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Effects of Biochar Particle Size on Methane Emissions from Rice Cultivation

Patikorn Sriphirom[1,2], Amnat Chidthaisong[1,2,3], Kazuyuki Yagi[1,2], Nimaradee Boonapatcharoen[4], Sudarut Tripetchkul[5] and Sirintornthep Towprayoon[1,2,3,*]

[1]The Joint Graduate School of Energy and Environment, King Mongkut's University of Technology Thonburi, Bangkok, 10140, Thailand
[2]Center of Excellence on Energy Technology and Environment, PERDO, Ministry of Higher Education, Science, Research and Innovation, Bangkok, 10400, Thailand
[3]Earth System Science Research Cluster, King Mongkut's University of Technology Thonburi, Bangkok, 10140, Thailand
[4]Excellent Center of Waste Utilization and Management, Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bangkok, 10150, Thailand
[5]School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok, 10150, Thailand
*Corresponding Author: Sirintornthep Towprayoon. Email: sirin@jgsee.kmutt.ac.th
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Abstract: Biochar amendment is generally recognized as an effective mitigation option of methane (CH_4) emissions from rice cultivation. Although its mitigation mechanisms are not well understood, the potential relevance of surface area and porosity of biochar has been discussed. This study aimed to evaluate the application of different biochar particle sizes on CH_4 production, oxidation, and emissions from rice cultivation in a clay loam soil, based on the assumption that porosity and surface area of biochar are directly related to its mitigation effects. Rice was grown under greenhouse conditions for two growing seasons, either with 0.5–2 mm (small, SB) or with 2–4 mm (large, LB) biochar. The results show that both sizes of biochar increased soil pH and redox potential (Eh) during rice growth. Soil dissolved organic carbon (DOC), nitrate (NO_3^-), and sulfate (SO_4^{2-}) also increased under both biochar amendments, but size effects were not observed. SB and LB suppressed the abundance of CH_4 producers (methanogens) but stimulated the abundance of CH_4 consumers (methanotrophs). The increase of soil Eh and electron acceptors (NO_3^- and SO_4^{2-}) indicated the increase in soil oxidation capacity is a barrier to CH_4 production by methanogens in both biochar treatments. Laboratory incubation experiments showed that CH_4 production activity was significantly ($p \leq 0.05$) reduced by 18.5% using SB and by 11.3% using LB compared to the control. In contrast, the stimulation of methanotrophs promoted greater CH_4 oxidation activity by 15.0% in SB and 18.7% in LB compared to the control. It shows that CH_4 production was reduced more by larger surface area biochar (SB), while a greater increase in CH_4 oxidation was found using larger pore volume biochar (LB). The effects on CH_4 production were more pronounced than those on CH_4 oxidation, resulting in a greater reduction of cumulative CH_4 emissions by SB than LB (by 26.6% and 19.9% compared to control, respectively).

Keywords: Biochar particle size; CH_4 production; CH_4 oxidation; CH_4 emission; rice cultivation

1 Introduction

Biochar is a by-product of the pyrolysis of biomass with potential utility in various environmental stewardship activities. Recent studies indicate that using biochar as a soil amendment can enhance soil carbon sequestration and mitigate greenhouse gas (GHG) emissions, particularly methane (CH_4), from rice cultivation [1–3]. While substantial GHG mitigation potentials (33.8–91.2%) have been widely reported [3–5], the process and mechanisms of interactions between biochar-soil-plant-microbes are largely unknown. Feng et al. [4], Wang et al. [6] and Wang et al. [7] have reported that CH_4 emissions are mitigated by biochar through reduction of the ratio of methanogens to methanotrophs. However, different effects were observed when biochar was applied to different soil types [4] or applied at different application rates [7]. Several studies have discussed the mechanism in relation to a unique property of biochar, specifically, the large surface area and porosity [3,8,9]. This feature can enhance the absorption and availability of various electron acceptors such as nitrate (NO_3^-), sulfate (SO_4^{2-}), manganese oxide (MnO_2), and iron (III) oxide (Fe_2O_3) [10–14]. These are the major electron acceptors used by microorganisms that compete with methanogens [15]. Biochar may also increase soil oxygen availability through improved aeration [16], thereby inhibiting CH_4 production [17,18]. Biochar's porosity and surface area can also provide habitat or refuge for soil microbes, particularly aerobes [19]. Feng et al. [4] applied biochar of various surface areas obtained under pyrolysis temperatures onto rice field soil. They found that among all treatments, the biochar with the highest surface area (produced at 500°C) provided the highest CH_4 mitigation. However, the relationship between the surface area of biochar and CH_4 mitigation was not elucidated. Wang et al. [20] also showed the effects of different surface areas and pore volumes produced at pyrolysis temperatures on CH_4 production in a soil incubation experiment. They reported a negative relationship between the surface area of biochar and CH_4 production, i.e., biochar amendments induced higher CH_4 production than the controls, leading them to conclude that biochar promoted higher CH_4 emissions. The inconsistencies among these results suggest the need for further study to clarify the interactions between biochar and mitigation effects of CH_4 in rice cultivation.

The possible mechanisms of biochar mitigation of CH_4 emissions are related to its porosity and surface area [20]. A large surface area is required to have large mitigation effects [4,20,21]. Biochar with a large surface area is usually produced at temperatures above 400°C, and surface area increases when pyrolysis temperature is increased [22]. In addition to the additional energy requirement, from an agricultural perspective, biochar produced at high temperatures has other disadvantages [22–24]. For example, high temperature (>800–900°C) reduces biochar yield [22] and reduces some desirable soil amendment features (cation exchange capacity [CEC] and nutrients). Gul et al. [24] and Yoo et al. [25] recommended approximately 600°C as the proper temperature to produce biochar to be used as a soil amendment with minimal labile carbon. Labile carbon can be used as a methanogenic substrate, thereby stimulating CH_4 formation [16,20]. Therefore, fixing the temperature of pyrolysis at 600°C may reduce CH_4 emissions and promote the use of biochar for this purpose. In addition, the biochar's surface area can also be manipulated through biochar particle size selection. Chen et al. [19] and Jaafar et al. [26] reported 49.6% and 98.6% surface area increases for Wundowie and bamboo biochar could be achieved when the particle size was changed from 2–4 mm to 0.5–1 mm and 1–2 mm to 0.05–1 mm, respectively. This manipulation is much easier to implement than modifying pyrolysis temperature, with no need for additional chemicals or energy inputs [27]. A straightforward method is needed for the adoption by biochar users (farmers). In this study, different surface areas and porosities occurring as a result of different particle sizes of biochar were evaluated for their ability to change CH_4 production and oxidation in rice cultivation soil and to explain the mechanisms of CH_4 mitigation by biochar in relation to its particle sizes.

2 Material and Methods

2.1 Soil and Biochar Properties

Soil samples were collected from Damnoen Saduak District, Ratchaburi Province (13˚31′07″ N, 99˚58′43″ E) and classified as Vertisols (Endoaquerts) (Ratchaburi: Rb Thai soil series) with a clay loam texture (Tab. 1). Fresh soil was directly transported from its original location to the study site and laboratory at the Bangkhuntien Campus of King Mongkut's University of Technology Thonburi (KMUTT), Bangkok, Thailand. Soil was transferred into a polyethylene bucket (60 cm [width] × 90 cm [length] × 60 cm [depth]) until its depth in a bucket was 50 cm. Biochar was produced from mangrove (*Rhizophora apiculata*) wood at 600°C by pyrolysis using a traditional kiln in the Yisan community, Amphawa District, Samut Songkhram Province, Thailand. The basic properties of this biochar are provided in Tab. 1. Biochar was crushed by a cutting mill and sieved to particle size groups of 0.5–2 mm (small) and 2–4 mm (large) prior to incorporation with soil in the bucket. Automated gas sorption analyzer (Quantachrome Autosorb iQ-MP/XR, USA) was used to determining surface area and pore volume of biochar. The specific surface areas of small and large size particles were 88.5 and 34.8 $m^2\ g^{-1}$, respectively, while the specific pore volumes were 0.61 and 1.01 $cm^3\ g^{-1}$, respectively, as shown in Tab. 1.

Table 1: The basic properties of biochar and soil prior to use with rice cultivation

Parameter (unit)	Soil	Biochar size group	
		0.5–2 mm	2–4 mm
pH [H_2O]	7.00	7.8	
OM (%)	1.03	–	
EC (dS m^{-1})	0.85	1.47	
CEC (cmol kg^{-1})	23.0	54.4	
Total C (%)	0.60	58.5	
Total N (%)	0.06	0.28	
Total P (%)	–	0.23	
Total K (%)	–	0.18	
Total Mg (%)	–	1.12	
Available P (mg kg^{-1})	9.47	–	
Available K (mg kg^{-1})	88.0	–	
Available Mg (mg kg^{-1})	309	–	
Bulk density (g cm^{-3})	1.54	–	
Specific surface area ($m^2\ g^{-1}$)	–	88.5 ± 3.16 a	34.8 ± 2.72 b
Specific pore volume ($cm^3\ g^{-1}$)	–	0.61 ± 0.02 b	1.01 ± 0.02 a
Specific micropores volume ($cm^3\ g^{-1}$)	–	0.07	0.03
Specific mesopores volume ($cm^3\ g^{-1}$)	–	0.29	0.02
Specific macropores volume ($cm^3\ g^{-1}$)	–	0.25	0.96
Average pore diameter (nm)	–	18.5	51.6

Different letters indicate the significant difference of specific pore volume and surface area of both particle sizes of biochar prior to the experiment conducting (±S.D. of n = 3)

2.2 Rice Cultivation and Experimental Design

Mangrove biochar at a rate equivalent to 10 t ha^{-1} $season^{-1}$ (0.90 kg) was thoroughly mixed with the soil in the bucket 16 days before rice cultivation. The seed of rice (*Oryza sativa* L.) cultivar Pathumthani 1 was sown at a rate of 62.5 kg ha^{-1} $season^{-1}$. Water was kept level to the soil surface 1–19 days after sowing (DAS), then flooded to 10 cm above soil surface until approximately 90 DAS when water was naturally evaporated to prepare for harvest. Compound fertilizer ($N–P_2O_5–K_2O$: 15–15–15) was applied at a rate of 233 kg ha^{-1} $season^{-1}$ at 20 DAS and urea (CH_4N_2O: 46–0–0) was applied for top dressing at a rate of 141 kg ha^{-1} $season^{-1}$ on 60 DAS. Rice was cultivated for two seasons (the first season was August to December 2018 and the second season, February to June 2019) inside a greenhouse. After harvesting the first season rice, aboveground biomass was removed from the bucket before the soil of the second season was added with biochar at the same rate, so the total biochar concentration in the soil during the second season was double that of the first season. The experiment was conducted with three treatments: control (CT) with no biochar, small particle size biochar (SB), and large particle size biochar (LB) in a randomized complete block design with three replications. All treatments were subject to the same practices throughout the cultivation seasons. The time from sowing to harvest of each crop was 110 days.

2.3 Soil Sampling and Analysis

Soils were sampled and analyzed for pH and redox potential (Eh) using an ion-selective electrode, pH/ORP combination sensor (YSI Professional Plus, USA) throughout the cultivation season. Soil samples were collected at three critical growth stages: tillering, maximum tillering and heading stages, for analysis of DOC, NO_3^-, and SO_4^{2-}. Triplicate soil samples were collected at 29, 45, and 70 DAS in the first season and 29, 45, and 66 DAS in the second season using a sterilized polyvinylchloride (PVC) pipe (internal diameter of 2.54 cm) at 0–15 cm depth. The soil was extracted by 0.5 mol l^{-1} K_2SO_4 and DOC concentration determined by an automated TOC Analyzer (Multi N/C 2100, Jena, Germany) with non-dispersive infrared detection [28,29]. For the analysis of soluble SO_4^{2-} and NO_3^-, the soil supernatant solution was separated by centrifugation at 8,000 rotations per minute (rpm) for 15 mins. The solution was analyzed for SO_4^{2-} and NO_3^- concentrations by ion chromatography (IC, Thermo Scientific, Dionex Integrion HPIC system 2016, USA) as described in Morales et al. [30].

2.4 Measurements of CH_4

2.4.1 CH_4 Production and Oxidation Activities

Fresh soil samples (10 g) were incubated in the laboratory to determine CH_4 production and oxidation activities. For the CH_4 production study, the soil was mixed with 10 ml of a 0.2 mM glucose solution in a 100 ml glass vial. The headspace of the vial was flushed with N_2 (99.999%) gas for 3 mins to remove O_2 and to simulate anaerobic conditions [29,31,32]. For the CH_4 oxidation study, the soil was mixed with 2.5 ml of distilled–sterile water under ambient conditions, and the vial headspace was injected with 5 ml of CH_4 (99.99% purity) gas according to the methods of Hanson [33] and Chan et al. [34]. All vials were covered with gas-tight butyl rubber septa and aluminum caps and incubated at room temperature (around 28–30°C) for 24 hours. The concentration of CH_4 was analyzed using a gas chromatograph (GC) (Shimadzu GC–2014, Japan) equipped with a flame ionization detector (FID) and a Unibead C Packed column (Shimadzu MTN–1, Japan). CH_4 production and oxidation were calculated by the increase and decrease of CH_4 concentration in the vial's headspace over the sampling time and presented in units of micromoles CH_4 per soil mass per unit of time [29,31,35].

2.4.2 CH_4 Emissions

CH_4 emissions from rice cultivation were determined by the closed rectangular chamber method [36]. The chamber was made from opaque black acrylic and covered with a 1.5 cm thick white polystyrene foam sheet for thermal insulation. Chambers 0.3 m wide and long with three heights (0.3 m, 0.6 m, and 1.2 m) were

used for different plant heights. An air sample inside the chamber's headspace was collected with a plastic syringe at 0, 5, 10, 15, and 20 mins after chamber closure. After sampling, air samples were immediately injected into the pre-evacuated vials and sealed with laboratory film. CH_4 concentrations in the samples were determined by the same GC method, as described above. The CH_4 flux was calculated by the equation given in Minamikawa et al. [36]. The seasonal accumulations of CH_4 emissions were computed by consecutive linear interpolation and numerical integration between gas sampling days [37].

2.5 Microbial Analysis

2.5.1 Soil DNA Extraction

DNA was extracted from 10 g soil samples according to the method described by Zhou et al. [38] and using magnetic beads (Agencourt AMPure XP; Beckman, USA) for DNA purification. The concentration and quality of extracted DNA were determined by the optical density (OD) technique at 260 nm using a Nanodrop ND–1000 spectrophotometer (Wilmington, USA) and checked by an agarose gel electrophoresis. The extracted DNA was then stored at –30°C until further use.

2.5.2 Real-Time Quantitative Polymerase Chain Reaction (qPCR)

The extracted DNA was analyzed for the abundances of methanogenic archaea and methanotrophic bacteria. The primer set of mcrA–f/mcrA–r [39,40] and A189–f/mb661–r [40,41] were used to amplify methyl coenzyme M reductase (*mcr*A) and particulate methane monooxygenase (*pmo*A) genes for the quantification of the copy number of methanogens and methanotrophs, respectively. The copy number of the target gene was quantified using an ABI Prism 7900HT system (Applied Biosystems, CA, USA). The real–time PCR amplification specifications for the *mcr*A gene were 95°C for 3 min; 5 cycles of 95°C for 25 s, 48°C for 45 s, and 72°C for 30 s; 35 cycles of 95°C for 15 s, 55°C for 30 s, and 72°C for 30 s. The thermal cycling conditions for the *pmo*A gene were 95°C for 3 min; 5 cycles of 95°C for 25 s, 65°C for 30 s, and 72°C for 30 s; 35 cycles of 95°C for 25 s, 55°C for 30 s, and 72°C for 30 s. Each reaction of real-time PCR was performed in a 20 µl mixture that contained 10 µl of 2 × SYBR Green PCR Master Mix (TaKaRa, Shiga, Japan), 0.4 µl each of the forward and reverse primers (0.4 µM), 1.0 µl of template DNA (20 ng), and 8.2 µl of sterile water. Standard curves were generated using 10–fold dilution series of plasmid DNA with the target genes covering five orders of magnitude from 10^3 to 10^8 copies of template per assay. The reaction efficiencies were 104.5–106.0% and the coefficient R^2 values were 0.997–0.999. The results were compared with the nucleotide sequence of each functional gene in the database of the National Center for Biotechnology Information (NCBI). The accession number (GenBank) of the methanogenic archaeal sequenced in this study was KF836867, while methanotrophic bacteria were AB538965 and U31650.

2.6 Rice Growth and Yield Measurement

The growth of rice was determined by manual height measurements (using a measuring tape) at each growth stage at the same time soil sampling was conducted. On the harvesting date, the roots length and aboveground biomass were measured. Grain yields from each bucket were collected and weighed. All parameters were measured in triplicate.

2.7 Statistical Analysis

One-way analysis of variance (ANOVA) was applied for the statistical analysis, and the significance of the difference was determined by applying the Tukey HSD test [42]. The statistical analysis was conducted using SPSS version 22 at a confidence level of 95%, and significance was assigned at $p \leq 0.05$. The data were presented as mean ± standard error.

3 Results and Discussion

3.1 Effects of Particle Sizes of Biochar on CH_4 Production

CH_4 is generally produced under an anoxic environment in the final step after the reduction of all electron acceptors [15] because methanogens are less capable of capturing the electron donors than other reducing bacteria [43,44]. The existence of other electron acceptors (or other reducing bacteria), therefore, inhibits CH_4 production [15]. In addition, CH_4 is produced within a specific pH range [45,46], approximately 6.5–8.0 [47]. Methanogens are reportedly sensitive to changes in soil pH [48]. In this study, however, soil pH changes occurring after both biochar amendments did not affect the activity of methanogenic archaea because they only increased soil pH by an average of 0.5 units, still within the optimal range for methanogen growth throughout both growing seasons. No significant difference in soil pH between SB and LB was observed (Figs. 1a and 1b).

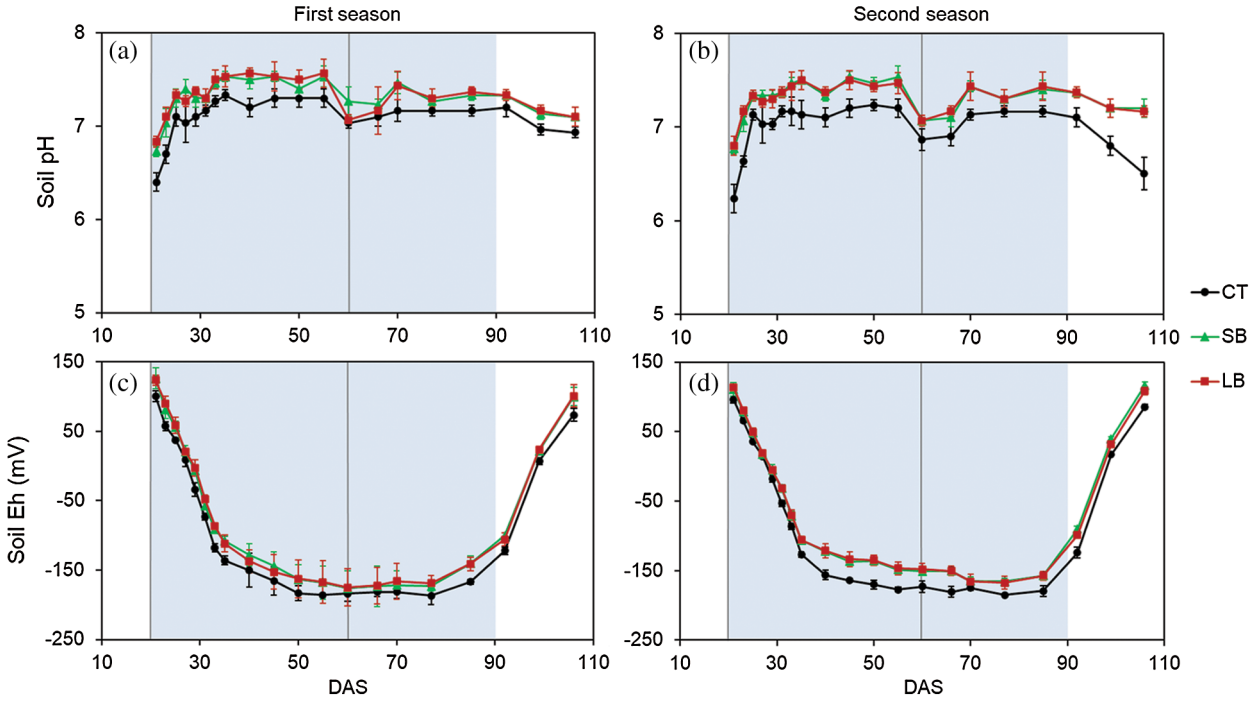

Figure 1: Soil pH (a,b) and Eh (c,d) throughout both cultivation seasons. Blue backgrounds indicate the flooded period. Vertical lines indicate the application timing of fertilizer. CT is soil with no biochar, SB is application of small particle size biochar, and LB is application of large particle size biochar

Similar to soil pH, both particle sizes of biochar affected soil redox potential (Figs. 1c and 1d). Soil Eh remained significantly ($p \leq 0.05$) higher in SB and LB than the CT in some of the rice growth stages. Significant ($p \leq 0.05$) differences in soil Eh between SB and LB were not found. The increase in soil Eh was consistent with the higher concentration of nitrates and sulfate (Figs. 2a–2d). The nitrate increase in the biochar treatment groups was significant ($p \leq 0.05$) on all sampling days in the first season and at 29 DAS in the second season compared to no biochar treatment. Similar results were also reported by Dong et al. [12], who found higher NO_3^- concentrations in the soil during rice cultivation season by biochar amendment. Biochar enhances the availability of electron acceptors by promoting the growth of oxidizing bacteria [19,49], thereby increasing concentrations of NO_3^- and SO_4^{2-} through the stimulation of nitrification and sulfur oxidation processes. The concentrations of these electron acceptors were slightly higher in SB than LB, but these differences were not statistically significant ($p \leq 0.05$). The

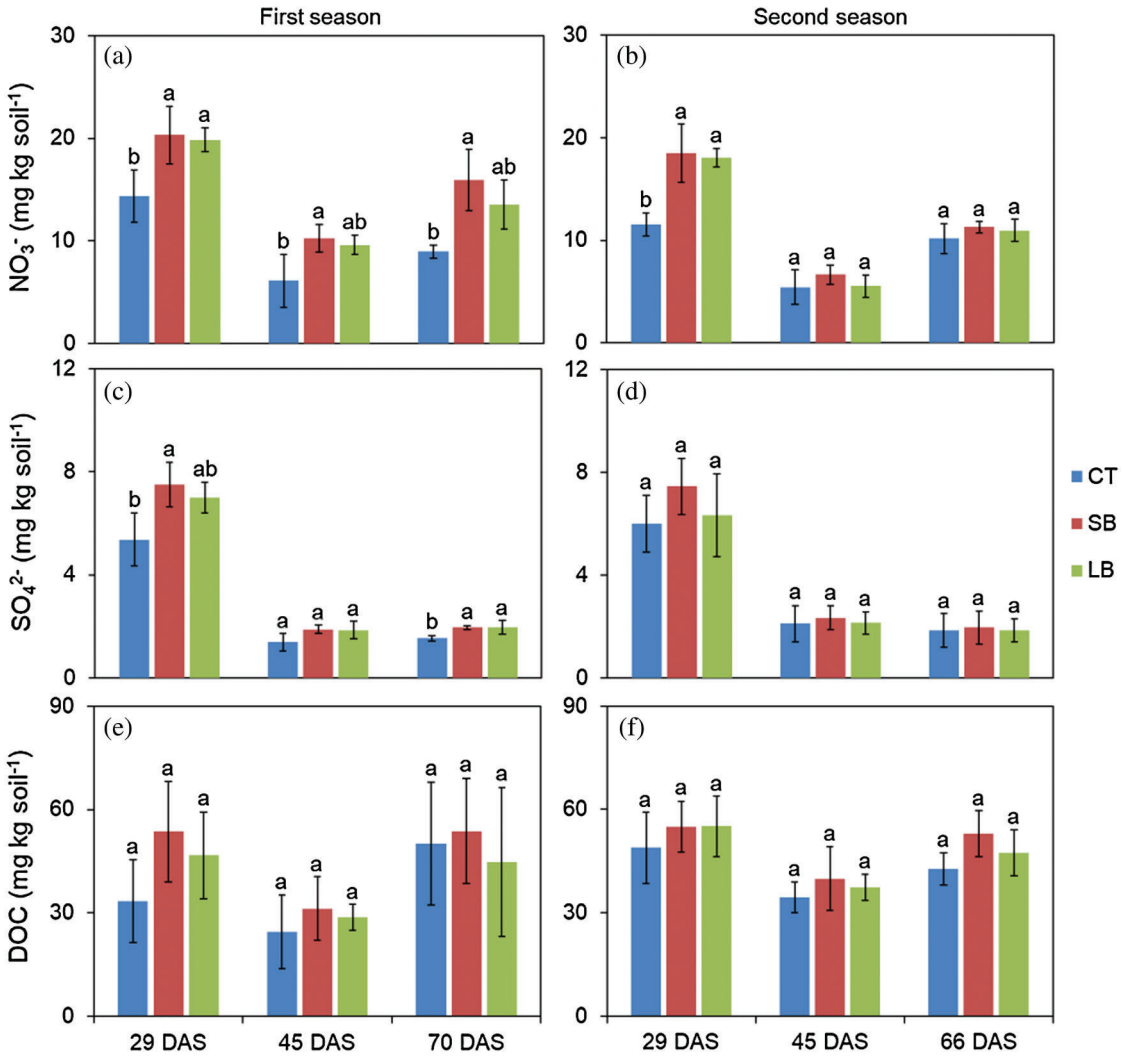

Figure 2: Concentration of NO_3^- (a,b), SO_4^{2-} (c,d), and DOC (e,f) in rice cultivation soil at each stage of rice growth. CT is soil with no biochar, SB is application of small particle size biochar, and LB is application of large particle size biochar. The different letters indicate significant difference among treatment in each stage of each season

larger specific surface area of SB may allow it to bind and absorb more of these ions than LB [27,50], although less of an effect was demonstrated in this study than we expected. Wang et al. [20] and Briones [51] reported that higher Brunauer–Emmett–Teller (BET) surface area of biochar creates more suitable conditions for electron transfer than lower BET surface area. Higher electron acceptor concentrations and soil Eh found in the biochar treatments are therefore considered one reason for lower CH_4 production.

The main substrate for CH_4 formation by methanogens in anaerobic soil is DOC [52]. To investigate whether biochar addition to soil affects soil carbon dynamics, we measured DOC in soil solution at three stages of rice growth. Due to high DOC variation (large error bars), we concluded that additions of biochar resulted in no statistical difference in DOC from the CT in both cultivation seasons (Figs. 2e and 2f). Previous studies indicate that biochar porosity promoted the decomposition of native soil carbon via stimulation of soil microbes [21]. Chen et al. [19] demonstrated that biochar addition could increase

microbial population abundances and their community structure. We found that DOC in biochar treatments might be utilized by other reducing bacteria rather than by methanogens because of the methanogen's low substrate affinity and low abundance, leading to low CH_4 formation. Despite this, we found high DOC concentrations in biochar treatments (compared to CT), which may be due to biochar's influence on the soil absorption-desorption capacity of DOC [12,13]. This finding is consistent with the study of Feng et al. [4], who proposed that higher DOC in biochar treatments is unlikely to affect CH_4 formation. The significantly ($p \leq 0.05$) lower abundance of methanogenic archaea (*mcr*A gene) in SB and LB than CT was shown in both cultivation seasons (Figs. 3a and 3b). However, Feng et al. [4] showed no change in methanogen abundance under biochar treatments. This result might be due to less favorable growth conditions for methanogens in soil with biochar compared to those in soils without biochar. Such conditions include the relatively higher oxidation-reduction conditions (higher Eh and abundance of electron acceptors, as described above). These effects resulted in less CH_4 production activity in soils with biochar than those

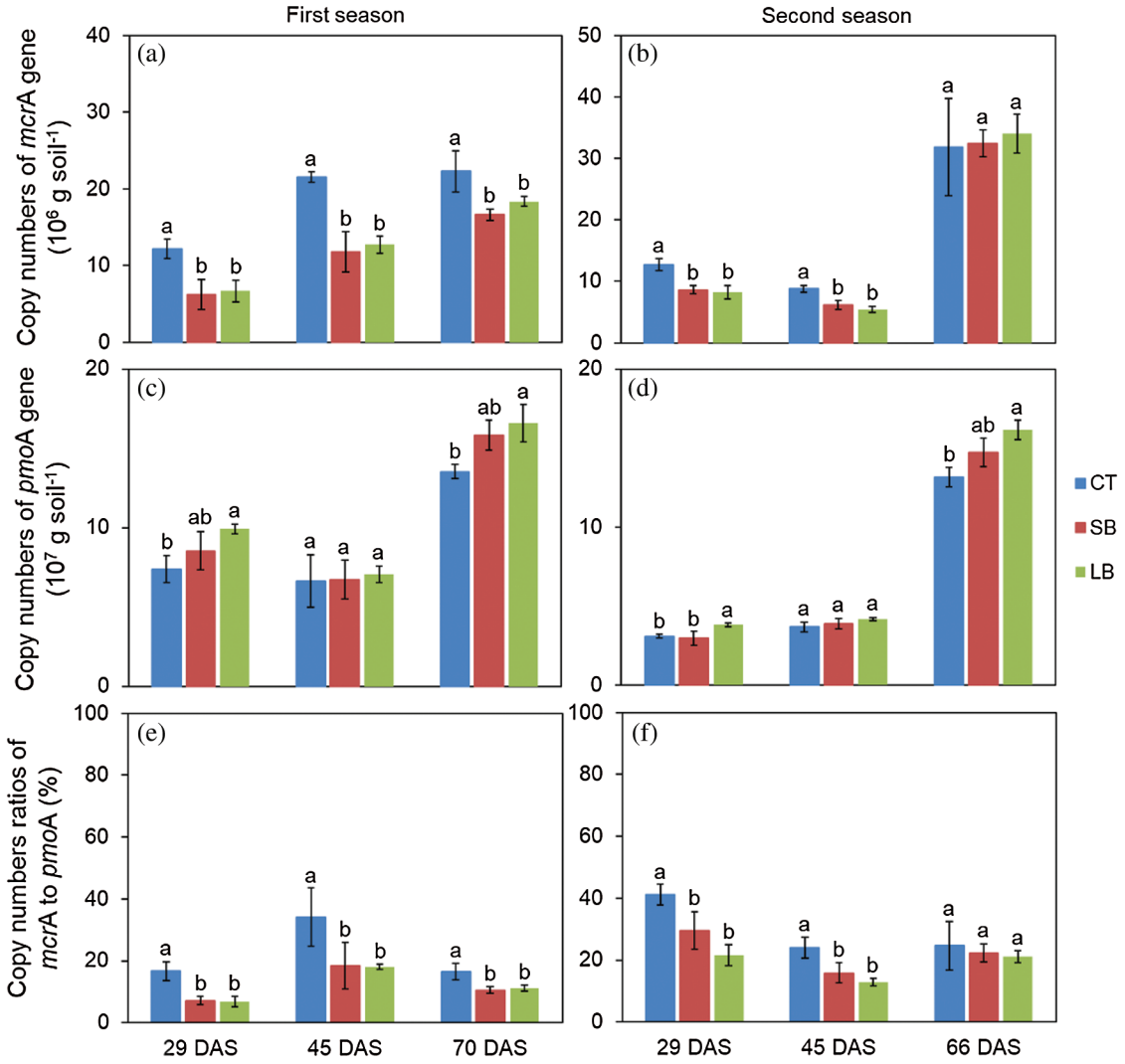

Figure 3: Abundances of methanogens (a,b), methanotrophs (c,d), and ratio of methanogens to methanotrophs (e,f) in the rice cultivation soil at each stage of rice growth. CT is soil with no biochar, SB is application of small particle size biochar, and LB is application of large particle size biochar. The different letters indicate significant difference among treatment in each stage of each season

without biochar. Relative to CT, SB reduced CH_4 production activity by 20.9% and 13.9% at the tillering stage, 19.3% and 22.1% at the maximum tillering stage, and 20.4% and 14.3% at the heading stage in the first and second season, respectively. LB reduced CH_4 production activity by 11.1% and 10.5% at the tillering stage, 12.0% and 17.8% at the maximum tillering stage, and 10.5% and 5.65% at the heading stage in the first and second season, respectively (Figs. 4a and 4b). The lower CH_4 production activity in SB than LB was thus attributed to the active reduction of electron acceptors in SB. The difference in CH_4 production between SB and LB indicates that higher surface area (in SB) resulted in lower CH_4 production activity in the rice cultivation soil; however, this difference was not statistically significant.

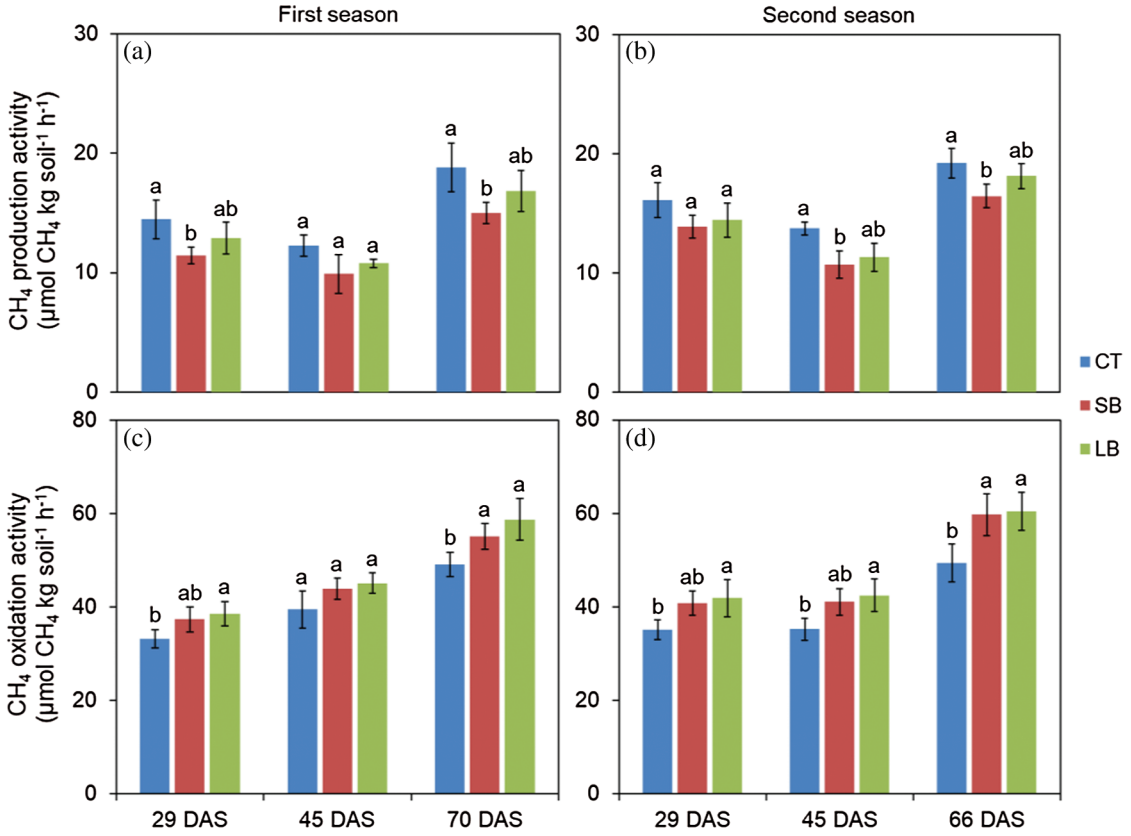

Figure 4: CH_4 production (a,b) and oxidation (c,d) activities in the rice cultivation soil at each stage of rice growth. CT is soil with no biochar, SB is application of small particle size biochar, and LB is application of large particle size biochar. The difference letters indicate significant difference among treatment in each stage of each season

3.2 Effects of Particle Sizes of Biochar on CH_4 Oxidation

Before CH_4 produced by rice cultivation soil is emitted into the atmosphere, some can be oxidized by methanotrophic bacteria in the rhizospheric zone [53,54]. Therefore, increasing CH_4 oxidation capacity can result in lower CH_4 emissions. Consistent with a previous study [4], the application of both biochar sizes significantly stimulated ($p \leq 0.05$) methanotroph growth (*pmo*A gene concentrations), in the tillering (29 DAS) and heading (66 or 70 DAS) stages in both cultivation seasons (see Figs. 3c and 3d). Soil aeration improvement upon biochar application was suggested to be a principal factor in stimulating CH_4 oxidation [4,6]. Another factor may be the stimulation of the growth of facultative methanotrophs, which can directly use DOC as a substrate [55,56]. Considering the impact of biochar sizes, LB was found to

promote a larger abundance of these bacteria than SB on 29 and 70/66 DAS in both growing seasons, although a significant difference was found only on 29 DAS in the second season. A higher methanotroph population in LB–mixed soil than in CT and SB could be explained by greater soil aeration resulting from larger pore volumes, particularly, macropores [20,21] (see Tab. 1). Macropores of biochar are suitable habitat for both methanotrophs and other aerobic bacteria [21,57]. The suppression of methanogens and the stimulation of methanotrophs in both biochar applications are reflected by significantly ($p \leq 0.05$) lower ratios of methanogens to methanotrophs in both cultivation seasons (except on 66 DAS in the second season) than in the CT (Figs. 3e and 3f).

The consumption activity of CH_4 was enhanced in SB and LB by an average of 12.5% and 16.0% on 29 DAS, 11.3% and 14.2% on 45 DAS, and 12.1% and 19.6% on 70 DAS, respectively, compared to the CT in the first season (Fig. 4c). For the second season, these values were 16.5% and 19.4% on 29 DAS, 16.6% and 20.5% on 45 DAS, and 21.1% and 22.7% on 66 DAS, respectively, compared to the CT (Fig. 4d). A higher contribution of CH_4 oxidation in the second season than the first season was probably a result of a higher concentration of biochar in the soil. A significant ($p \leq 0.05$) increase in CH_4 oxidation under both sizes of biochar application was found in the heading stage for both cultivation seasons. This study shows the significant ($p \leq 0.05$) change on 29 and 70 DAS in the first season and all stages in the second season for LB compared to CT, while in SB this increase was significant ($p \leq 0.05$) only on 70 DAS in the first season and on 66 DAS in the second season. A previous study of Han et al. [9] demonstrated similar results of higher CH_4 oxidation under biochar application in the heading stage of rice growth.

Another possible explanation of higher CH_4 oxidation activity was in the development of rice growth. Roots are largely responsible for the oxygen transfer from the atmosphere into the soil after it is transferred through stems. The variations in root growth and stem size may contribute to the variations in CH_4 oxidation [58–61]. Therefore, more development of stems might result in greater oxygen transfer and, thus, the promotion of more CH_4 oxidation [62]. Taller plants under both biochar treatments in the second season (see Tab. 2) might be one of the reasons for higher CH_4 oxidation activity than those in the first season. However, CH_4 oxidation was not statistically different between the biochar sizes.

3.3 Effects of Particle Sizes of Biochar on CH_4 Emission

The major pathway by which CH_4 is released from rice soils into the atmosphere is through aerenchyma tissue [45]. Higher emissions of CH_4 are usually found in the tillering and reproductive stages because of the high availability of substrates and transport conduits [63,64]. In the current study, CH_4 emissions were reduced when both sizes of biochar were applied. Consistent with Feng et al. [4], we found that CH_4 emission reduction was a result of the increase in CH_4 oxidation. Besides enhancing oxidation, the reduction of CH_4 emissions upon biochar application decreased CH_4 production, increasing our understanding of this process. Relative to CT, SB significantly ($p \leq 0.05$) reduced cumulative CH_4 emissions by 30.4%, 22.7%, 16.8%, and 24.0% in the first season (Fig. 5a) and by 35.0%, 34.0%, 29.1%, and 29.3% in the second season (Fig. 5b) at 29 DAS, 45 DAS, 70/66 DAS, and the whole season, respectively. LB significantly ($p \leq 0.05$) reduced cumulative CH_4 emission by 22.5%, 16.1%, 10.3%, and 17.1% in the first season (Fig. 5a) and by 32.0%, 27.4%, 23.3%, and 22.8% in the second season (Fig. 5b) at 29 DAS, 45 DAS, 70/66 DAS, and the whole season, respectively. Greater mitigation effects of biochar in the second season than those in the first season were due to the presence of double the biochar in the soil. The results also indicated that SB slightly reduced CH_4 emissions more than LB in all the periods of rice growth for both cultivation seasons, although this reduction was not significantly different (Fig. 5). The SB mitigation effects by an average of 8.40% compared with those of LB are consistent with the reduction percentage of CH_4 production activity in SB (8.25% against LB). In contrast, the increased percentage of CH_4 oxidation activity of LB was only 2.93% higher than that of SB. Therefore, the abatement of CH_4 emissions from rice cultivation in SB was mainly attributed to the inhibition of CH_4 production [20].

Table 2: Rice height at each stage of the growth and yield, root length, and aboveground biomass at harvesting date

Growth and yield	CT	SB	LB
First season			
Height at 29 DAS (cm)	31.7 ± 2.08 a	33.0 ± 3.00 a	33.3 ± 2.31 a
Height at 45 DAS (cm)	71.7 ± 1.53 a	71.8 ± 1.61 a	70.8 ± 1.04 a
Height at 70 DAS (cm)	114 ± 1.15 a	117 ± 1.53 a	114 ± 2.08 a
Height at harvesting (cm)	119 ± 0.58 b	122 ± 0.58 a	120 ± 1.00 b
Aboveground biomass (t ha^{-1})	12.5 ± 0.57 a	13.0 ± 0.21 a	12.8 ± 0.19 a
Yield (t ha^{-1})	6.12 ± 0.13 a	6.25 ± 0.16 a	6.21 ± 0.18 a
Root length (cm)	21.3 ± 0.15 a	21.4 ± 0.37 a	21.3 ± 0.34 a
Second season			
Height at 29 DAS (cm)	31.3 ± 2.08 a	33.0 ± 1.73 a	33.3 ± 1.53 a
Height at 45 DAS (cm)	69.3 ± 1.04 b	72.2 ± 0.58 a	72.3 ± 0.29 a
Height at 66 DAS (cm)	106 ± 1.26 b	110 ± 1.00 a	109 ± 0.29 a
Height at harvesting (cm)	119 ± 0.58 b	123 ± 0.58 a	123 ± 0.58 a
Aboveground biomass (t ha^{-1})	12.5 ± 0.36 b	13.1 ± 0.17 a	12.9 ± 0.12 ab
Yield (t ha^{-1})	6.19 ± 0.10 b	6.57 ± 0.10 a	6.42 ± 0.06 a
Root length (cm)	21.3 ± 0.08 a	21.5 ± 0.14 a	21.4 ± 0.08 a

Different letters indicate the significant difference among treatment in each parameter (±S.D. of n = 3)

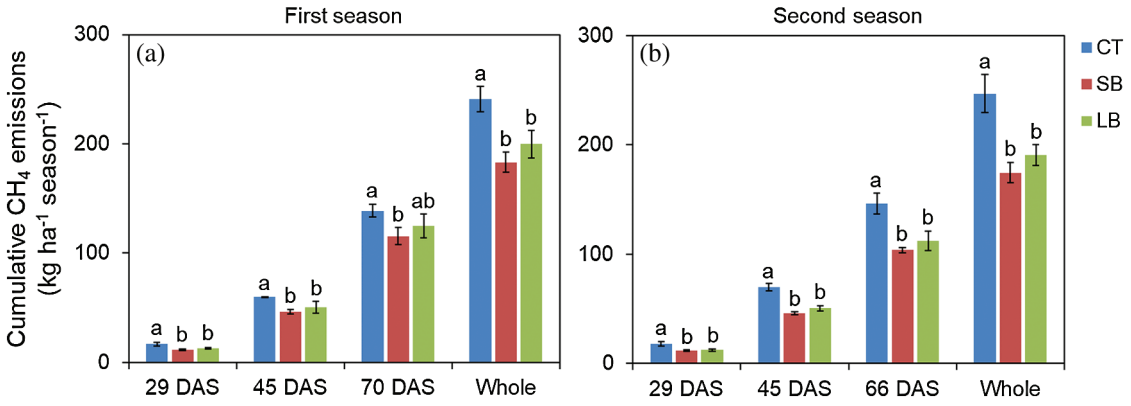

Figure 5: Cumulative CH_4 emissions at each stage and for whole season of rice cultivation in first (a) and second season (b). CT is soil with no biochar, SB is application of small particle size biochar, and LB is application of large particle size biochar. The different letters indicate significant difference among treatment in each stage of each season

3.4 Effects of Particle Sizes of Biochar on Rice Growth and Yield

In addition to the mitigation of CH_4 emissions, biochar can also influence rice growth and yield [3]. In this study, the effects of biochar on plant height were not significant in the first season. However, significant effects of biochar application on rice growth were found after 45 DAS in the second season. It seems that the application rate of biochar in the first season may not have been sufficient to stimulate rice growth

significantly. There was no difference in plant height between SB and LB (Tab. 2). However, compared to CT, both SB and LB groups demonstrated higher aboveground biomass. Similar to plant height, significant ($p \leq 0.05$) effects of biochar application on rice yield were found only during the second season. Koyama et al. [65] also demonstrated that high application rates of biochar were required to significantly ($p \leq 0.05$) influence rice yield. Rice yield in this study increased by 6.14% in SB and 3.72% in LB in the second season. Although it was a small increase, it can be said that biochar application positively promotes yield production. The greater rice growth and yield could be explained by improvements in soil conditions and nutrient cycling, as described above [66–68].

4 Conclusions

The results of the current study indicate that the application of both sizes of biochar could mitigate CH_4 emissions from rice cultivation. Seasonal emissions were reduced an average of 26.7% by the small size and 20.0% by the large size of biochar relative to the soil with no amendment. This reduction further increased when larger amounts of biochar were applied. The CH_4 mitigation effects could be explained by the suppression of CH_4 production and the stimulation of CH_4 oxidation in the soil. CH_4 production activities were inhibited by the increase in soil oxidation activities and the number of electron acceptors in the soil. The effects were stronger with the small size of biochar, probably because their surface area was higher than that of the large size of biochar. CH_4 oxidation activities in the rice soil were promoted by the stimulation of methanotroph growth as a result of soil aeration improvement. In contrast to CH_4 production, CH_4 oxidation was promoted more by the application of large size biochar, probably because of larger pore volume and thus greater soil aeration. However, the effects of biochar application on CH_4 production activity was more pronounced than those on CH_4 oxidation. As a consequence, we conclude that the small particle size biochar likely has higher CH_4 emissions mitigation potential than the large particle size biochar. A broader range of different biochar particle sizes (more than two size groups), particularly those <0.5 mm, should be included in further studies to increase our knowledge on this topic, particularly the presence of CH_4 oxidation in the rice soil, and promote the use of biochar for emission mitigation.

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