Wheat straw biochar reduces N_2O emission by increasing denitrification in alkaline and acidic submerged paddy soils

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Abstract

Paddy fields are one of the most important N sinks in terrestrial ecosystems, and considerable N loss is caused by denitrification. Biochar has been recognized as useful soil amendment to paddy field in mitigating nitrous oxide (N_2O) emission. However, the key mechanisms responsible for the reduced N_2O emissions by biochar in paddy soils are still obscure. Here, using two paddy soils with contrasting pH, the denitrification and N_2O emission were investigated in soil amened with different amounts of biochar (0%, 0.5% and 5%) via soil slurry incubation combined with N_2/Ar technique. The results showed that biochar amendment significantly increased the pH values both in the alkaline and acidic soils. Biochar at 5% amendment rate significantly increased denitrification and significantly decreased N_2O emission in soils. In the alkaline soil, biochar at 0.5% amendment rate significantly increased denitrification, but had no effect on N_2O emission. Conversely, in the acidic soil, biochar at 0.5% amendment rate did not affect denitrification, but significantly reduced N_2O emission. The $N_2O/(N_2+N_2O)$ ratio was significantly reduced by biochar amendment irrespective amendment rate both in alkaline and acidic soils. In the alkaline soil, biochar at 5% amendment rate significantly increased the abundance of *nosZ* genes, whereas biochar had no effect on the abundance of *nosZ* genes in the acidic soil irrespective of amendment rate. Our results suggested biochar effects in the alkaline soil were attributed to increase of denitrifying community, whereas biochar effects in the acidic soil was attributed to increase of pH.

Key Words

rice paddy, black carbon, denitrification, N_2/Ar technique, nitrous oxide

Introduction

Rice yield in China has increased substantially during the last three decades because of the rapidly increasing use of synthetic nitrogen (N) fertilizers (Ju et al., 2009). The N use efficiency in paddy field is generally low ranging from 30% to 40% (Zhu and Chen, 2002). The inefficiency of N use in the rice paddy field is mainly caused by denitrification, ammonia volatilization, and N leaching, among which denitrification is the predominant pathway for N loss way (Figure 1) (Xing and Zhu, 2000; Zhu and Chen, 2002). Also, substantial amounts of the applied synthetic N fertilizers in paddy soils are lost as nitrous oxide (N_2O) (Cai et al., 1997), a powerful greenhouse gas, which also contribute to the stratospheric ozone destruction (Ravishankara et al., 2009).

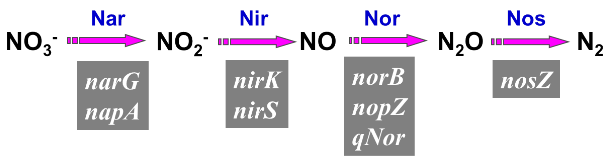

Figure 1. Sequential reductive pathway of denitrification with the location of enzymes i.e., nitrate reductase Nar (encoding *narG* and *napA* genes), nitrite reductase Nir (encoding *nirK* and *nirS* genes), nitric oxide reductase Nor (encoding *norB*, *norZ*, and *qNor* genes), and nitrous oxide reductase Nos (encoding *nosZ* gene).

It has been demonstrated that biochar application to paddy soils can significantly reduce emissions of N_2O in most previous studies (Zhang et al., 2010; Wang et al., 2011). Because in submerged paddy soils, N2O is mainly produced through denitrification as an obligate intermediate product (Majumdar, 2013), the lower N_2O in the paddy soil amended with biochar can be attributed to a reduction in the total N denitrified or a stimulation of the reduction of N_2O to N_2 (Baggs, 2011; Cayuela et al., 2013). However, the key mechanisms responsible for the reduced N_2O emissions by biochar in paddy soils are still obscure. Also little is known about effects of biochar application on denitrification rate and $N_2O/(N_2+N_2O)$ ratio in paddy soils.

In the present study, effects of biochar application on denitrification rate, N_2O emission and denitrifiers in one acidic and one alkaline paddy soils were investigated. We hypothesized that biochar application increases denitrification rates both in acidic and alkaline paddy soils, consequently leading to reduced N_2O emissions. The abundance of denitrifier gene (*nosZ* gene) was also investigated to prove our hypothesis.

Methods

Soil and biochar

The alkaline and acidic rice paddy soils were collected from the plough layers (0–20 cm in depth) of fields under a rice–wheat rotation in Jiangdu (119° 42′ E, 32° 35′ N), Jiangsu province and Yingtan (28° 15′ N, 116° 55′ E), Jiangxi province, respectively. Biochar was produced from wheat straw in a muffle furnace under oxygen-limited conditions. Briefly, the starting pyrolysis temperature was set at 100 °C, subsequently elevated to 500 °C at 5 °C min-1 and was held constant for 8 h.

Incubation experiment

All the incubation experiments were performed in 500 mL glass beaker, in which 150 g of soil (dry weight) were amended with different quantitites of biochar resulting inthree treatments: 0% biochar amendment (control), 0.5% biochar amendment, and 5% biochar amendment. The water content of all soil samples was adjusted to 60% water holding capacity (WHC). All the beakers were covered by parafilm with pin holes and incubated at 25 °C for 5 days, after which, soil pH, inorganic N contents of the soil-biochar matrix were determined. After 5 days aerobic incubation, subsamples (2.5 g) of the soil or soil-biochar matrix in the beakers was transferred into 12 mL glass tubes (Labco, UK) and 3 mL He-purged water was added, homogenized with the soil and standed for 5 min. Then another 6 mL He-purged water to result in soil slurry. For each treatment, forty glass tubes were prepared. Subsequently, all the tubes were sealed and preincubated underwater at 25 °C for 2 h. In the following 8 h incubations, eight replicate tubes at each sampling time (2 h interval) were removed and preserved with 200 µL saturated $HgCl_2$ solution, followed by centrifugation for 10 min (1800 g/25 °C). Then four replicate tubes were used to analyse dissolved N_2 by MIMS and N_2/Ar technique (Kana et al., 1994), while another four replicates were used to analyse dissolved N_2O by injecting 5 mL supernatant into 15 mL viacuum serum vials according to the method of Terry et al. (1981) and the N_2O concentration in the supernatant was determiend by gas chromatography (GC). In our experiments, for simplicity, the denitrification rate was refer as the net N_2 flux because other processes which also generated N_2 play minor role compared with denitrification. DNA was extracted from soil samples at the last time point for analysing functional gene abundances (*nosZ* gene) relevant to N_2O reduction by *q*PCR (Shan et al., 2016).

Data analysis

All statistical analyses were performed with software Sigmaplot 11.0 and the significantly level was set at $P < 0.05$. The one way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test was performed to evaluate the differences among means of different biochar amendment treatments.

Results

Figure 2 presents the effects of biochar amendment (0%, 0.5%, and 5%) on pH and abundance of *nosZ* genes in the alkaline and acidic paddy soils. In the alkaline soil, 5% biochar amendment significantly increased soil pH values and size of *nosZ* gene abundance. Whereas, in the acidic soil, biochar amendment regardless of amendment rate significantly increased soil pH values and had no effect on size of *nosZ* gene abundance (Figure 2).

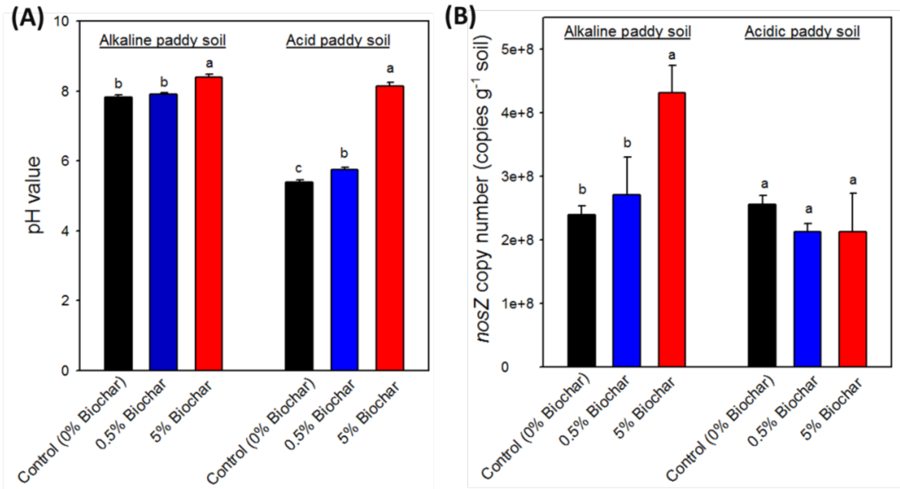

Figure 2. Effects of biochar amendment (0%, 0.5%, and 5%) on pH (A) and abundance of *nosZ* gene (B) in the alkaline and acidic paddy soils. Values are means with standard deviations (*n* = 4). The different letters above the bars indicate significant differences among the treatments.

Figure 3 shows the net N_2 flux, N_2O emission and $N_2O/(N_2+N_2O)$ ratio in the soils as affected by biochar amendment. In the alkaline soil, thenet N_2 flux (24.1 nmol N_2-N g^{-1} h^{-1}) was significantly increased by biochar amendment and the increasement was 155.0% and 185.7% for 0.5% and 5% biochar amendment, respectively. The N_2O emission in the alkaline soil (0.29 nmol N_2O-N g^{-1} h^{-1}) was significantly reduced by 5% biochar amendment (0.15 nmol N_2O-N g^{-1} h^{-1}) and the $N_2O/(N_2+N_2O)$ ratio (1.2%) was significantly reduced by biochar amendment (0.47% and 0.33% for 0.5% and 5% biochar amendment, respectively) regardless of amendment rate (Figure 3). In the acidic soil, the net N_2 flux (17.7 nmol N_2-N g^{-1} h^{-1}) was significantly increased by 5% biochar amendment (25.4 N_2-N g^{-1} h^{-1}) and the increasement was 143.5%. The N_2O emission (0.040 nmol N_2O-N g^{-1} h^{-1}) was significantly reduced by biochar amendment (0.0084 and 0.010 nmol N_2O-N g^{-1} $h^{-1}$for 0.5% and 5% biochar amendment, respectively) irrespective of amendment rate. Similar to the alkaline soil, the $N_2O/(N_2+N_2O)$ ratio in the acidic soil (0.22%) was significantly reduced by biochar amendment (0.045% and 0.039% for 0.5% and 5% biochar amendment, respectively) regardless of amendment rate (Fig. 3).

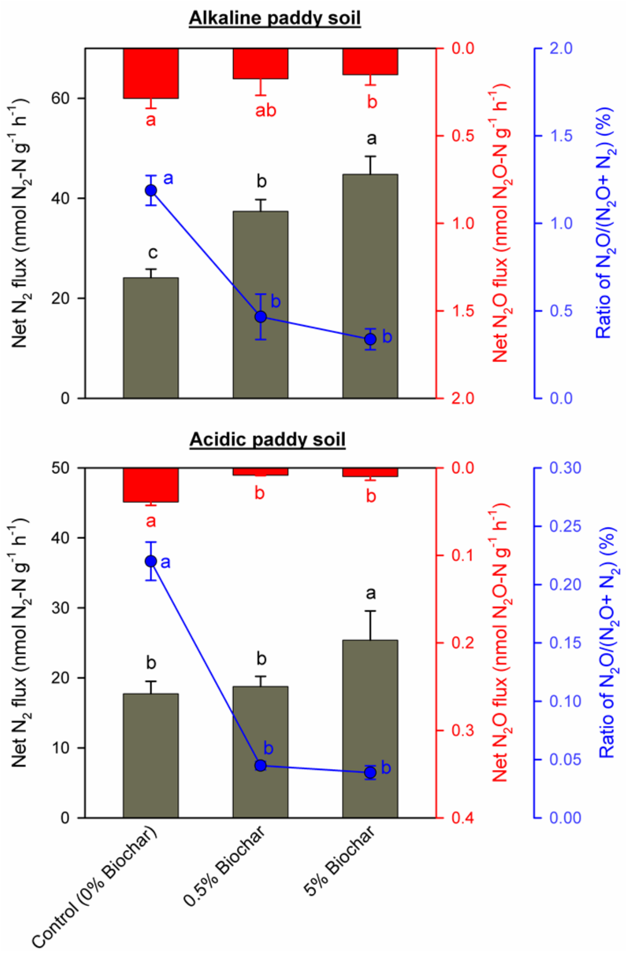

Figure 3. Effects of biochar amendment (0%, 0.5%, and 5%) on denitrification, N_2O emission and ratio of N_2O to N_2 and N_2O in the alkaline and acidic paddy soils. Values are means with standard deviations (n = 4). The different letters above the bars indicate significant differences among the treatments.

Conclusion

Our results indicated that effects of biochar on denitrification and N_2O emission were soil-specific and depends on the biochar amendment rate. In the alkaline soil, biochar reduced N_2O emission by increasing denitrification being attributed to increase of *nosZ* gene transcription. Whereas, in the acidic soil biochar reduced N_2O emission by increasing denitrification being attributed to increase of pH.

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