Biochar decreases nitrogen oxide and enhances methane emissions via altering microbial community composition of anaerobic paddy soil

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HIGHLIGHTS

• Rice straw biochar was amended to paddy soil for anaerobic incubation.
• Soil N₂O emission was suppressed mainly due to increased soil pH.
• Soil CH₄ emission was enhanced mainly due to increased soil DOC.
• The influence depended on biochar properties.
• Denitrifying bacteria abundance decreased while Fe-reducing bacteria increased.

GRAPHICAL ABSTRACT

Abstract

Biochar application to agricultural soil is an appealing approach to mitigate nitrous oxide (N₂O) and methane (CH₄) emissions. However, the underlying microbial mechanisms are unclear. In this study, a paddy soil slurry was incubated anaerobically for 14 d with biochar amendments produced from rice straw at 300, 500, or 700 °C (B300, B500, and B700) to study their influences on greenhouse gas emissions. Illumina sequencing was used to characterize shift of soil bacterial and archaeal community composition. After peaking at day 1, N₂O emission then sharply decreased to low levels while CH₄ started to emit at day 3 then continually increased with incubation. Compared to control soil (57.9 mg kg⁻¹ soil), B300, B500, and B700 amendments decreased N₂O peak emission to 17.9, 1.28, and 0.59 mg kg⁻¹ soil, mainly due to increased soil pH. In contrast, the amendments enhanced CH₄ production from 58.2 to 93.4, 62.6, and 63.4 mg kg⁻¹ at day 14 due to increased soil dissolved organic carbon. Abundance of denitrifying bacteria (e.g., Bacilli, 7.07–13.6 vs. 16.9%) was reduced with biochar amendments, especially with B500 and B700, contributing to the decreased N₂O emissions. However, larger pore size of B500 and B700 (surface area of 68.1 and 161 m² g⁻¹) than B300 (4.40 m² g⁻¹) favored electron transfer between bacteria and iron minerals, leading to increased abundance of iron-reducing bacteria (e.g., Clostridia, 48.2–50.6 vs. 33.3%), which competed with methanogens to produce CH₄, thereby leading to lower increase in

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CH₄ emission. Biochar amendments with high pH and surface area might be effective to mitigate emission of both N₂O and CH₄ from paddy soil.

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1. Introduction

Paddy soil is an important source of greenhouse gas emissions including nitrous oxide (N₂O) and methane (CH₄) (Zhang et al., 2010). In China, the annual emissions of N₂O and CH₄ from paddy soils are ~93 Gg and ~7.8 Tg (Khan et al., 2013; Liu et al., 2010). Increasing amounts of N₂O and CH₄ in the atmosphere are main contributors to climate changes (Kumar et al., 2007). Therefore, to slow down global warming, strategies are needed to mitigate emissions of these gases from paddy soil. Due to its low cost, feedstock availability, and favorable properties, biochar can be used to mitigate microbial growth and reduce greenhouse gas emissions (Khan et al., 2013; Xu et al., 2014).

As a carbon-rich solid, biochar is produced through pyrolysis of biomass under limited oxygen supply (Khan et al., 2013). Due to its characteristics such as high pH and surface area, it has been used as a beneficial soil amendment (Butnan et al., 2015; Cao et al., 2011; Lehmann et al., 2011). In addition, biochar application can mitigate emissions of N₂O and CH₄ from paddy soils (Feng et al., 2012; Nelissen et al., 2014). However, the results are inconsistent. Some studies show no effects (Cheng et al., 2012) while others show increased emissions (Clough et al., 2010; Troy et al., 2013). At present, how biochar property controls its influences on N₂O/CH₄ emissions remain unclear.

In addition to the inconsistent effects of biochar on N₂O/CH₄ emission, the underlying microbial mechanisms remain unclear. It is possible that N₂O/CH₄ emissions from paddy soils involve complicated biological processes, which are related to microbial communities (Braker and Conrad, 2011; Schütz et al., 1988). For example, both nitrification and denitrification, the predominant processes of soil N₂O emission, are performed by soil microbial communities (Braker and Conrad, 2011). Meanwhile, methanogenic archaea, including acetoclastic and hydrogenotrophic methanogens, affects CH₄ emission in anoxic habitats (Angel et al., 2011). Marker genes for nitrogen (N) and carbon (C) cycling have been determined prior to soil characteristics measurements. Soil pH and contents of total C and N were determined using procedures similar to biochar characterization (Weber et al., 2006b), supplying more substrates to promote N₂O production. For C cycling, Fe oxide reduction causes competition for H₂ or acetate between methanogens and ferric Fe reducers, suppressing CH₄ emission. In addition, the stability and high surface area of biochar serve as a stable platform for biofilm formation, supporting electron shuttling between microbes and insoluble electron acceptors such as Fe oxides (Kappler et al., 2014). Therefore, Fe reducing bacteria might play an important role in N and C cycling, especially in paddy soil with biochar amendments. However, studies on the composition of microbial community participating in dissimilatory Fe⁶⁺ reduction are limited because of the unavailability of universal functional gene markers for Fe reduction.

Despite biochar’s chemically recalcitrant structure, studies suggest that biochar addition to soil stimulates microbial activity (Gomez et al., 2013; Santos et al., 2012) and alters microbial community composition (Elzobair et al., 2016; Gal et al., 2015; Mitchell et al., 2015). As biochar has been considered unlikely to be consumed by soil microbes, it cannot directly influence soil microbial community (Lehmann and Joseph, 2009). However, with higher surface area and pore volume, it could serve as a habitat, protecting microorganisms from predators (Quilliam et al., 2013), as well as improving soil properties such as water holding capacity, nutrient availability, and pH buffering capacity (Ameloot et al., 2013; Basso et al., 2013), thus indirectly affecting N₂O/CH₄ emissions. Therefore, more work is needed to understand the potential effects of biochar on soil microorganisms, especially those underpinning N₂O/CH₄-related transformation processes.

The barcoded Illumina paired-end sequencing (amplicon sequencing) method provides a cost-effective way to study microbial community, monitoring rapid changes in soil microbial populations in response to environmental change and providing information about the abundance of microbial taxa including denitrifying bacteria, methanogens, and Fe reducing bacteria (Xu et al., 2014; Wang et al., 2015). Therefore, the Illumina sequencing technology can be applied to better understand the microbial mechanisms of biochar-induced changes in N₂O/CH₄ emissions from paddy soil.

The objective of this study was to explore the microbial mechanisms of biochar-induced changes in N₂O/CH₄ emissions. Three types of biochar, which were produced through pyrolysis of rice straw at 3 different temperatures (300, 500, and 700 °C), were incorporated into an anaerobic paddy soil. The specific objectives of this study were to 1) determine the effect of biochar on N₂O and CH₄ emissions from a paddy soil under anaerobic conditions; 2) investigate the responses of archaeal and bacterial community diversity and composition to biochar addition; and 3) explore the relationships between microbial composition changes and N₂O/CH₄ emissions.

2. Materials and methods

2.1. Biochar and paddy soil

Biochar was prepared through pyrolysis of rice straw at 300, 500, and 700 °C (i.e., B300, B500, and B700) in a furnace with nitrogen gas (N₂) for 4 h (Khan et al., 2013). Following preparation, biochar was measured for pH and EC in a water solution with biochar:water ratio of 1:10 (w/v), and content of total carbon (C) and nitrogen (N) using an element analyzer (CHN-O-Rapid, Heraeus, German). The Brunauer–Emmett–Teller surface area and pore volume were determined using a surface area and porosity analyzer (Micromeritics, ASAP 2020). Biochar images were obtained using a scanning electron microscope (SEM, Hitachi, S-4800).

A paddy soil (0–10 cm depth) was collected from a typical paddy filed from Yingtan, Jiangxi, China. After transported to the laboratory, a portion of the soil was air-dried, sieved (2 mm mesh) and homogenized prior to soil characteristics measurements. Soil pH and contents of total C and N were determined using procedures similar to biochar analyses. Another portion of the soil was kept fresh prior to the anaerobic incubation experiment with biochar amendments.

2.2. Biochar amendment and anaerobic slurry incubation

Amendments of B300, B500, and B700 were performed at an application rate of 3% w/w (referred to as biochar/soil) with homogenous mixing of biochar and soil. Following mixing, 10 g of control and amended soils were weighed into 100-mL serum vials in triplicate, and then saturated with 15 mL of sterile ultrapure water (~2 cm water depth). After sealing with butyl rubber septa and crimping with aluminum caps, headspaces of vials were then saturated with 15 mL of sterile ultrapure water (~2 cm water depth). After sealing with butyl rubber septa and crimping with aluminum caps, headspaces of vials were flushed with ultrahigh purity He to achieve anaerobic conditions. All bottles were incubated at 25 °C for 14 days.

At time intervals of 0.5, 1, 3, 5, 7, 10, or 14 d, concentrations of N₂O and CH₄ in headspace air of the vials were analyzed using a robotized sampling and analyzing system (Molstad et al., 2007), followed by destructive sampling of soil slurry by separating soil and solution in a
23. Soil archael and bacterial community analyses using Illumina sequencing

To identify the role of soil microbial community compositions in the greenhouse gas emissions with biochar application, genomic DNA in the soil samples collected at day 14 was extracted using a FastDNA SPIN Kit. Archaeal 16S rRNA gene amplifications were performed utilizing primers [519F (5′-CAGCCCGCCGCCTAA-3′), 806R (5′-GGACTACNSGGTMTCTTAA-3′)] that target V4 region with the thermal profile of an initial denaturation step at 95 °C for 5 min, 40 cycles of 95 °C for 30 s (denaturing), 60 °C for 30 s (annealing), 72 °C for 1 min (extension), followed by a final extension step for 5 min at 72 °C. Bacterial 16S rRNA gene amplifications were completed using primers [515F (5′-GTG CCACCMGCCGCGG-3′), 907R (5′-CCGTCAATTCCMTTTRAGTTT-3′)] that target V4-V5 region with the thermal profile of an initial denaturation step at 94 °C for 5 min, 30 cycles of 94 °C for 30 s (denaturing), 58 °C for 1 min (annealing), 72 °C for 1 min (extension), followed by a final extension step for 5 min at 72 °C. Equal amounts of PCR products from different samples (barcoded) were mixed, purified, and quantified prior to Illumina sequencing at Beijing Genomics Institute, China (Wang et al., 2015).

2.4. Data processing and statistical analyses

Illumina sequencing data were processed according to Caporaso et al. (2010). Operational Taxonomic Units (OTUs) were clustered, and then the overall differences in microbial community composition between biochar amendments were determined using the unweighted UniFrac metric (Lozupone et al., 2005; Wang et al., 2007).

To find out the most important soil parameters that controlled archaeal and bacterial community, redundancy analysis (RDA) was conducted using R software (version 2.14.0) and the community ecology package vegan (2.0–4) (Oksanen et al., 2013). Variance test of significance and enivft function with 999 Monte Carlo permutations were used to identify environmental variables significantly contributed to the variance of soil archaeal and bacterial community. Pearson correlations between gas emissions and soil characteristics were established. A one-way analysis of variance (ANOVA) was conducted to test the differences in soil characteristics among treatments. All statistical analyses employed the IBM SPSS Statistics 23.0.

3. Results

3.1. Basic characteristics of biochar and soil

Biochar varied considerably in characteristics depending on the pyrolysis temperature (Table 1). Biochar pH, EC, surface area, and pore volume generally increased with increasing pyrolysis temperature. For biochar B300, the pH, EC, surface area, and pore volume were 6.93, 20.2 μm² g⁻¹, 4.40 m² g⁻¹, and 0.011 cm³ g⁻¹, respectively, while they increased to 10.8, 87.3 μm² cm⁻¹, 161 m² g⁻¹, and 0.093 cm³ g⁻¹ in biochar B700. The increase in surface area and pore volume was due to increasing amounts and size of micropores from B300 to B700 (Fig. 1). No difference in N content (p > 0.05, average 1.12%) was observed within B300, B500, and B700, while C content decreased from 45 to 36%.

Compared to the biochar, the paddy soil was much lower in pH (5.90 vs. 6.93–10.8) and contents of C (1.13% vs. 35.7–45.0%) but much higher in EC (159 vs. 20.2–87.3 μm² cm⁻¹) (Table 1).

3.2. Changes in soil properties

During the 14-d anaerobic incubation, soil pH remained stable for all treatments (Fig. 2A). However, NH₄⁺, DOC, and Fe³⁺ concentrations increased with incubation time (Fig. 2B–D) while NO₃⁻ decreased to low levels quickly (Fig. S1A). Soil reducible Fe³⁺ concentration gradually declined during the incubation (Fig. S1B).

Compared to control soil, biochar amendments significantly increased soil pH, NH₄⁺, and DOC content, which depended on pyrolysis temperature. Higher pH was observed for soils amended with B500 (7.35–7.58) and B700 (7.67–7.92) than B300 (6.60–6.97) (Fig. 2A). Similarly, amendments of B500 and B700 led to higher NH₄⁺ (32.6–40.6 and 30.7–41.5 mg L⁻¹) in soil solution than B300 (23.1–32.0 mg L⁻¹) (Fig. 2B). The increased soil DOC and NH₄⁺ with biochar amendments may result from the biochar. For DOC, although biochar addition generally increased its concentration, the difference in DOC between amended and control treatments (ΔDOC) showed different temporal variation for different biochars. ΔDOC for B300 increased with incubation from 12.2 mg L⁻¹ at 0.5 d to 22.1 mg L⁻¹ at 14 d, while ΔDOC for B500 and B700 amendments decreased from 27.9 to 8.32 and from 19.1 to 0.23 mg L⁻¹ (Fig. 2C).

Unlike pH, NH₄⁺, and DOC, different biochars had inconsistent influences on soil Fe³⁺ during the incubation period, B300 significantly enhanced Fe³⁺ concentration; however, B500 and B700 addition decreased Fe³⁺ concentration, especially towards the end (Fig. 2D). Biochar had no influence on soil NO₃⁻ or reducible Fe³⁺ (Fig. S1).

3.3. Changes in N₂O and CH₄ emissions

For both control and amended treatments, soil N₂O production peaked at day 1, followed by sharp decrease to low levels with further incubation (Fig. 3A). The production of N₂O was related with available NO₃⁻ concentration. During the initial anaerobic incubation, high levels of NO₃⁻ could be reduced to N₂O by denitrification. However, soon after day 1, abundance of residential nitriﬁers would be sharply decreased, which could not supply NO₃⁻ to maintain N₂O production over a longer time via denitrification. After day 1, the available NO₃⁻ decreased to low levels (Fig. S1A), thus leading to sharp decrease in N₂O emission.

Compared to the control treatment, amendments of B300, B500, and B700 signiﬁcantly decreased the peak N₂O emission from 57.9 to 17.9, 1.28, and 0.59 mg kg⁻¹, respectively, with higher decrease for B500 and B700 (45.4- and 97.7-fold) than B300 (3.23-fold).

In contrast to N₂O, soil CH₄ emission occurred after day 3, then continually increased to 58.2, 93.4, 62.6, and 63.4 mg kg⁻¹ at the end of incubation for control soil, and soils amended with B300, B500, and B700,
respectively (Fig. 3B). Emission of CH4 after day 3 might be related with release of DOC during the anaerobic incubation (Fig. 2C). Low levels of DOC in the early incubation period might not support growth of methanogens.

Compared to control, soil CH4 production was generally enhanced with biochar addition, and the increase was significantly (p < 0.05) higher for B300 (1.60–2.25 times) than B500 (1.07–1.39 times) and B700 (1.05–1.66 times) amendments.

3.4. Influence of biochar on bacterial and archaeal community composition

The major compositions of archaeal community in the paddy soil were MCG (Miscellaneous Crenarchaeota Group), Thaumarchaeota, and Methanobacteria at class level (Fig. 4A). Compared to control soil (21.4%), B300, B500, and B700 amendments increased the abundance of MCG to 26.5, 28.8, and 45.7%, respectively. In contrast, abundance of Methanobacteria decreased from 64.0 to 33.2%.

The major compositions of bacterial community were Actinobacteria, Bacilli, and Clostridia at class level, with Clostridia being the dominant (Fig. 4B). B300, B500, and B700 amendments increased the abundance of Clostridia from 33.3% for control soil to 40.6, 48.2, and 50.6%, while they decreased the abundance of Bacilli from 16.9 to 13.6, 10.0, and 7.07%, respectively.

The major compositions of archaeal and bacterial community at genus level are shown in Fig. S2. Candidatus Nitrososphaera and Methanobacterium were the dominant archaea, while Bacillus and Symbiobacterium were the dominant bacteria. Biochar amendment decreased the abundance of Methanobacterium from 64.1% for control soil to 58.2, 58.7, and 33.2% for B300, B500, and B700 amendments, while the abundance of Symbiobacterium increased from 23.0 to 28.5, 39.2, and 40.7%, respectively.

Principal component analysis (PCA) based on bacterial and archaeal abundances at genus level showed that biochar amended soils were separated from control soils (Fig. S3), indicating biochar amendment significantly changed soil archaeal and bacterial community composition.

Based on envfit function (999 permutations), pH, NH4+, and Fe2+ were identified as variables that significantly contributed to the variance of soil archaeal and bacterial community. RDA analysis showed that these factors together explained 70.4% and 85.9% variation of soil archaeal and bacterial community composition, respectively (Fig. 5).

Table 1

<table>
<thead>
<tr>
<th>Soil/biochar</th>
<th>pH</th>
<th>Electrical conductivity (μS cm⁻¹)</th>
<th>C (%)</th>
<th>N (%)</th>
<th>BET surface area (m² g⁻¹)</th>
<th>Pore volume (cm³ g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>5.90 ± 0.01ab</td>
<td>159 ± 1.20b</td>
<td>1.13 ± 0.01b</td>
<td>0.11 ± 0.00b</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B300</td>
<td>6.93 ± 0.11b</td>
<td>20.2 ± 2.30c</td>
<td>45.0 ± 0.01a</td>
<td>1.11 ± 0.02a</td>
<td>4.40</td>
<td>0.011</td>
</tr>
<tr>
<td>B500</td>
<td>10.5 ± 0.15a</td>
<td>28.4 ± 1.11a</td>
<td>43.7 ± 0.02b</td>
<td>1.13 ± 0.01a</td>
<td>68.1</td>
<td>0.054</td>
</tr>
<tr>
<td>B700</td>
<td>10.8 ± 0.14a</td>
<td>87.3 ± 3.23a</td>
<td>35.7 ± 0.01c</td>
<td>1.11 ± 0.02a</td>
<td>161</td>
<td>0.093</td>
</tr>
</tbody>
</table>

Different upper letters indicate significant (p < 0.05) differences.

4. Discussion

4.1. Variation in biochar characteristics with pyrolysis temperature

In this study, we observed that surface area and pore volume of biochar increased with increasing pyrolysis temperature, consistent with previous observations (Chun et al., 2004; Keiluweit et al., 2010). The increased surface area and pore volume provided more adsorption sites and space for growth of bacteria involved in electron transfer between bacteria and insoluble electron acceptor, thus serving as a stable platform for biofilm formation to support electron transfer (Briones, 2012). The increase in biochar EC with increasing pyrolysis temperature further implied biochar produced with higher temperature was more effective in electron transfer. In addition, pH of biochar increased with temperature, consistent with previous studies (Jie et al., 2015; Subedi et al., 2016). This was due to release of alkali salts from feedstock during the pyrolysis process. Yuan et al. (2011) showed that carbones were the major alkaline components in the biochar generated from crop straws at high temperature. With increasing pyrolysis temperature from 300 to 700 °C, contents of total base cations, soluble base cations, exchangeable base cations, and carbones in biochar increased, contributing to increased pH of biochar. Acid-base titration curves of the
biochar showed increased HCl consumption with pyrolysis temperature (Yuan et al., 2011). In addition, decrease of acidic functional groups such as —COOH with pyrolysis temperature is another contributor to the increased pH of biochar.

4.2. Effects of biochar amendments on N2O emission

In this study, biochar amendment significantly suppressed N2O emission at day 1 (Fig. 3A). However, studies on the effects of biochar on N2O emission are inconsistent, with decreased N2O emission being reported in some (Martin et al., 2015; Nelissen et al., 2014; Rondon et al., 2016).

4.2.1. Nitrous oxide (N2O) production

Fig. 3. Emissions of nitrous oxide (N2O, A) and methane (CH4, B) during 14-d anaerobic incubation of paddy soil without (Control) and with biochar produced from rice straw at 300, 500, and 700 °C (B300, B500, and B700). Each point represents the mean and standard deviation of triplicate analyses.

In this study, biochar amendment significantly suppressed N2O emission at day 1 (Fig. 3A). However, studies on the effects of biochar on N2O emission are inconsistent, with decreased N2O emission being reported in some (Martin et al., 2015; Nelissen et al., 2014; Rondon et al., 2016).

4.2.2. Methane (CH4) production

4.2.3. Nitrous oxide (N2O) and methane (CH4) production

Fig. 4. Class distribution of archael (A) and bacterial (B) community composition in soil samples collected after 14-d anaerobic incubation of paddy soil without (Control) and with biochar produced from rice straw at 300, 500, and 700 °C (B300, B500, and B700). Other for archael includes the taxonomically-unassigned sequences at class level. Other for bacteria includes the low abundance bacteria (≤0.6%) and the taxonomically-unassigned sequences at class level. MBGA represents Marine Benthic Group A; MCG represents Miscellaneous Crenarchaeota Group. Each column represents the mean value of triplicate analyses.
N2O can be the final or intermediate product of denitrification, as well as improved soil properties (Ameloot et al., 2013) as well as protecting microorganisms from predators and other environmental stresses. Biochar with higher surface area and pore volume could serve as a habitat, protecting microorganisms from predators and other environmental stresses. However, biochar addition in paddy soils could alter microbial community composition and regulate microbial N-turnover, thus affecting the microbial community composition with biochar addition significantly increased N2O reducing bacteria (such as nosZ-denitrifying bacteria), leading to reduction in N2O emission by enhancing its reduction to N2. Further analysis of functional genes controlling N cycling could help to understand the microbial mechanisms of the biochar influence.

4.3. Effects of biochar amendments on CH4 emission

In this study, CH4 emission was higher with biochar amendments, especially with biochar produced at low temperature (Fig. 3B). This is consistent with the report of 37% increase with biochar amendment (Wang et al., 2012), but different from the report of decreased CH4 emission (Karhu et al., 2011). The reduction in CH4 emission in Karhu et al. (2011) was because that biochar increased soil aeration, thereby increasing CH4 oxidation through stimulating the growth of methanotrophs, an aerobic proteobacterial group utilizing CH4 as the sole C source (Van Zwieten et al., 2009). However, in this study, anaerobic incubation was used, which could not support the growth of methanotrophs.

Methane emission from anaerobic paddy soil is the net result of CH4 production and oxidation, which can be regulated by various factors including microbial community composition (Farrell et al., 2015; Mer and Roger, 2001; Mitchell et al., 2015; Prayogo et al., 2014). The obvious increase in CH4 emission for B300 treatment might be related with its higher soil DOC (Fig. 2C), which can act as potential substrates for methanogens to produce CH4 (Tiedje, 1982). However, Xu et al. (2014) reported that biochar amendment significantly increased N2O reducing bacteria, as well as the net N2O emission (Fig. 3A). An important factor regulating soil N2O emission is the availability of soil labile organic C, which serves as the energy and C source for heterotrophic denitrifiers (Gärdenäs et al., 2011). In addition, NH4 + and Fe2+ are also important factors controlling soil N2O emission by serving as available substrates for microbial growth (Chu et al., 2007). However, in this study, soil amended with biochar showed higher available substrates (e.g., DOC, NH4 +, and Fe2+) for denitrifying microbes, but with decreased N2O emission, suggesting that these available substrates might not be the main factors controlling N2O emission. However, a negative association between N2O emission and soil pH was observed, suggesting that biochar-induced pH increase might be the main contributor to the reduced N2O emission. In previous studies, pH is a primary variable in controlling soil microbial community (Shen et al., 2013). Increased soil pH could stimulate the N2O reductive enzymatic activity of denitrifying bacteria (Liu et al., 2010), while suppressing the activity of reductase enzymes responsible for conversion of NO3 − and NO2 − to N2O (Zhang et al., 2010). In this study, biochar treatment significantly decreased the abundance of Bacilli, several are known as denitrifying bacteria to produce N2O (Tiedje, 1982). However, Xu et al. (2014) reported that biochar amendment significantly increased N2O reducing bacteria, as well as the labile organic C source (Van Zwieten et al., 2009). However, in this study, anaerobic incubation was used, which could not support the growth of methanotrophs.
A possible inhibition mechanism for methanogens might be competition for H₂ or acetate between methanogens and ferric iron reducers when biochar with higher surface area was added (Lovley and Phillips, 1987b). Following biochar addition, a significant increase in relative abundances of Fe₃₈⁻ reducing bacteria with biochar was observed, such as Clostridium (Weber et al., 2006a), and the increase was higher for B500 and B700 (Fig. 4B). The higher surface area of biochar produced at higher temperature had a greater ability to support electron shuttling between microbes and insoluble electron acceptors such as Fe₃₈⁻ (oxyhydr)oxide and promote Fe₃₈⁻ reduction (Kappler et al., 2014), which might compete for electron with CH₄ production, leading to decreased CH₄ emission. However, lower FeII concentrations in soil amended with B500 and B700 treatments than control treatment were observed (Fig. 2D). It was possible that the released FeIIO ions were re-adsorbed by the biochar. Metal sorption capacity of biochar has been shown to increase with increased surface area (Ahmad et al., 2013; Xu et al., 2013). In addition, previous studies have shown that biochar can sorb CH₄ given its larger surface area (Chun et al., 2004; Schimmelpfennig and Glaser, 2012). The higher surface area of B500 and B700 might be another factor leading to lower CH₄ emission for B500 and B700 than B300 treatments.

5. Conclusion

Biochar amendment played a vital role in controlling soil microbial community and emissions of greenhouse gases from an anaerobic paddy soil. The present study showed that the biochar-induced changes in N₂O/CH₄ emissions depended on biochar, helping to understand the impact of biochar amendment on soil microbial composition and environmental functionality. Further quantitative analysis on the interaction between microbial communities and elements (C, N, and Fe) cycling in paddy soils might better predict the change in the microbial community and N₂O/CH₄ emissions from paddy soils. In addition, to mitigate emissions of both N₂O and CH₄ from anaerobic paddy soil, amendment of biochar with higher surface area and pore volume produced at higher temperature is recommended. This was mainly due to its greater inhibition of N₂O emission with higher pH, promoting DOC consumption by Fe-reducing bacteria. However, higher temperature implies greater energy cost. Considering that B500 and B700 did not differ significantly in their effects on N₂O and CH₄ emission, biochar produced at 500 °C might be a best choice for field application. In short, biochar can be promising to mitigate greenhouse gas emissions from paddy soil.

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Appendix A. Supplementary data

The changes in soil NO₃⁻ and reducible FeIII, abundances of archaeal and bacterial at genus level, and principle component analysis of archaeal and bacterial community composition are provided in the Supplementary data. Supplementary data associated with this article can be found in the online version, at 10.1016/j.jsctenv.2016.12.181.

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