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Biochar prepared at different pyrolysis temperatures affects urea-nitrogen immobilization and rice productivity in paddy fields

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Food safety has become a major issue , with serious environmental pollution resulting from losses of nitrogen (N) fertilizers. Transformation of agricultural waste (e.g., straw) into biochar to amend soil has been suggested as a globally applicable green method of field management. However, due to high variability in the quality of biochar, its application has varying effects on N loss and crop productivity. In this study, we examined the effects of pyrolysis temperature on the quality and applicability of straw-derived biochar used for paddy soil amendment in an area of japonica rice production in North China. Pot experiments were performed to determine nitrous oxide (N_2O) emissions and ^{15}N recovery using a ^{15}N tracer across the rice growing season. Biochar was prepared at two pyrolysis temperatures (400 and 700℃) and applied at three rates (0, 0.7, and 2.1%, w/w), with or without N fertilization (0, 168, and 210 kg N ha^{-1}). The results showed that biochar significantly decreased soil bulk density, while increasing soil porosity, irrespective of pyrolysis temperature and N level. Low-(B400) and high-temperature (B700) biochar treatment reduced the N loss rate by up to 66.42 and 68.90%, respectively, following co-application of 2.1% biochar and 168 kg ha^{-1} N fertilizer. Compared with the non-biochar control, 2.1% biochar plus 210 kg ha^{-1} N fertilizer significantly decreased N fertilizer-induced N_2O emission factor under both B400 and B700. Overall, B700 treatment reduced rice biomass and yield compared with B400. In conclusion, irrespective of pyrolysis temperature, biochar had multiple effects on fertilizer N recovery in the rice-soil system, and N_2O emissions, rice biomass and yield in the paddy field; however, these effects were dependent on N fertilizer level, biochar application rate, and their interactions.

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ABSTRACT

Food safety has become a major issue, with serious environmental pollution resulting from losses of nitrogen (N) fertilizers. Transformation of agricultural waste (e.g., straw) into biochar to amend soil has been suggested as a globally applicable green method of field management. However, due to high variability in the quality of biochar, its application has varying effects on N loss and crop productivity. In this study, we examined the effects of pyrolysis temperature on the quality and applicability of straw-derived biochar used for paddy soil amendment in an area of japonica rice production in North China. Pot experiments were performed to determine nitrous oxide (N_2O) emissions and ^{15}N recovery using a ^{15}N tracer across the rice growing season. Biochar was prepared at two pyrolysis temperatures (400 and 700°C) and applied at three rates (0, 0.7, and 2.1%, w/w), with or without N fertilization (0, 168, and 210 kg N ha^{-1}). The results showed that biochar significantly decreased soil bulk density, while increasing soil porosity, irrespective of pyrolysis temperature and N level. Low-(B400) and high-temperature (B700) biochar treatment reduced the N loss rate by up to 66.42 and 68.90%, respectively, following co-application of 2.1% biochar and 168 kg ha^{-1} N fertilizer. Compared with the non-biochar control, 2.1% biochar plus 210 kg ha^{-1} N fertilizer significantly decreased N fertilizer-induced N_2O emission factor under both B400 and B700. Overall, B700 treatment reduced rice biomass and yield compared with B400. In conclusion, irrespective of pyrolysis temperature, biochar had multiple effects on fertilizer N recovery in the rice-soil system, and N_2O emissions, rice biomass and yield in the paddy field; however, these effects were dependent on N fertilizer level, biochar application rate, and their interactions.

Keywords Pyrolysis temperature, Straw-derived biochar, Nitrogen immobilization, ^{15}N labeling, Recovery efficiency, Nitrous oxide emissions

INTRODUCTION

Food safety has become a shared global concern. With the rapidly growing population, which is predicted to reach 9.8 billion by the year 2050, there is huge demand for more food (King et al.

2017). Rice is the major staple in Asia, where per capita consumption is expected to increase from 84.9 kg in 2012 to 86.8 kg in 2024 (*OECD/FAO, 2015*). To increase the production of grains, fertilizer nitrogen (N) application has increased. The global average N use efficiency is 59%; however, in China, the average is below 40%, indicating that nearly 40–50% of N input is lost (*Liu et al., 2010;M Vitousek et al., 2009*). N losses exert pressure on the ecological environment, causing water pollution and soil acidification (*Gruber & Galloway, 2008*). However, meeting the demands for increased food production while minimizing adverse environmental impacts through improved N recovery remains a challenge (*Fixen & West, 2002*). Innovation and technologies aimed at understanding the recovery of fertilizer N in paddy systems is therefore required to provide data for higher N use efficiency (*Zhang et al., 2012*).

The application of biochar is considered an effective way of mitigating the negative impacts of agricultural production, improving nutrient uptake and conditioning reactive N in agricultural systems (*Sun et al., 2017; Woolf et al., 2010*). Biochar is a solid carbon-rich product obtained via the pyrolysis conversion of biomass in an oxygen-limited environment (*Initiative, 2012; Lehmann & Joseph, 2009*). Biochar, as a method of soil amendment, can increase the fertility and quality of barren soil by improving soil physico-chemical properties, mainly due to its large surface area, elevated pH, higher ash content, total surface charge, and high porosity (*Biederman & Harpole, 2013*). Biochar has therefore received increasing attention due to its contribution to agricultural practices (*Gale et al., 2016*) and effect on soil carbon (C) storage (*Nguyen et al., 2016*) and N conversion (*Clough et al., 2013; Riaz et al., 2017*). For example, *Thangarajan et al. (2018)* revealed 23 and 43% reductions in gaseous N emissions, respectively, from organic and inorganic N sources when biochar was applied to Andisol soil. Moreover, *Cayuela et al. (2014)* reported a decrease in soil N_2O emissions of 49±5% after biochar application under field conditions based on a meta-analysis involving pasture soil, hydroagric stagnic anthrosol, and loamy soil. Biochar was also found to mitigate greenhouse gas emissions at high N levels and promote nutrient uptake without fertilizer N supplementation (*Sun et al., 2017*), while aged biochar increased N use efficiency by reducing leaching or gaseous N losses in sandy soil (*Mia et al., 2017*).

The biochar effects depend on a number of factors, such as its characteristics and application rate (*Cayuela et al., 2014; Li et al., 2019; Oladele et al., 2019; Restuccia et al., 2019*). Temperature and pyrolysis conditions affect the characteristics of biochar, having an indirect impact on soil properties, and therefore, crop growth (*Ahmad et al., 2012; Angın, 2013; Hagner et al., 2016; Keiluweit et al., 2010; Purakayastha et al., 2015*). For example, *Zhou et al. (2017)* showed that low-temperature biochar (250–350°C) had a more positive effect on soil N than high-temperature biochar because of the more stable aromatic structure and higher hydrogen (H) and oxygen (O) contents. However, when prepared at a low temperature (300°C), birch biochar had a negative effect on the germination and biomass of lettuce, unlike higher-temperature (375 and 475°C) samples (*Hagner et al., 2016*). The pyrolysis temperature of biochar therefore seems to play a significant role in nutrient uptake by crops. However, little is known about effect of different pyrolysis temperatures and biochar application rates on urea-N fixation and N_2O emissions in paddy systems.

In this study, a pot experiment study was carried out, with two different pyrolysis temperatures and three biochar application rates. Three levels of stable isotope ^{15}N-traced fertilizer were then used to monitor N immobilization in a rice-soil system and N uptake by rice plants, to examine the following hypotheses: (i) pyrolysis temperature indirectly affects soil N retention for plant uptake and rice yield by affecting the quality of biochar; and (ii) higher-temperature biochar has a larger suppressive effect on soil N_2O emissions compared with biochar produced at a lower temperature.

MATERIALS AND METHODS

Biochar production and characterization

Maize straw was collected from Shenyang Agricultural University (Shenyang, Liaoning Province, China). After oven-drying (85°C, 24 hours) then cooling, the straw was cut into small pieces (< 2 mm) and stored in sealed plastic bags. The straw samples were then transferred to a rectangular porcelain container (150 × 100 × 50 mm) and placed in a muffle furnace for pyrolysis at a heating rate of 15°C min^{-1}. Temperature was first raised to 200°C and then to a final temperature of 400 or 700°C, maintained for 1 h then cooled to room temperature. To minimize the oxygen content in the reaction, the container was filled with straw and tightly sealed. The biochar was then stored at room temperature until analysis and experimentation. Basic properties of straw-derived biochar samples obtained at each different temperature are shown in Table 1. Each sample was analyzed in triplicate.

Site description

Paddy soil was collected (ca. 0–20 cm surface layer) from an experimental field (41°50′ N, 123°24′ E) managed by the Rice Institute of Shenyang Agricultural University (Shenyang, Liaoning Province, China). The soil was classified as silt loam according to the United States Department of Agriculture (USDA) soil taxonomy. The basic properties of the soil prior to the experiment were as follows: initial pH = 6.8 (1:2.5, water/soil, w/v) (HANNA HI2221, Italy), bulk density = 1.46 g cm^{-3}, total N = 1.87 g kg^{-1}, and total C = 15.39 g kg^{-1}. The site experiences a typical semi-humid temperate continental monsoon climate, with a mean annual temperature and precipitation of 8.3°C and 500 mm, respectively, and 183 frost-free days. The accumulated temperature (>10°C) is 3300–3400°C. Annual precipitation is concentrated, and the annual air temperature differs (*Sui et al., 2016*). Air temperature and precipitation data were recorded during the rice growing season (June to October 2016, Fig. 1).

Experimental design

The experiment followed a 2 × 3 × 3 factorial completely randomized design. There were two pyrolysis temperatures (400 and 700°C; B400 and B700, respectively), three biochar application rates (0, 0.7, and 2.1% w/w; C0, C0.7, and C2.1, respectively), and three N fertilizer levels (0, 168, and 210 kg N ha^{-1}; N0, N168, and N210, respectively), with three replications each (n = 54). Each treatment set included three PVC pots (30 cm diameter × 25 cm height), giving a total of 162 pots. A total of 14 kg of soil (air-dried, 2-mm sieved) was packed into each pot at a depth of 20 cm.

The rice (*Oryza sativa* L.) *japonica* variety 'Shennong 265' was cultivated in 2016. Two seedlings at the three-leaf stage were transplanted from the nursery bed to each pot on 29 May 2016. In the experiment, 36% total urea was applied before transplanting (^{15}N-labeled urea as base fertilizer), with 24% at the active tillering stage (unlabeled urea as tillering fertilizer) and 40% at the ear primordial stage (unlabeled urea as panicle fertilizer). The ^{15}N-labeled urea (10.18 atom% ^{15}N abundance) was provided by Shanghai Research Institute of Chemical Industry (Shanghai, China). In addition, all treatments received the same amounts of phosphorus (615 kg P_2O_5 ha^{-1} as triple super phosphate) and potassium (200 kg K_2O ha^{-1} as potassium chloride) as base fertilizers. The soil remained flooded to a depth of 5 cm except for aeration at the top-tillering stage to control effective tillering. Pots were kept outdoors. To reduce the effects of precipitation, a mobile steel-framed plastic canopy (800 cm long × 300 cm wide × 200 cm high) was used.

At maturity (14 October, 2016), all plants were harvested and separated into grains and straw then oven-dried to a constant weight at 70°C for 48 h and weighted to determine total yield. Grain moisture was determined using a hand-held moisture tester after drying (John Deere, Moline, IL, USA), and grain yield was estimated with a 14.5% moisture content.

^{15}N analysis

Dry plant samples (grain and straw) were ground and sieved (0.15 mm) to analyze total N and ^{15}N content (% in atoms) by isotope ratio mass spectrometry. ^{15}N analyses were performed using elementar ISO prime 100 (Isoprime Ltd., Germany). Stable nuclides and the natural abundance differ from the atom% excess of the element. The background value (atom %) was subtracted from the experimental value to give the atom% excess. The natural ^{15}N abundances of the plants and soil were estimated by averaging the values of all experimental treatments, respectively. Plant N uptake, N use efficiency, and the percentage of plant N derived from urea fertilizer were calculated using the following equations:

$$\text{Plant N content} = \text{Total N concentration in dry biomass} \times \text{weight of dry biomass} \quad (1)$$

$$\text{Ndff} = \frac{Af\% - Acf\%}{Au\% - Acf\%} \times TN\ (plant\ or\ soil) \quad (2)$$

$$Ndfs = TN - Ndff \qquad (3)$$

where Ndff is the N in the plant or soil derived from ^{15}N-labeled urea fertilizer (mg pot^{-1}) and Ndfs is the N in the plant derived from the soil (mg pot^{-1}), TN is the total N content in the plant or soil (mg pot^{-1}), and Au, Acf, and Af are the ^{15}N abundance in the ^{15}N-labeled urea fertilizer (10.18 atom%), natural ^{15}N abundance in the plant or soil, and total ^{15}N abundance in the plant or soil, respectively.

The recovery of ^{15}N-labeled urea in the plant tissue or percentage retained in the soil was derived at the harvest stage using the following equation (*Bronson et al., 2000*):

$$REN\ (\%) = \frac{Ndff}{F} \times 100 \qquad (4)$$

where F is the amount of ^{15}N-labeled urea applied (mg pot^{-1}).

Soil analyses

Three soil samples were obtained using a hand-operated core sampler (inner diameter = 3.5 cm, 20 cm deep) from each pot after harvest (October 2016). Soil samples were sealed in plastic bags and maintained on ice in an insulated box then transported to the laboratory where they were stored at -20°C until use. Each sample was divided into two parts, one for analysis of soil water content and another for soil physical and chemical analyses. Soil water content was determined after oven-drying ca. 5 g soil samples at 105°C for 48 h. For analysis of other soil properties, samples were ground using a Wiley millto and passed through a 2 mm sieve to remove root detritus prior to analysis. Subsamples were further passed through a 0.15 mm sieve for determination of total N using an Elementar Variomax CNS Analyzer (German Elementar Company, 2003). Soil inorganic N (NH_4^+-N) was extracted with 2.0 M KCl solution filtered by Whatman No. 1 filter paper, and quantitated with a Continuous Flow-injection Analyzer AA3 (German SEAL Company, 2011). Bulk density was determined using a cylinder (100 cm³) with additional samples collected at a 0–10 cm soil depth. Soil porosity was calculated as follows: soil porosity (%) = (1 – bulk density / specific gravity of soil) × 100.

Gas sampling and analysis

N_2O emission fluxes were measured across the rice growing season (June to October) using static opaque chambers (*Wang et al., 2011*). The size of the chambers (32 cm diameter × 7[illegible]0 cm height) was adapted to rice growth. The chambers were also wrapped in aluminum foil to reduce internal temperature changes, and equipped with circulating fans to ensure complete gas mixing during gas sampling. During the growing season, gas fluxes were measured approximately every two weeks then once more after each fertilization or water control practice. This measurement frequency was adjusted to capture the period of most active N loss in more detail.

Chambers were placed on stainless steel pedestals during the period of each flux measurement. The edge of the stainless steel pedestals had a groove filled with water to seal the gas chamber during gas collection. Gas samples were collected using a 50 mL air-tight syringe (*Singla & Inubushi, 2014*) at 15-min intervals (0, 15, 30 and 45 min) after the chambers were closed. N_2O flux measurements were conducted between 8 and 10 a.m. (*Zou et al., 2005*). N_2O concentrations were analyzed using a gas chromatograph (Agilent 7890A, Agilent Technologies, Santa Clara, CA, USA) and hourly emissions of N_2O were determined from the slope of the mixing ratio change with four sequential samples. Quality checks were applied and N_2O flux measurements were discarded if the r^2 of the linear regression of the fluxes was < 0.90. Cumulative emissions of N_2O during the growing season were calculated using trapezoidal integration to interpolate fluxes between successive sampling days (*Millar et al., 2018*).

The N fertilizer-induced N_2O emission factor was calculated by the difference in cumulative N_2O-N emission during the rice growing season between treatments with or without N fertilization, divided by the fertilizer N applied (Eq. 5):

$$\text{EF}\ (\%) = \frac{Cumulative\ N_2O - N\ (fertiliztion) - cumulative\ N_2O - N\ (unfertilized\ control)}{Fertilizer\ N\ applied} \times 100 \quad (5)$$

where EF is the N fertilizer-induced N_2O emission factor.

Statistical analysis

Treatment effects were assessed by three-way analysis of variance (ANOVA) using SPSS Statistics 18.0 (IBM, Somers, NY, USA), and significance was expressed at $P < 0.05$. All data are expressed as the mean ± standard error ($n = 3$). Multiple comparisons of means were based on Fisher's least significant difference (LSD) test at a 5% significance level unless stated otherwise.

RESULTS

Soil physical properties

A significant decrease in soil bulk density was observed with increasing biochar application, irrespective of pyrolysis temperature and N level. In contrast, biochar application led to an average increase in soil porosity of 12.19 and 10.37% across the two pyrolysis temperatures (B400 and B700, respectively) with and without N fertilization. Biochar had no significant effects on capillary porosity or air-filled porosity under all treatments (Table 2).

Under high-N treatment (N210), soil NH_4^+-N decreased slightly with increasing biochar rate, irrespective of pyrolysis temperature. The lowest soil NH_4^+-N concentration was observed at 3.79 μg g^{-1} under B400 and 3.71 μg g^{-1} under B700 (Fig. 2).

^{15}N recovery

A higher biochar rate resulted in lower N uptake from ^{15}N-labeled urea in rice, irrespective of pyrolysis temperature and fertilizer level. Following N fertilization alone at 210 kg ha^{-1}, N uptake from ^{15}N-labeled urea reached 316.03 and 306.00 mg pot^{-1} under B400 and B700, respectively. Compared with the no-biochar treatment (C0), N uptake from ^{15}N-labeled urea decreased by 55.03 and 44.03% under B400 and B700, respectively, at 2.1% biochar (C2.1). Similarly, N uptake from the soil also decreased with increasing biochar rate (Table 3).

The recovery rate of ^{15}N-labeled urea in rice was 12.57 and 13.11% under B400 and B700, respectively, following co-application of 0.7% biochar and 168 kg ha^{-1} N fertilizer. With increasing biochar rate, the recovery rate of ^{15}N-labeled urea in rice decreased significantly across the two pyrolysis temperatures, irrespective of N level (Table 3). Overall, 540.56 and 500.77 mg pot^{-1} of ^{15}N was recovered in the plant-soil system under B400 and B700, respectively, following co-application of 2.1% biochar and 168 kg ha^{-1} N fertilizer. Corresponding N recovery rates in the plant-soil system under these conditions were 33.58 and 31.10%, ranking highest among all treatments (Table 4).

A large proportion of ^{15}N in the rice-soil system was presumably lost, and lowest ^{15}N loss rates were found under B400 (66.42%) and B700 (68.90%) following co-application of 2.1% biochar and 168 kg ha^{-1} N fertilizer. Under B400 treatment, a smaller ^{15}N loss rate was observed following N fertilization at 168 kg ha^{-1} compared with 210 kg ha^{-1}, irrespective of biochar rate. Similar results were also observed under B700. Concerning biochar rate alone, a high application rate resulted in a lower N loss rate (71.82 and 72.94%) than a low application rate (78.00 and 79.69%) under both B400 and B700. In the presence of 168 kg ha^{-1} N fertilizer, 2.1% biochar rate under B700 had a significant effect on the ^{15}N content of the rice plants compared with non-biochar treatment (P <0.05). Both B400 and B700 enhanced soil ^{15}N retention at the 2.1% biochar rate, with a larger soil ^{15}N value under B400 compared with B700 (Table 4).

N_2O emissions

N_2O emissions from the soil were significantly affected by pyrolysis temperature, biochar rate, N level, and their interactions (Table 5). N_2O emissions fluxes initially peaked on day 2 then decreased on day 20 after transplanting, except under co-application of 2.1% biochar and 168 kg ha^{-1} N fertilizer. Under B400, co-application of 2.1% biochar and 210 kg ha^{-1} N fertilizer resulted in wide fluctuations in N_2O emissions after water control and then a slight decrease after topdressing with N, while under B700 a sharp decrease was observed (Fig. 3). Cumulative N_2O emissions ranged from 0.75 to 3.75 kg ha^{-1} and significantly decreased with increasing biochar rate, regardless of pyrolysis temperature and fertilizer level. The application of 2.1% biochar without N resulted in the least cumulative N_2O emissions under both B400 and B700. Meanwhile, 2.1% biochar treatment caused a notable decrease in cumulative N_2O emissions compared with 0.7% biochar treatment ($P < 0.05$; Fig. 4).

The N fertilizer-induced N_2O emission factor, calculated as the percentage of total N supplied through urea, ranged from 0.09 to 0.77%. The lowest emission factor was obtained under B400 with 2.1% biochar plus 210 kg ha^{-1} N fertilizer, while the highest was observed under B700 with 0.7% biochar plus 168 kg ha^{-1} N fertilizer. The average emission factor under high-biochar treatment (C2.1) was 0.14%, which was significantly lower than that under all other biochar treatments.

Rice biomass and yield

The aboveground biomass (including straw and grain) of rice plants at harvest was significantly higher under high-N treatment alone compared with all other treatments ($P < 0.05$). Meanwhile, co-application of 2.1% biochar (B400) and a high N level decreased the aboveground biomass by 21.58% compared with no-biochar treatment, whereas a low N level decreased the aboveground biomass by only 10.15%. Under B700, the rice biomass under high-N treatment alone was significantly higher than that under co-application of 2.1% biochar and 168 kg ha^{-1} N fertilizer. Moreover, under 2.1% biochar (B700), the high N level caused an increase in biomass of 19.07 g pot^{-1} compared with the low N level (Table 5).

Grain yield was markedly higher under 0.7% biochar treatment compared with 2.1% biochar treatment, but under a low pyrolysis temperature only. The average yield with 0.7% biochar was 62.64 and 62.37 g pot^{-1} under B400 and B700, respectively. Three-way ANOVA revealed the significant effects of biochar rate and N level on rice biomass and grain yield (Table 5).

DISCUSSION

Different preparation conditions can alter the characteristics of biochar (*Angın, 2013*), with certain types of higher value in terms of comprehensive utilization (considering energy consumption during the pyrolysis process). In this study, two biochar samples prepared at different pyrolysis temperatures (B400 or B700) from maize straw were used due to their availability and utilization potential. The biochar properties varied considerably depending on the pyrolysis temperature (Table 1), suggesting that biochar effects on soil physical properties, fertilizer N immobilization, and crop growth also differ.

Biochar application reduces N loss in the rice-soil system

Following biochar application, fertilizer N can be lost as NH_4^+-N; however, the amount is very small compared with NO_3^--N, and therefore, the nitrate content in paddy soil is very low and barely detectable (*Cheng et al., 2017*) . In the present study, soil NH_4^+-N concentrations were low across treatments (3.71–6.07 μg g^{-1}). Interestingly, the soil NH_4^+-N concentration did not respond to increasing rates of biochar application alone (N0); however, a small decrease was observed with increasing biochar application following 210 kg ha^{-1} N fertilization (N210), irrespective of pyrolysis temperature (Fig. 2). *Ding et al. (2010)* previously observed a slight decrease in the cumulative loss of NH_4^+-N in the 0–20 cm surface layer of sandy silt soil after bamboo charcoal application, which was attributed to the NH_4^+-N sorption capacity of biochar. In our study, a lower soil NH_4^+-N concentration was observed under high-temperature biochar treatment (B700) than low-temperature treatment (B400) with 0.7% biochar application only (Fig. 2). The difference in the NH_4^+-N sorption ability of biochar is thought to be due to (1) differences in the ash content between biochar samples prepared at different temperatures; and (2) differences in the amount of certain functional groups on the biochar surface (*Zheng et al., 2013*). In addition, biochar tends to include anionic sites, which increase the ability of soil to adsorb and reserve NH_4^+ (*Sohi et al., 2010*).

Another pathway of N loss is gas emission (e.g., N_2O), which is a major problem encountered in paddy fields (*Team et al., 2014*). Throughout the study period (i.e., the rice growing season), biochar treatment caused notable decreases in cumulative N_2O emissions compared with non-biochar treatment (Fig. 4), confirming that biochar application to agriculture soil can efficiently mitigate N_2O emissions (*Dicke et al., 2015; Hagemann et al., 2017; Sun et al., 2017*). However, a recent study also found that corn straw-derived biochar application to alkaline clay soil has no effect on N_2O emissions (*Wu et al., 2018*). Consensus has yet to be reached on how and why biochar reduces N_2O emissions (*Cayuela et al., 2014*). In our study, N_2O emissions were higher under B400 treatment than B700 treatment (Fig. 3), suggesting that the enhanced N immobilization and NH_4^+ sorption ability of low-temperature (400ºC) biochar increases N_2O emissions (*Clough et al., 2013*). *Cayuela et al. (2015)* and *Harter et al. (2016)* suggested that lower N_2O emissions following biochar application were due to microbial reduction of N_2O to N_2 via *nosZ* gene-containing microorganisms. In this study, we did not verify *nosZ* gene abundance throughout the N_2O monitoring period. Further studies are therefore needed to quantify the *nosZ* gene under different biochar treatments and thereby determine the microbiological mechanisms of N_2O emission reduction.

As previously reported, biochar may affect the soil N content (*Clough et al., 2013*). However, our hypothesis that pyrolysis temperature indirectly affects soil N availability for plant uptake was not supported by the experimental results, with no differences in the recovery of ^{15}N in rice plants between B400 and B700 treatment. *Zhou et al. (2017)* revealed that biochar prepared with mixed materials at different temperatures caused an increase in both residual soil ^{15}N and subsequent plant ^{15}N uptake in an agricultural field in Canada (*Zhou et al., 2017*). These results suggest that biochar immobilizes soil nutrients onto its surface, thereby increasing soil ^{15}N concentrations at higher application rates through increased porosity and surface area (*Atkinson et al., 2010; Liang et al., 2006*).

The relationship between residual soil ^{15}N and the pyrolysis temperature of biochar observed here might be explained by the fact that high-temperature biochar favors soil fungal and bacterial colonization, in turn enhancing gaseous N losses and decreasing N retention (*Gul et al., 2015; Nguyen et al., 2016*). Complementation experiments further suggested that applying a low rate of high-temperature biochar (>450ºC) resulted in more correlations between microbial taxa, with a large number of microorganisms appearing to influence soil N retention (*Nelissen et al., 2014*). The higher residual soil ^{15}N content observed under B400 treatment was therefore not simply the function of a single factor, and further analysis of long-term biochar application in the field is therefore required to determine the agricultural-environmental win-win benefits and improve crop yield. The effects of biochar on soil microbial biomass N content, microbial activity, and N fixation processes of key factors in various soils also need to be studied. Consequently, the use of straw-derived biochar remains challenging, requiring an accurate pyrolysis temperature and application standards. Further research is therefore needed to support the universal use of straw-derived biochar in agriculture.

Biochar application decreases rice productivity in short-term pot experiments

In the present study, the rice biomass response to biochar varied with pyrolysis temperature, application rate, and N fertilizer level, with biochar application worsening plant growth. Treatment with 2.1% biochar, regardless of N fertilizer level, resulted in a ca. 13.35% decrease in rice biomass under B400 compared with no-biochar treatment (Table 5). This could be attributable to (1) the high application rate of biochar, which increased soil pH and therefore resulted in a decrease in nutrient availability (*Gonzaga et al., 2018*); and (2) the large surface area of biochar which immobilized inorganic N in soil. Our results are therefore contrary to those of *Zhao et al. (2014)*, who suggested that rice straw biochar applied at 9 t ha^{-1} had a positive effect on rice/wheat growth. These growth improvements could be explained by an increase in soil C and bioavailable NH_4^+-N and NO_3^--N levels after application of straw biochar (*Sui et al., 2016; Schulz et al., 2013*). *Rajkovich (2010)* found a small increase or no change in aboveground biomass following soil application with varying rates of feedstock biochar obtained at different pyrolysis temperatures, whereas *Dao et al. (2013)* observed a 3-fold increase in aboveground biomass after application of 80 t ha^{-1} biochar compared with the no-biochar control. Overall, therefore, the plant biomass response to biochar depends not only on the characteristics of the biochar, the application rate and crop species, but also on the experimental set-up and original soil conditions *(Biederman & Harpole, 2013; Chan et al., 2008; Lehmann et al., 2003)*.

Crop biomass directly affects grain yield. The results obtained from our pot experiment suggest that co-application of biochar with N fertilizer could significantly decrease rice yield under B400, but not B700, compared with the no-biochar control (Table 5). These findings suggest that our low-temperature biochar has a larger surface area and lower porosity compared with high-temperature biochar, as explained by the adsorption of organic molecules by the biochar surface, affecting soil pH and reducing rice yield. Wheat-straw biochar (12 t ha^{-1}) was previously found to have no significant effect on rice production in the first season (*Xie et al., 2013*). However, *Zhang et al. (2012)* revealed that application of wheat straw biochar (20 t ha^{-1}) resulted in a 10% increase in rice yield in the first cycle and more than 9.5% increase in the second cycle. Such sustainable yield-increasing effects of biochar were also found in other experimental studies on crops. Together, these data suggest that pyrolysis temperature has only a small effect compared with biochar application rate on the short-term yield potential in paddy soil.

CONCLUSIONS

This study confirms that application of biochar as an approach to recycle straw and reduce N loss in rice-soil systems requires thorough evaluation, both in terms of pyrolysis temperature and application rate. The results suggest that biochar application could enhance base fertilizer ^{15}N retention in the soil over the rice growing season, while negatively affecting urea-N uptake and biomass production in the first year. Meanwhile, soil bulk density and rice yield decreased after application of both low-temperature and high-temperature biochar, although N_2O emissions from the paddy soil were markedly reduced throughout the growing season. Moreover, the biochar effects were not proportional to pyrolysis temperature. The lowest N loss rate was obtained under low-temperature treatment with application of 168 kg ha^{-1} urea plus 2.1% biochar. In conclusion, the findings suggest that application of low-temperature biochar may be an effective strategy for mitigation of N losses in paddy fields in Northeast China.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare that they have no conflict of interest.

Author Contributions

•Jiping Gao conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, wrote the paper, authored or reviewed drafts of the paper, approved the final draft.

•Yanze Zhao performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

•Wenzhong Zhang conceived and designed the experiments, contributed reagents/ materials/ analysis tools, authored or reviewed drafts of the paper, approved the final draft.

•Yanghui Sui conceived and designed the experiments, analyzed the data, prepared figures and/or tables, contributed analysis tools, wrote the paper, reviewed drafts of the paper.

•Dandan Jin analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

•Wei Xin performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

•Jun Yi analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

•Dawei He performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw data are available as Supplemental Files.

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Table 1 (on next page)

(on next page)

Properties of the two biochar samples produced by pyrolysis at temperatures of 400°C (B400) and 700°C (B700).

Table 1 Properties of the two biochar samples produced by pyrolysis at temperatures of 400°C (B400) and 700°C (B700).

Biochar	Total C (g kg^{-1})	Total N (g kg^{-1})	Surface area (m^2 g^{-1})	Ash content (%)	Average pore size (nm)	pH (H_2O)	C/N
B400	624.2±6.3	20.0±0.0	34.9±1.2	14.8±0.5	43.2±0.9	9.8±0.3	31.2±0.3
B700	665.5±7.6	16.4±0.7	12.1±0.7	19.3±0.4	77.7±1.0	10.4±0.4	40.6±1.2

Table 2(on next page)

Basic physical properties of soil under different biochar treatments with or without nitrogen fertilization.

Table 2 **Basic physical properties of soil under different biochar treatments with or without nitrogen fertilization.**

Treatment†		Bulk density (g cm^{-3})						Soil porosity (%)						Capillary porosity (%)						Air-filled porosity (%)					
		B400			B700			B400			B700			B400			B700			B400			B700		
		Mean	Std. error	SL	Mean	Std. error	SL	Mean	Std. error	SL	Mean	Std. error	SL	Mean	Std. error	SL	Mean	Std. error	SL	Mean	Std. error	SL	Mean	Std. error	SL
N0	C0	1.49	0.03	a	1.42	0.05	bc	43.83	1.17	b	46.23	2.03	c	34.73	1.24	a	34.33	0.68	ab	9.09	1.12	a	11.90	2.09	bcd
	C0.7	1.42	0.07	ab	1.42	0.01	bcd	46.32	2.71	ab	46.58	0.34	bc	35.29	0.68	a	37.29	2.75	a	11.03	2.10	a	9.28	2.45	d
	C2.1	1.26	0.17	b	1.27	0.06	e	52.54	6.52	a	52.05	2.18	a	33.17	2.56	ab	34.98	0.22	ab	19.37	9.05	a	17.07	1.95	a
N210	C0	1.40	0.10	ab	1.49	0.00	a	47.20	3.60	ab	43.62	0.15	d	29.43	2.56	b	30.10	1.53	c	17.77	6.07	a	13.51	1.58	bc
	C0.7	1.38	0.02	ab	1.43	0.04	ab	48.09	0.71	ab	46.07	1.45	c	35.15	5.19	a	34.14	1.12	b	12.94	5.84	a	11.93	2.18	bcd
	C2.1	1.31	0.07	b	1.35	0.04	d	50.43	2.70	a	49.09	1.46	b	34.41	2.70	ab	35.30	0.13	ab	16.02	4.73	a	13.78	1.54	abc
N168	C0	1.41	0.06	ab	1.43	0.03	ab	46.70	2.36	ab	45.96	1.22	c	32.94	0.69	ab	32.97	1.20	b	13.76	3.05	a	12.99	2.41	bc
	C0.7	1.35	0.08	ab	1.38	0.05	bcd	48.95	3.04	ab	47.92	1.77	bc	32.68	2.78	ab	37.30	2.60	a	16.27	5.30	a	10.62	0.92	cd
	C2.1	1.28	0.03	b	1.36	0.06	cd	51.54	1.11	a	48.77	2.17	b	34.37	1.39	ab	33.97	1.20	b	17.17	2.48	a	14.80	3.36	ab

†B400 and B700 represent biochar prepared by pyrolysis at temperatures of 400 and 700°C, respectively; C0, C0.7, and C2.1 represent biochar application rates of 0, 0.7%, and 2.1% (w/w), respectively; and N0, N210, and N168 represent urea-nitrogen fertilizer levels of 0, 168, and 210 kg N ha^{-1}, respectively. SL = significant level. Lowercase letters within each column are significantly different at $P < 0.05$.

Table 3 (on next page)

Nitrogen uptake from ^{15}N-labeled urea in rice plants under different biochar treatments at the harvest stage.

Table 3 Nitrogen uptake from ^{15}N-labeled urea in rice plants under different biochar treatments at the harvest stage.

Temperature†	Treatment‡		Ndff (mg pot^{-1})			Ndsf (mg pot^{-1})			REN of ^{15}N-labeled urea in rice (%)		
			Mean	Std. error	SL	Mean	Std. error	SL	Mean	Std. error	SL
B400	N210	C0	316.03	13.47	a	2054.31	53.95	a	15.80	0.67	a
		C0.7	248.08	31.50	b	1749.50	129.86	ab	12.40	1.57	bc
		C2.1	142.13	42.86	de	1528.75	249.03	bc	7.11	2.14	d
	N168	C0	245.59	2.39	b	1636.33	98.99	bc	15.25	0.15	ab
		C0.7	202.32	4.08	bc	1560.99	25.51	bc	12.57	0.25	bc
		C2.1	113.63	18.83	e	1350.94	158.09	c	7.06	1.17	d
B700	N210	C0	306.00	25.56	a	2046.47	81.88	a	15.30	1.28	ab
		C0.7	230.51	54.28	b	1765.17	109.24	ab	11.53	2.71	c
		C2.1	171.26	57.10	cd	1606.81	337.90	bc	8.56	2.86	d
	N168	C0	245.51	21.15	b	1820.15	135.12	ab	15.25	1.31	ab
		C0.7	211.12	24.25	bc	1584.70	143.52	bc	13.11	1.51	abc
		C2.1	109.21	21.22	e	1378.55	216.23	c	6.78	1.32	d

†B400 and B700; ‡C0, C0.7, and C2.1; and N0, N210, and N168 are defined in **Table 2** footnote. Ndff = N content in the plant or soil derived from the ^{15}N-labeled urea, Ndfs = N content in the plant derived from the soil, and REN = recovery of ^{15}N-labeled urea in the plant tissue. SL = Significant level (different lowercase letters in a column indicate significant difference at P <0.05).

Table 4(on next page)

Recovery and loss of ^{15}N-labeled urea in the rice-soil system under different biochar treatments at the harvest stage.

Table 4 **Recovery and loss of ^{15}N-labeled urea in the rice-soil system under different biochar treatments at the harvest stage.**

Temperature[†]	Treatment[‡]		Recovery of ^{15}N in rice (mg pot^{-1})		Residual soil ^{15}N content (mg pot^{-1})		Residual soil ^{15}N rate (%)		Recovery of ^{15}N in rice-soil system (mg pot^{-1})		Recovery rate in rice-soil system (%)		^{15}N lost (mg pot^{-1})		^{15}N loss rate (%)	
			Mean	Std. error	Mean	Std. error	Mean	Std. error	Mean	Std. error	Mean	Std. error	Mean	Std. error	Mean	Std. error
B400	N210	C0	316.03	13.47	169.24	134.51	8.46	6.73	485.27	125.44	24.26	6.27	1514.73	125.44	75.74	6.27
		C0.7	248.08	31.50	159.68	18.06	7.98	0.90	407.76	19.31	20.39	0.97	1592.24	19.31	79.61	0.97
		C2.1	142.13	42.86	313.59	146.55	15.68	7.33	455.72	176.95	22.79	8.85	1544.28	176.95	77.21	8.85
	N168	C0	245.59	2.39	78.19	27.59	4.86	1.71	323.78	27.74	20.11	1.72	1286.22	27.74	79.89	1.72
		C0.7	202.32	4.08	177.97	29.45	11.05	1.83	380.30	33.09	23.62	2.06	1229.70	33.09	76.38	2.06
		C2.1	113.63	18.83	426.93	268.08	26.52	16.65	540.56	249.26	33.58	15.48	1069.44	249.26	66.42	15.48
B700	N210	C0	306.00	25.56	246.63	37.83	12.33	1.89	552.63	15.52	27.63	0.78	1447.37	15.52	72.37	0.78
		C0.7	230.51	54.28	213.57	74.53	10.68	3.73	444.08	41.30	22.20	2.06	1555.92	41.30	77.80	2.06
		C2.1	171.26	57.10	288.98	23.15	14.45	1.16	460.24	66.52	23.01	3.33	1539.76	66.52	76.99	3.33
	N168	C0	245.51	21.15	92.85	26.85	5.77	1.67	338.36	31.18	21.02	1.94	1271.64	31.18	78.98	1.94
		C0.7	211.12	24.25	85.53	27.89	5.31	1.73	296.64	52.14	18.43	3.24	1313.36	52.14	81.57	3.24
		C2.1	109.21	21.22	391.56	97.98	24.32	6.09	500.77	118.94	31.10	7.39	1109.23	118.94	68.90	7.39

[†]B400 and B700; [‡]C0, C0.7, and C2.1; and N0, N210, and N168 are defined in **Table 2** footnote.

Table 5

(on next page)

Biomass, grain yield, and N_2O emission factor of rice for the treatment factors of biochar applications, pyrolysis temperature, and fertilizer and their interaction.

Table 5 Biomass, grain yield, and N_2O emission factor of rice for the treatment factors of biochar applications, pyrolysis temperature, and fertilizer and their interaction.

Temperature[†]	Treatment[‡]		Dry matter (g pot^{-1})			Grain yield (g pot^{-1})			N_2O emission factor (%)		
			Mean	Std. error	SL	Mean	Std. error	SL	Mean	Std. error	SL
B400	N0	C0	48.64	2.93	c	19.72	0.86	d			
		C0.7	51.84	3.70	c	22.01	1.79	d			
		C2.1	53.44	1.69	c	24.97	0.75	d			
	N210	C0	202.94	8.42	a	98.62	2.57	a	0.0071	0.0008	a
		C0.7	174.50	8.70	ab	86.80	5.47	abc	0.0027	0.0008	bc
		C2.1	159.15	34.24	b	80.34	11.22	bc	0.0009	0.0005	e
	N168	C0	168.49	9.07	b	85.91	4.25	abc	0.0075	0.0018	a
		C0.7	159.71	9.23	b	79.10	6.65	bc	0.0012	0.0010	cde
		C2.1	151.39	22.11	b	74.72	9.58	c	0.0010	0.0005	e
B700	N0	C0	53.35	7.27	c	21.50	4.14	d			
		C0.7	47.04	0.98	c	19.05	0.17	d			
		C2.1	47.20	6.78	c	20.27	4.71	d			
	N210	C0	202.73	12.54	a	97.02	5.32	a	0.0013	0.0003	cde
		C0.7	174.02	1.07	ab	83.61	4.54	abc	0.0042	0.0012	b
		C2.1	173.23	42.64	ab	88.73	21.39	abc	0.0012	0.0002	de
	N168	C0	184.48	21.49	ab	91.01	12.07	ab	0.0033	0.0007	b
		C0.7	171.12	7.47	ab	84.45	2.77	abc	0.0077	0.0013	a
		C2.1	154.16	31.52	b	77.32	14.43	bc	0.0027	0.0005	bcd
Effect		df	F	P		F	P		F	P	
B		2	6.37	**<0.01**		4.57	**0.02**		52.91	**<0.01**	
N		2	300.72	**<0.01**		359.58	**<0.01**		13.09	**<0.01**	
T		1	0.76	0.39		0.28	0.60		0.01	0.93	
B×N		4	1.86	0.14		1.85	0.14		0.13	0.88	
B×T		2	0.09	0.92		0.11	0.90		89.01	**<0.01**	

N×T	2	0.55	0.58	0.64	0.53	22.26	**<0.01**
B×N×T	4	0.33	0.86	0.52	0.72	4.70	**0.02**

†B400 and B700; ‡C0, C0.7, and C2.1; and N0, N210, and N168 are defined in **Table 2** footnote. Significant level: Different lowercase letters in a column indicate significant difference (P <0.05). B = biochar application rate, N = nitrogen fertilizer level, and T = pyrolysis temperature.

Figure 1 (on next page)

Daily mean air temperature and precipitation in the study area during the rice growing season (June to October 2016) .

Figure captions

Figure 1 Daily mean air temperature and precipitation in the study area during the rice growing season (June to October 2016).

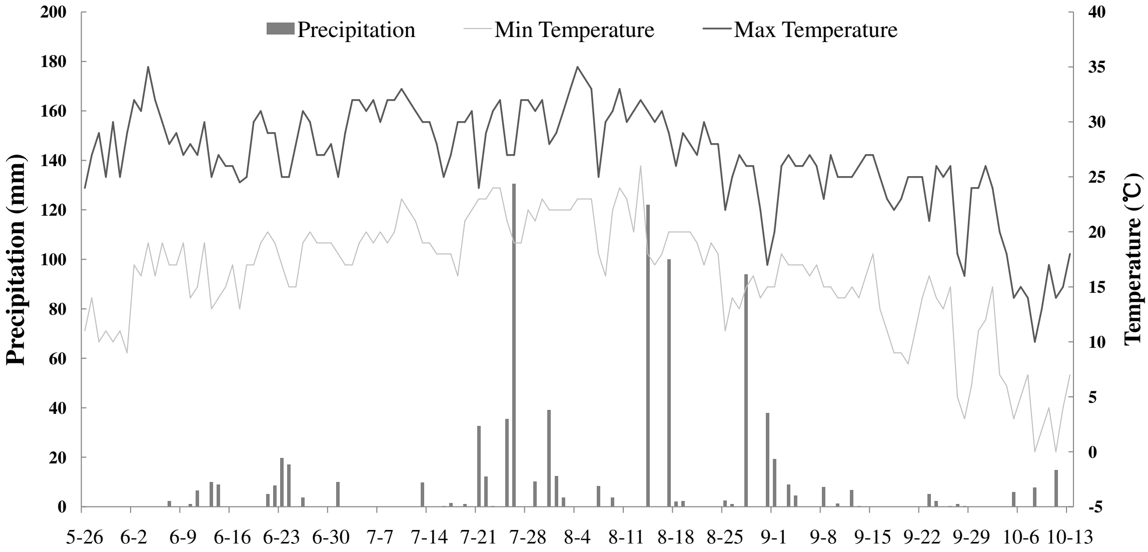

Figure 2 (on next page)

Effects of different biochar treatments with or without nitrogen fertilization on NH_4^+-N concentration in paddy soil.

Figure captions

Figure 2 Effects of different biochar treatments with or without nitrogen fertilization on NH_4^+-N concentration in paddy soil. B400 and B700 represent biochar prepared by pyrolysis at temperatures of 400 and 700°C, respectively; C0, C0.7, and C2.1 represent biochar application rates of 0, 0.7%, and 2.1% (w/w), respectively; and N0, N210, and N168 represent urea-nitrogen fertilizer levels of 0, 168, and 210 kg N ha^{-1}, respectively.

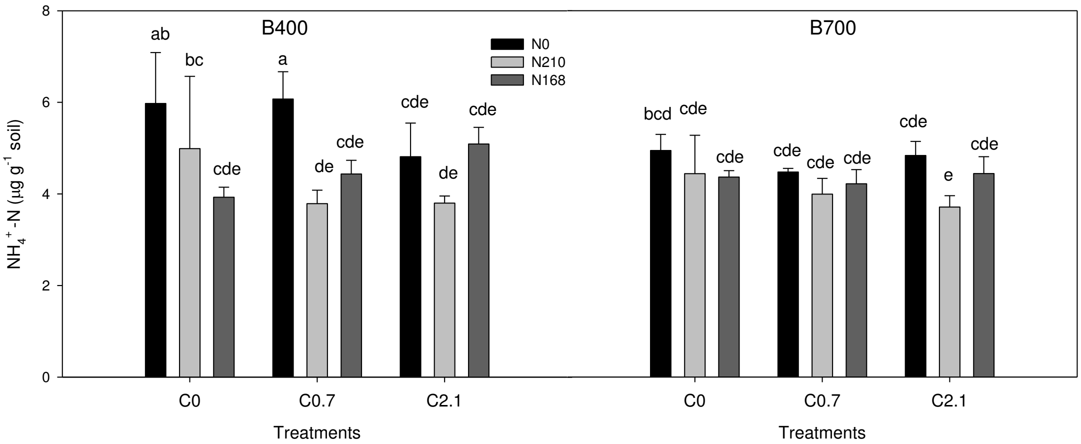

Figure 3 (on next page)

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Time series of daily N_2O emissions from paddy soil under different biochar treatments during the rice growing season.

Figure captions

Figure 3 Time series of daily N_2O emissions from paddy soil in different biochar treatments during the rice growing season. B400 and B700; C0, C0.7, and C2.1; and N0, N210, and N168 are defined in Figure 2 caption. Solid arrows indicate water controlling, and dot arrows indicate nitrogen fertilization. Error bars represent the standard error.

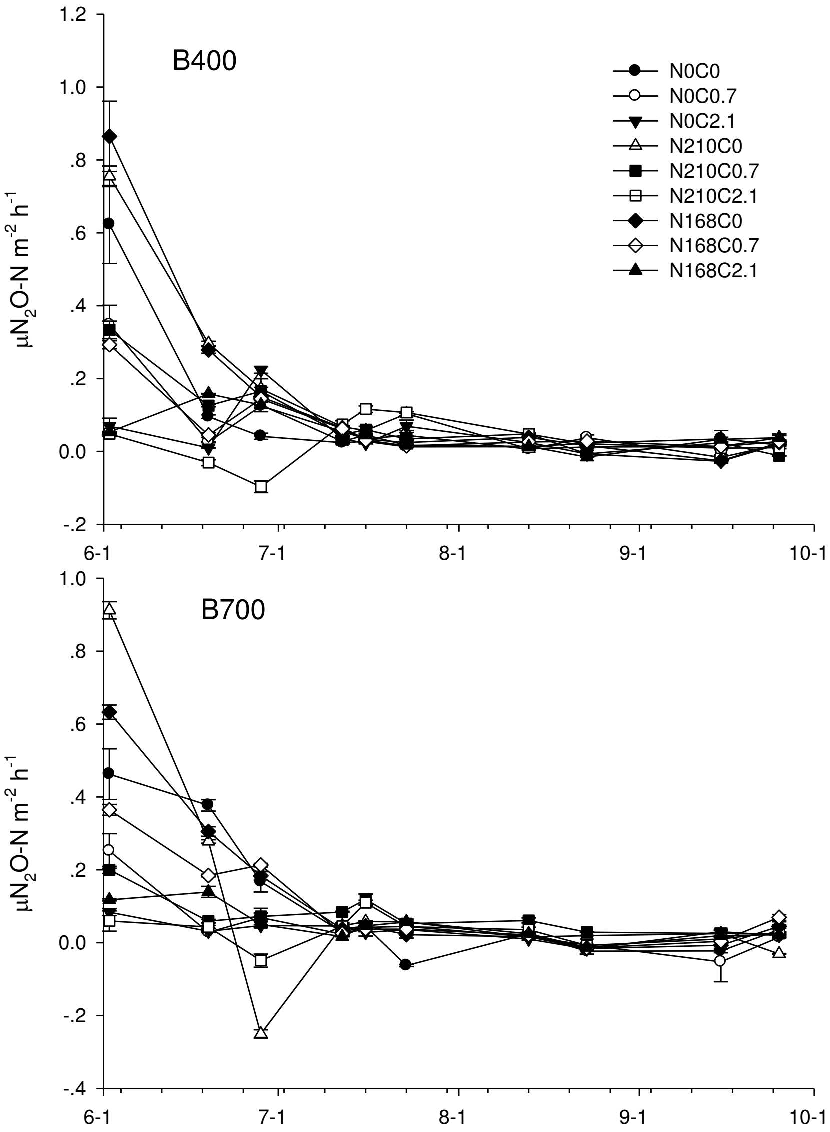

Figure 4 (on next page)

Cumulative N_2O emissions from paddy soil under different biochar treatments during the rice growing season.

Figure captions

Figure 4 Cumulative N_2O emissions from paddy soil in different biochar treatments during the rice growing season. B400 and B700; C0, C0.7, and C2.1; and N0, N210, and N168 are defined in Figure 2 caption.