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Wheat straw-derived biochar amendment stimulated N_2O emissions from rice paddy soils by regulating the *amoA* genes of ammonia-oxidizing bacteria

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ABSTRACT

Biochar amendment of upland soil has been generally accepted to mitigate nitrous oxide (N_2O) emissions. However, this is not always the case in rice paddy soil, and the underlying mechanisms are not well understood. To evaluate how biochar amendment affects N_2O production and emissions in paddy soil, an incubation experiment was designed including six treatments: wheat straw-derived biochar (slow pyrolyzed at 400 °C) amendment at rates of 0% (Control), 1% and 4% soil mass (w/w), inorganic nitrogen (N) fertilizer amendment (with urea), and N fertilizer plus 1% biochar and 4% biochar. The application of 4% biochar significantly increased N_2O emissions from N-unfertilized and fertilized soils during the 45-day incubation, by 291% and 256%, respectively, while 1% biochar amendment significantly increased soil N_2O emissions when accompanied by N fertilizer addition. On day 14, when the N_2O emission peaks occurred, N_2O flux was significantly correlated with soil pH in all treatments. Biochar addition also enhanced the abundance of ammonia-oxidizing bacteria (AOB) *amoA* genes, which was significantly related to soil pH. Among all detected N_2O-forming and reducing microbial genes, the abundance of AOB *amoA* genes was most closely related to N_2O flux. On biochar addition, the AOB community structure shifted from *Nitrosospira*-dominated toward *Nitrosomonas*, and the diversity of AOB was significantly increased. Compared with the control, biochar amendment decreased, albeit not significantly, the abundance of the nitrous oxide reductase encoding gene *nosZ*, but did not alter the abundance of nitrite reductase encoding genes *nirK* and *nirS*. Our study suggests that wheat straw-derived biochar amendment of paddy soils increased soil pH, which in turn increased the abundance and diversity of AOB and N_2O emissions.

1. Introduction

Increasing demand for nitrogen (N) fertilizer in agriculture impacts global biogeochemical N-cycling and causes many environmental problems, such as increased N_2O emissions (Duce et al., 2008; Galloway et al., 2008). Agricultural ecosystems are widely accepted to be an important source of N_2O and annual direct and indirect N_2O emissions from fertilizer N applied in arable soils equaled 4 Tg N globally (Bouwman et al., 2010). Rice paddy soil is regarded as an important source of atmospheric N_2O, although its N_2O emission intensity is not as high as that in upland soils (Yu and Patrick, 2004; Akiyama et al., 2005). Establishing effective agricultural management practices for mitigation of greenhouse gas (GHG) emissions is urgent.

Biochar amendment of agricultural soils has been proposed as a potential way to reduce GHG emissions (Case et al., 2012), enhance soil carbon sequestration (Lehmann and Joseph, 2012), improve soil fertility (Novak et al., 2009; Van Zwieten et al., 2010) and increase crop productivity (Jeffery et al., 2011; Huang et al., 2013; Liu et al., 2013). Meta-analysis of data reported previously showed that biochar amendment generally reduced N_2O emissions from upland soils (Cayuela et al., 2014). However, there is conflicting evidence in the literature about the effect of biochar on N_2O emissions from paddy soils. Liu et al. (2012) found that biochar amendment of three

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paddy fields across south China reduced N_2O emissions by 0%–60.9%. In contrast, biochar has also been found to stimulate N_2O emission from paddy fields (Liu et al., 2014; Shen et al., 2014).

N_2O is primarily produced by nitrification and denitrification (Wrage et al., 2001; Pihlatie et al., 2004). Previous study suggested that increased N_2O emission under biochar application was due to additional N input within the biochar (Shen et al., 2014) or increased denitrification resulting from biochar-derived labile organic C in paddy soils (Liu et al., 2014). However, biochar application has also been determined to increase soil pH (Wang et al., 2012) and improve soil aeration (Zhang et al., 2010); such factors are associated with the abundance and community structure of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (Chen et al., 2011; French et al., 2012; Li et al., 2015). For example, long-term fertilization (Wang et al., 2009, 2014; Wu et al., 2011) and increased soil pH (Cytryn et al., 2012; Hu et al., 2013) could increase AOB abundance and change the community structure of the AOB. It is not clear how biochar amendment regulates the abundance and community structure of ammonia oxidizers and subsequently N_2O emissions.

N_2O production during denitrification is regulated by NO-producing nitrite reductase, which is encoded by the genes *nirS* or *nirK*, and is reduced to N_2 by nitrous oxide reductase, encoded by *nosZ* (Cheneby et al., 1998; Thomson et al., 2012). Morales et al. (2010) found that *nirS* gene targets minus *nosZ* gene targets have a strong correlation with N_2O emissions, while Németh et al. (2014) reported that N_2O flux is only associated with the abundance of *nosZ*, and in acidic upland soil, biochar amendment reduced N_2O emissions by increasing *nosZ* gene abundance due to increased pH (Van Zwieten et al., 2014; Xu et al., 2014).

In the present study, we designed a microcosm experiment supplied with different amounts of wheat straw-derived biochar to monitor the variation of N_2O flux and the abundance of N_2O-forming and reducing functional genes. The objective of this study was to evaluate (1) whether wheat straw-derived biochar amendment influences the abundance of functional genes of N_2O-forming and reducing microorganisms such as bacterial and archaeal *amoA*, *nirS*, *nirK* and *nosZ*, and (2), if so, how the abundance of functional genes of N_2O-forming and reducing microorganisms is related to N_2O emissions in biochar-amended paddy soils.

2. Materials and methods

2.1. Soil sampling and biochar production

Surface soil (0–20 cm) samples were collected from a rice (*Oryza sativa* L.) and winter wheat (*Triticum aestivum* Linn.) rotation field in Dongshan Town, Suzhou City, Jiangsu Province, China (31°24′N, 120°25′E). Rice cultivation has been carried out in this area for several thousand years (Xu, 2001), and it is considered to be one of the most productive regions for rice in China. Derived from lacustrine deposits, paddy soil is classified as a hydroagric Stagnic Anthrosol (Gong, 1999) and an entic Halpudept (Soil Survey Staff, 1994). The moist soil was air-dried and ground to pass through a 2-mm mesh sieve. Biochar was produced in "no-oxygen" conditions using a slow-pyrolysis process. Before pyrolysis, wheat straws were oven-dried for 12 h at 80 °C, and then transferred into the biochar reactor. The reactor was heated in a stepwise procedure. The temperature was initially set at 200 °C, and then elevated to 250 °C, 300 °C, 350 °C and 400 °C, respectively. At each temperature step (except for 400 °C), the process was maintained for 1.5 h. The reactor was flushed by N_2 throughout the process, which was terminated after about 13 h when there was no visible smoke emission from the gas vent. The biochar produced was ground to pass through a 2-mm sieve. Table 1 shows physicochemical properties of the soil and biochar.

2.2. Laboratory incubation experiment

A series of 250-mL Erlenmeyer flasks were supplemented with 50 g of soil (on an oven-dried basis). Deionized water was added using a minipipette to adjust the ratio of soil to water to 1:1. All flasks were covered with aluminum foil with needle-punched holes to maintain aerobic conditions, and incubated at 25 °C in the dark for 3 days to activate microorganisms. The experiment included six treatments (20 flasks for each): wheat straw-derived biochar amendment at rates of 0% (Control), 1% (1% biochar) and 4% soil mass (4% biochar) (w/w), inorganic N fertilizer amendment (i.e. urea) (Urea), and N fertilizer plus 1% biochar (1% biochar + urea) or 4% biochar (4% biochar + urea). Biochar application at the rate of 1% and 4% soil mass (on an oven-dried basis) was equivalent to a field application rate of 20 and 80 t ha^{-1} in the 0–20 cm ploughed layer, respectively. Added biochar was mixed well with the soil using a glass rod. A solution of urea was added to designated (i.e. fertilized) flasks at the application rate of 200 mg N kg^{-1}. The ratio of soil to water was adjusted to 1:2 with approximately 2 cm standing water in all flasks. All flasks were covered with aluminum foil with needle-punched holes and incubated at 25 °C in the dark. To maintain the soil water content, deionized water was added with a minipipette every other day by weighing flasks during the 45-day incubation.

2.3. Measurement of soil N_2O flux, mineral N and dissolved organic carbon (DOC)

Four replicate flasks from each treatment were used to measure N_2O fluxes on after 1, 3, 5, 7, 10, 14, 18, 21, 25, 35 and 45 days. Before gas sampling, the headspace air in the flasks was thoroughly flushed with fresh air. The flasks were capped immediately with silicone rubber stoppers. An additional 20 mL fresh air was injected into the flasks using a syringe and completely mixed with the headspace gas. The same volume of gas was sampled and injected into pre-evacuated vials; this was the time-zero sample for analysis. The flasks were returned to the incubator for 2 h, and another 20 mL headspace gas was sampled from the flasks. After gas sampling, the stoppers were removed and aluminum foil was used to cover the flasks again. The N_2O concentration was determined on a gas chromatograph (GC; Agilent 7890, Agilent technologies, Santa Clara, CA, USA) equipped with an electron capture detector (ECD). N_2O fluxes were calculated from the N_2O accumulated between time zero and after incubation for 2 h N_2O gas standards were supplied by the National Research Center for Certified Reference

Table 1
Selected properties of soil and biochar.

	pH (H_2O)	Total C (g C kg^{-1})	Organic C (g C kg^{-1})	Total N (g N kg^{-1})	Bulk density (g cm^{-3})	NH_4^+-N (mg N kg^{-1})	NO_3^--N (mg N kg^{-1})
Soil	5.95	–	23.59	1.68	1.00	10.26	0.89
Biochar	10.74	492.67	–	11.64	ND	0.76	0.66

ND indicates not determined.