N, soil microbial C (MBC) and N (MBN), and three extracellular enzymes (BG: β-1,4-glucosidase; XYL: β-xylosidase; NAG: β-1,4-*N*-acetylglucosaminidase) with the rhizosphere priming effect for CO_2 (RPE_{CO2}) and CH_4 (RPE_{CH4}).

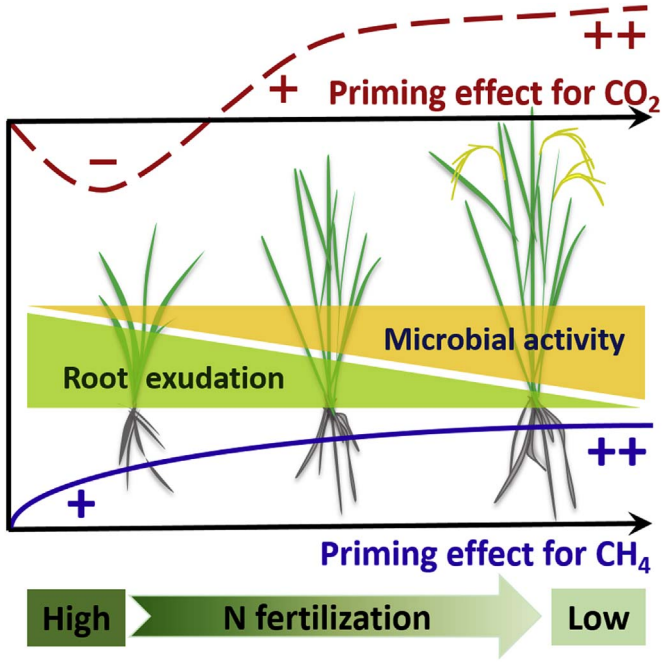

Fig. 6. Conceptual diagram of rhizosphere priming effect for CO_2 and CH_4 depending on rice growth an N fertilization.

increase of SOM-derived CH_4 at the latter sampling points (Fig. 2). Furthermore, N fertilization is known to decrease microbial mining of SOM for nutrients (Fontaine et al., 2011; Sinsabaugh and Follstad Shah, 2012; Chen et al., 2014). N fertilization increased the mineral N in planted soil and released microbes from N limitation, which reduced

the SOM-derived CO_2 emission and subsequently lowered the RPE for CH_4. These results were supported by the negative relationship between mineral N and RPE for CH_4 and the positive correlation between NAG and RPE for CH_4 (Fig. 4).

CH_4 emission from soils depends on the balance of CH_4 production and consumption due to oxidation (Dorodnikov et al., 2011), which is controlled by the relative intensity of activity of methanogens for CH_4 production and methanotrophs for CH_4 consumption (Cai et al., 2010; Shrestha et al., 2010). Previous studies reported that N fertilization stimulated methanotrophic bacteria and increased CH_4 uptake in soil (Prasanna et al., 2002; Shrestha et al., 2010). De Visscher and Van Cleemput (2003) reported that NH_4^+ could stimulate CH_4 oxidation at high CH_4 concentrations; this might imply that N fertilization could ultimately reduce CH_4 production and emission in paddy soil.

CH_4 production is also affected by the presence of electron acceptors (Megonigal et al., 2004; Jungkunst and Fiedler, 2007). N fertilization has various effects related to electron acceptors: 1) Increased root biomass and associated secreted O_2 increase Eh, thereby lowering generally CH_4 production and the RPE for CH_4 (Ye et al., 2015; Yuan et al., 2014). This was supported by the negative relationship between Eh and RPE for CH_4 (Fig. 5). 2) N added by fertilization as nitrate (NO_3^-) is additional electron acceptor by reduction to NH_4^+ (Liu and Greaver, 2009) and so, reduce CH_4 production. 3) N addition might increase the activity of dissimilatory reducing bacteria and promote Fe^{3+} and SO_4^{2-} reduction, which consump electrons and so, lead to decrease of CH_4 production (Gauci et al., 2008; Ye et al., 2015). Because of these mechanisms, CH_4 emission is reduced by N fertilization in paddy soils (Fig. 6). These results implied that applying mineral N fertilizers could mitigate greenhouse gas emissions (both CO_2 and CH_4) in rice cropping paddy systems.

5. Conclusions

The effects of rice rhizodeposits on total and SOM derived CO_2 and CH_4 emissions were measured in paddy soil by continuous $^{13}CO_2$ labelling. Rice root growth and rhizodeposits affected the direction of the RPE for CO_2, which changed from negative (before 40 days) to positive (after 52 days). The RPE for CH_4 was positive, gradually increased with rice growth (correspondingly to amount of rhizodeposits), and was well correlated with the decrease of soil redox potential. N fertilization reduced N competition between rice roots and microorganisms, provided additional electron acceptors, decreased extracellular enzyme activities, and lowered the magnitude of RPE for both CO_2 and CH_4. Overall, N fertilization and rice growth affected the RPE for CO_2 and CH_4 by altering microbial activity in paddy soil. Thus, optimized N fertilization is necessary to mitigate greenhouse gas emissions from rice field ecosystems by maintaining high C input by roots and so, high C sequestration.

Acknowledgments

This study was supported by the National Natural Science Foundation of China [grant numbers 41671292, 41501321, 41771337], the National Key Research and Development program [grant number 2016YFE0101100], the Youth Innovation Team Project of Institute of Subtropical Agriculture, Chinese Academy of Sciences [grant number 2017QNCXTD_GTD], and the Open Fund of Key Laboratory of Agro-ecological Processes in Subtropical Region, Chinese Academy of Sciences [grant number ISA2017301]. We thank Public Service Technology Center, Institute of Subtropical Agriculture, Chinese Academy of Sciences for technical assistance. The publication was prepared with the support of the “RUDN University program 5-100”.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.11.001.

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