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Methane emissions and associated microbial activities from paddy salt-affected soil as influenced by biochar and cow manure addition

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ABSTRACT

Although previous studies showed that biochar can mitigate methane emissions through microbial activities from paddy fields, little is known about its effects on salt-affected soil. The current study aims to examine the interactive effect of biochar and cow manure (CM) on (1) methane emissions from salt-affected soil and (2) methanogenic and methanotrophic activities to identify potential mechanisms for the emissions. A two-factor pot experiment was set up, following a randomized complete block design with four replicates. Paddy rice (*Oryza sativa L.*) was grown on six treatments (no CM + no biochar; no CM + rice husk biochar; no CM + rice straw biochar; CM + no biochar; CM + rice husk biochar; CM + rice straw biochar). Methane emissions from salt-affected soil were relatively low and CM addition significantly increased CH_4 emissions (by 801%). For the CM treatments, biochar addition significantly reduced methane emissions (by 28 to 680%), but for the non-CM treatments, biochar addition showed no clear effect. The increase in methane emissions by CM could be involved in the improved relative abundance of methanogenic and methanotrophic genes. In contrast, the reduction of methane emissions by biochar was likely related to the reduced abundance of methanogenic and increased that of methanotrophic genes. In brief, low methane emissions from salt-affected soil were increased by CM addition and biochar addition could mitigate the CM's effect. Balancing methanogenic and methanotrophic activities could serve as a major mechanism in determining methane emissions from salt-affected soil added with cow manure and biochar.

1. Introduction

Salt-affected soil occupies a large portion, around 6%, of the total land all over the world (Arora, 2017). Having a high concentration of salts such as chlorides, bicarbonates, sodium, and magnesium (Rengasamy, 2006), salt-affected soil adversely influences plant growth through osmotic pressure, toxic effects of ions such as Na^+, and Cl^-, and nutritional imbalance (Rengasamy, 2006; Munns, 2002). Rice crop (*Oryza sativa L.*), an important food crop producing around 480-million tone of spilled grains annually (Muthayya et al., 2014), is suffered from salt-affected soil with poor growth and yield (Shereen et al., 2002; Ahmed and Haider, 2014).

As a result, the reclamation of salt-affected soil has been often implemented to improve soil quality and crop productivities. Leogrande and Vitti (2018) documented that the amendment of organic matter, including animal manure, can be an effective way to reclaim the salt-affected soil. With the organic amendment, the physical and chemical properties of the degraded soil could be improved, stimulating plant growth and microbial activities. Chaganti et al. (2015) found that saline-sodic soil added with the organic amendment was significantly lowered in EC (Electrical Conductivity), ESP (Exchangeable *Sodium* Percentage), and SAR (Sodium Adsorption Ratio), relative to that added without amendment. In nature, salt-affected soil had a low CH_4 emission rate, compared to the other soils, and associated reasons were involved in the adverse effect of the high-concentration salts on methane-producing bacteria (Vo et al., 2018; Theint et al., 2016; Gon and Neue, 1995). Various soils were reported to have higher methane emissions as a result of the organic amendment (Song et al., 2019; Brenzinger et al., 2018; Kim et al., 2014a). Consequently, salt-affected soil, added with the organic amendment, could also have an increased

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Table 1
Basic properties of soil, rice-husk and rice-straw biochar, and cow manure used in the experiment. Ash percentage of rice-husk biochar and rice-straw biochar is 23.3 and 27.9 (%), respectively. The numbers in the parenthesis are standard deviations of the mean.

Materials	Particle size distribution (%)			TOC	pH	EC	Cl^-	Na^+
	Clay	Silt	sand	(%)		(dS m^{-1})	(mg kg^{-1})	
Soil	35.2	54.1	10.8	1.9	6.5	5.7	7921	1633
	(2.79)	(1.20)	(1.59)	(0.1)	(0.1)	(0.5)	(141)	14
Cow manure				34.8	7.1	1.4	5140	959
				(0.5)	(0.1)	(0.1)	(50)	32.0
Rice husk biochar				47.5	7.6	0.7	638	163
				(1.3)	(0.4)	(0.2)	(100)	2.2
Rice straw biochar				43.7	9.4	4.0	9385	884
				(0.9)	(0.3)	(0.8)	(313)	76.2

CH_4 emission rate because the amendment could improve the physical and chemical properties of the soil and also provide available organic subtract for the methane-producing bacteria (Vo et al., 2018). Nevertheless, such the effect was reported and discussed from the literature insufficiently.

Methane is an important greenhouse gas, having global warming potential of 28 times higher than carbon dioxide has on a 100-year scale, contributing around 20% to global warming (IPCC, 2013; Schaefer, 2019). Methane is a stable carbon compound, formed from the decomposition of organic matter in anaerobic conditions with the help of methanogenic bacteria. An increase in the atmospheric CH_4 concentration from pre-industrial time to present (Kurniawati et al., 2018) was demonstrated to contributively alter global climate regime. Around 15–30% of total global CH_4 emissions could be derived from soil source, of which paddy field may contribute up to 12% of CH_4 emissions (Yanan et al., 2018). Consequently, lowering CH_4 emissions from paddy fields is an important strategy and studied often recently in the context of global climate change (Ali et al., 2019).

Recent studies reported that biochar can be used in paddy fields to mitigate CH_4 emissions (Yanan et al., 2018; Pratiwi and Shinogi, 2016). Biochar is an organic carbon (C)-rich material and can be produced from plant residues through a pyrolysis process in a limited-oxygen environment (Lehmann and Joseph, 2009; Zhang et al., 2017). The reasons for reduced CH_4 emissions by biochar included (1) increased soil pH thereby enhancing the biological oxidation process of CH_4, (2) high C/N ratio of biochar, lowering quality of organic matter serving as a food source for methane-producing bacteria, and (3) low labile fraction of biochar (low availability of organic-C substrate for microbial activities). In addition, the mechanisms for reduced CH_4 emissions could be involved in the growth of the methanogenic and methanotrophic communities in the biochar-added soil. Methanogenic bacteria are anaerobes generating methane as the major product from their energy metabolism under anaerobic conditions, while methanotrophic bacteria are a subset of methylotrophs and are unique in utilizing methane as a sole carbon and energy source (Whitman et al., 2006; Evans et al., 2019). Nevertheless, two contrastive trends of biochar effect on either stimulating or suppressing the growth of methanogenic communities were reported from previous studies. Yuan et al. (2018) found that rice-husk biochar stimulated methanogenesis with a CH_4 emission rate of 11 folds higher than the non-biochar added soil. In contrast, Han et al. (2016) concluded that biochar addition decreased the activity of methanogens, while increased the abundance of methanotrophs. Similarly, Liu et al. (2011) found that biochar produced from bamboo chips and rice straw significantly reduced methanogenic activities, compared to the non-biochar treatments. These two contrastive findings, possibly due to the difference in their experimental conditions (soil types, biochar properties, duration, and environmental conditions), indicate that more studies focusing on the microbial mechanisms responsible for CH_4 emissions are needed.

Consequently, it could be expected that the application of animal manure and biochar on salt-affected soil could reclaim the detrimental effect of the soil while reducing CH_4 emissions additionally caused by the applied animal manure. Therefore, the current study was conducted to examine the interactive effect of biochar and cow manure on (1) CH_4 emissions from salt-affected soil and (2) activities of methanogenic and methanotrophic bacteria to identify potential mechanisms for the emissions.

2. Materials and methods

2.1. Soil and organic amendments

Paddy soil, biochar, and cow manure were used for the current pot experiment in a net-house. Soil samples were collected from a paddy field in Phu Tan commune, Tan Phu Dong District, Tien Giang province in southwestern Vietnam (10° 16′N and 106° 35′E)), which was located on a shrimp-rice farm. The shrimp-rice farming system is commonly applied throughout the salt-affected areas in the Mekong Delta (Brennan et al., 2002). The soil is classified as *Haplic Solonchaks* (FAO/UNESCO), which is saline-sodic soil with EC = 5.7 dS m^1, and exchangeable-sodium percentage (ESP) = 42.7. The soil is a silty clay loam with the proportion of clay, silt, and sand of 35.2%, 54.1%, and 10.8%, respectively. The soil sample was collected from a 0–10 cm surface layer from ten points over the selected field. After sampling, soil material was transported to the experimental net house, air-dried, and ground to pass a 2-mm sieve before the experiment. Biochar was produced from rice husk and straw at around 350–400 °C using a small biochar-producing kiln. Rice husk and straw were collected, air-dried, and cut into 3–5 cm segments (for rice straw) before loading to the kiln. Dry cow manure and inorganic fertilizers were bought from the commercial market located close to the sampling field (some selected properties of the experimental materials (soil and organic amendments) such as organic carbon, pH, EC, etc. shown in Table 1).

2.2. Experimental setup

A two-factor pot experiment was set up following a randomized complete block design. Three biochar treatments (no-biochar, rice-husk biochar, and rice-straw biochar) were fully crossed with two cow manure treatments (with cow manure and without cow manure), making six experimental treatments in total. The detailed treatments included T1: no manure and no biochar, T2: no manure and rice-husk biochar, T3: no manure and rice-straw biochar, T4: cow manure and no biochar, T5: cow manure and rice-husk biochar, and T6: cow manure and rice-straw biochar. For treatments (T5) and (T6), biochar and manure were mixed at 1:1 (w/w) ratio. The experiment was replicated 4 times and planted with paddy rice (*Oryza sativa* L) for one season (3 months).

The organic amendment (biochar and manure) rate was used at 2.5% (w/w, dry soil), and around five kg of the mixture (organic amendment and soil) were packed into a 20 cm × 30 cm (diameter x height) plastic pot (total 24 pots). The mixture in the pots was watered to 5 cm depth for ten days before rice seed sowing. Rice seeds of variety OM 6162 were germinated in a Petri dish lined with two Whatman No.1 filter papers and added with 5 ml of distilled water. Individual pots were sown with ten germinated seeds, and five seedlings on each pot were maintained after two weeks from sowing. All pots were applied with the same practices of rice cultivation and with inorganic fertilizer rate following treatment three in Luu and Nguyen (2006) (equal 100 kg nitrogen (applied in three splits), 30 kg P_2O_5 (all applied before the sowing day), and 30 kg K_2O (applied in two splits) per ha). After ten days, all pots were watered with tap water to 5 cm depth, which was maintained constant throughout the experimental period.

2.3. Measurements

2.3.1. Chemical properties

Before the experiment, three sub-samples were taken from the collected experimental materials (soil, biochar, and cow manure) and after the experiment, soil from all 24 experimental pots was sampled, air-dried, ground to pass 2-mm sieve to measure some basic properties. pH and electrical conductivity (EC) were measured from the extract of the solution of 1:2 ground soil to distilled water (w/w) ratio, using pH meter (Thermo Fisher Scientific) and EC meter (Oakton). Organic carbon was measured using the Walkley–Black method (Walkley and Black, 1934). Cl^- concentration was measured using the $AgNO_3$ titration method (Ren et al., 2018). The exchangeable concentration of sodium was measured using the Barium chloride method (Carter and Gregorich, 2008). The measurements were carried out by weighing 0.5 g of each sample into a centrifuge tube, adding 30.0 mL of 0.1-mol L^{-1} $BaCl_2$, and analyzing with Inductively coupled plasma - optical emission spectrometry (ICP-OES). In addition, the ground soil was analyzed for particle size distribution (Carter and Gregorich, 2008).

2.3.2. Methane emissions

Methane emissions were determined using the closed chamber method by Minamikawa et al. (2015). A closed chamber, made of the plastic cover with a diameter equal to the diameter of the experimental pot and a 0.8-m height, was used for air sampling. At the time of sampling, the closed chambers were superimposed over the experimental pots and the exhaust gas was accumulated in the chamber before being taken. A 50-mL syringe was used to sample air within the closed chamber at 0, 10 and 20 min from superimposing the chambers. The air sample, after collection, was pumped into a pre-vacuumed glass vial with a volume of 30 mL. Sampling was carried out from 8 am to 11 am, every week, starting from the 20th day after sowing. Air samples were transferred to the analytical laboratory of the Cuu Long Delta Rice Research Institute on the same day to analyze for CH_4 concentration, using *Gas Chromatography-Flame Ionization Detector* (Qi et al., 2018). The CH_4 emission rate was calculated by taking the slope of the linear regression equation between the CH_4 concentrations of the three air samples with corresponding sampling time and shown in mg $CH_4/m^2/h$.

2.3.3. Activities of methanogenic and methanotrophic bacteria

After the experiment, soil samples from individual experimental pots were taken, but to reduce analysis cost, 18 soil samples from the first three replicates were analyzed for the relative abundance of the methanogenic and methanotrophic genes. Around 0.5-gram soil was used for DNA extraction with a FastDNA® SPIN Kit for soil (MP Biomedical, LLC, OH, USA) according to the manufacturer's instructions. Real-time PCR *(polymerase chain reaction)* method was applied to quantify the relative abundance of methanogenic 16S rRNA and methanotrophic pmoA genes, using primer pairs 0357 F/0691 R56 and A189f/mb661r57, respectively (Han et al., 2016). The PCR reactions were conducted in triplicate using a Bio-Rad CFX1000 Thermal Cycler and more details about the PCR method can be found in Han et al. (2016). The relative abundance of these targeted genes was calculated using the ΔΔ CT method (Livak and Schmittgen, 2001) by converting the threshold cycle (Ct) values into relative quantities (the highest Ct value for the 16 S rRNA genes set to 1, while the lowest Ct value for the pmoA genes set to 1) for relative comparison. In addition, the activities of the CH_4-generating bacteria (methanogens) were quantified using the method by Liu et al. (2008). In brief, around 10-g fresh soil samples collected from 24 pots after the experiment were weighed into a 100-ml glass bottle, which was also added with a 0.2 mmol sterile glucose solution. After mixing, the bottles were injected with O_2-free N_2 gas for 3 min, sealed with butyl rubber lids and aluminum crowns, and incubated at 28 °C for 24 h. Gas cumulated in the headspace of the 24 bottles was collected for CH_4 measurement using *Gas Chromatography-Flame Ionization Detector*.

2.4. Statistical analyses

All data from the current study were statistically analyzed using Analysis of Variance (ANOVA) procedure following the two-factor randomized complete block design. The overall ANOVA model is $\gamma_{ijek} = \mu + \beta_i + \alpha_j + \beta\alpha_{ij} + \delta_e + \in_{ijek}$, where γ_{ijke} is the response of individual treatment; μ is overall mean; β_i is a fixed effect of the i^{th} level of cow manure; α_j is the fixed effect of the j^{th} level of biochar factor; $\beta\alpha_{ij}$ is the interactive effect of manure and biochar factors; δ_e is the fixed effect of e^{th} block; and $\in_{ijek}$ is the random error with mean zero and having a normal distribution (Ott and Longnecker, 2011). If ANOVA showed significant, Tukey's Honest Significant Difference test was applied to classify the treatment mean. Linear regression analysis was performed to examine the dependent pattern of methane emissions on the relative abundance of methanogenic and methanotrophic genes. The regression analysis and figures were performed using Sigmaplot 12 (Systat Software Inc.) and ANOVA was conducted, using JMP 10 (SAS Institute Inc., North Carolina, USA).

3. Results

3.1. Methane emissions

In general, CH_4 emissions from salt-affected soil were relatively low and cow-manure addition significantly increased CH_4 emissions from the soil (Fig. 1a, b, and c). Fig. 1a showed that CH_4 emissions increased rapidly from the first measurement day (20 days from sowing day) to the 34th day from the sowing day. The CH_4 flux rates measured in the maximum day were 73.5, 40.7, and 12.6 (mg CH_4-C m^{2-} day^{-1}) for T4, T5, and T6, respectively, and were 9.0, 6.8, and 6.2 (mg CH_4-C m^{2-} day^{-1}) for T1, T2, and T3, respectively. Afterwards, the emission rates of the 6 treatments were reduced and leveled up since the 62nd day. Although the general pattern of CH_4 flux was relatively similar among six treatments, the maximum emission rate of the T4 was the highest and these of the non-cow manure treatments were the lowest.

An interactive effect of the two experimental factors (biochar and cow manure) was observed on the cumulative quantity of CH_4 emitted from the tested soil (Fig. 1b and c). For the three treatments added with cow manure (T4, T5, and T6), biochar addition significantly lowered CH_4 emissions (T6, 731), compared to the non-biochar treatment (T4, 4285 mg CH_4-C m^{-2} 90 day^{-1}). Likewise, T6 applied with cow manure and biochar had cumulative CH_4 emissions per 1 kg soil C for 90 days (117) significantly lower than T4 applied with manure and without biochar (902 mg CH_4-C kg^{-1} soil-C 90 day^{-1}). Meanwhile, for the three treatments applied without cow manure (T1, T2, and T3), biochar addition did not have a clear effect on CH_4 emissions, varying from 262 (T3) to 299 (mg CH_4-C m^{-2} 90 day^{-1}, T1) and 5.2 (T3) to 6.3 (mg CH_4-C kg^{-1} soil-C 90 day^{-1}, T1).

3.2. Methane emissions-related bacteria and selected soil properties

The relative abundance of the 16S sRNA and the pmoA genes

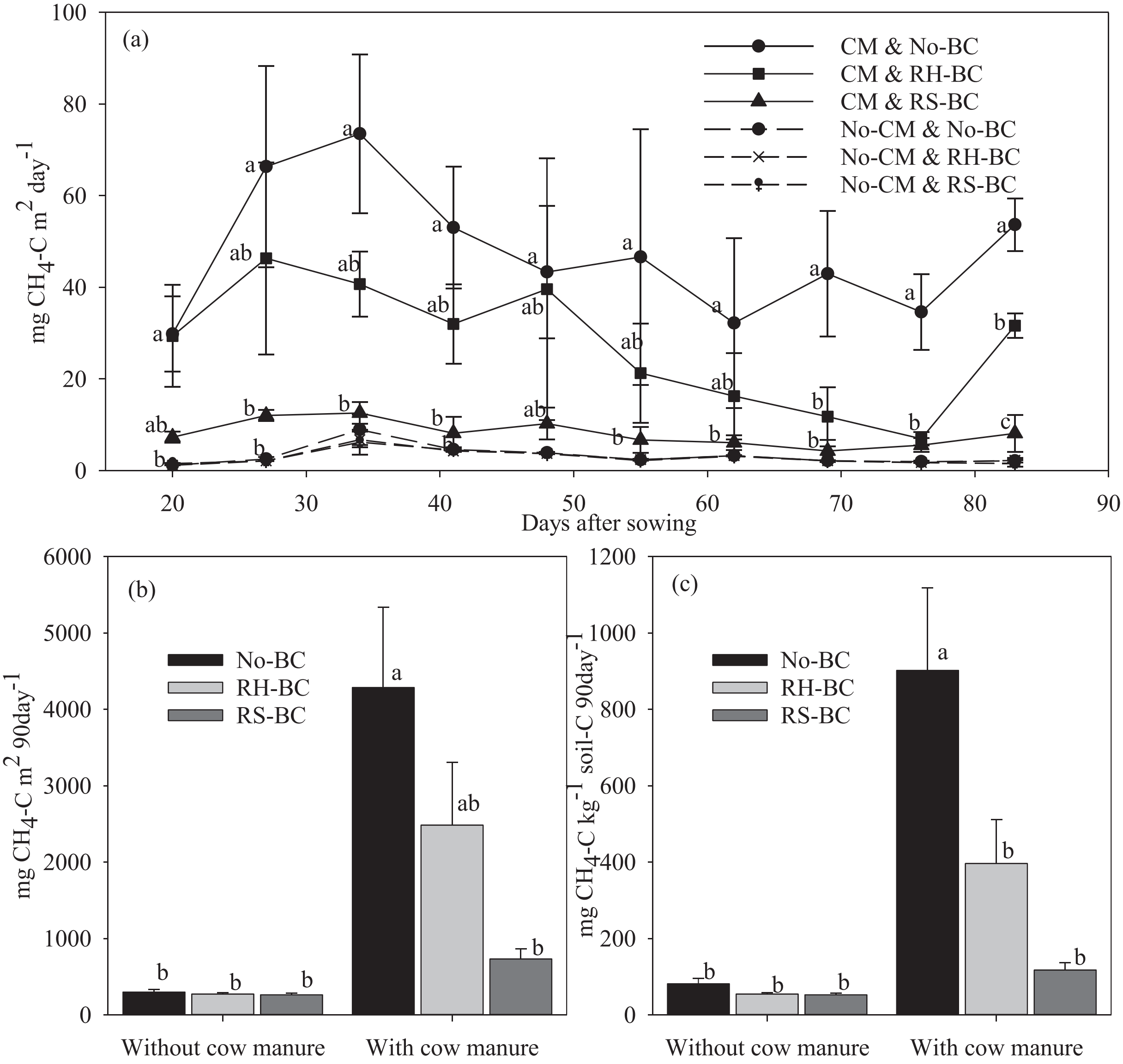

Fig. 1. Dynamics of CH_4 flux (a), cumulative CH_4 emissions over 1 m^2 for 90 days (b), and cumulative CH_4 emissions over 1 kg soil-C for 90 days (c) of six experimental treatments. Error bars are the standard deviation of the mean. Within each panel, data attached with the same letters are not significantly different from the other at $P \leq .05$. (Note: CM = Cow Manure; BC = biochar, RH = Rice Husk; RS = Rice Straw).

measured using the relative quantitative real-time PCR method was shown in Fig. 2a, b, c, and d. The interactive effect of biochar and cow manure was not significant on the relative abundance of these targeted genes. Nevertheless, the relative abundance of these genes was significantly affected by biochar treatments and cow manure treatments separately. Biochar addition significantly reduced the relative abundance of the 16S rRNA genes, while increased that of the pmoA genes. Cow-manure addition increased the relative abundance of these measured genes, relative to the no cow-manure addition.

An additional test was conducted to examine the activities of the methanogenic community in the 24 soils collected after the experiment and the results were shown in Fig. 3. Methane emissions from the experimental soils were significantly affected by the interaction of the two experimental factors. For the three treatments applied with cow manure, biochar addition significantly reduced CH_4 emissions (T6, 4.2), compared to the non-biochar addition (T4, 26.0 µmole CH_4-C kg^{-1} dws h^{-1}). For the three treatments applied without cow manure, biochar addition did not induce a clear effect on the activities of methanogenic communities, with CH_4 emissions varying from 0.53 to 1.37 (µmole CH_4-C kg^{-1} dws h^{-1}).

The organic carbon concentration of the after-experiment soils was not significantly affected by the interaction of the two experimental factors but was significantly affected by each factor separately (Fig. 4a and b). Overall, after the experiment the organic carbon concentration was increased, compared to the initial value. The two biochar treatments had a similar soil organic C concentration (3.01 and 3.07, %), significantly higher than the non-biochar treatments (2.1%). The organic C concentration of soil was significantly higher in the cow-manure treatments (3.1) than in the no-cow manure treatments (2.4%). In addition, soil pH and EC were not significantly affected by the interaction and by individual factors of biochar and cow manure. Soil EC of six treatments varied from 4.10 to 4.70 with an overall mean of 4.5 (dS m^{-1}); soil pH was from 6.9 to 7.1 with an overall mean of 7.0.

3.3. Relationships between methane emissions with microbial abundance and with soil organic C concentration

Fig. 5a and c showed that the cumulative CH_4 emissions were not significantly correlated with the relative abundance of 16S rRNA genes and soil organic carbon concentration, respectively. Meanwhile, the

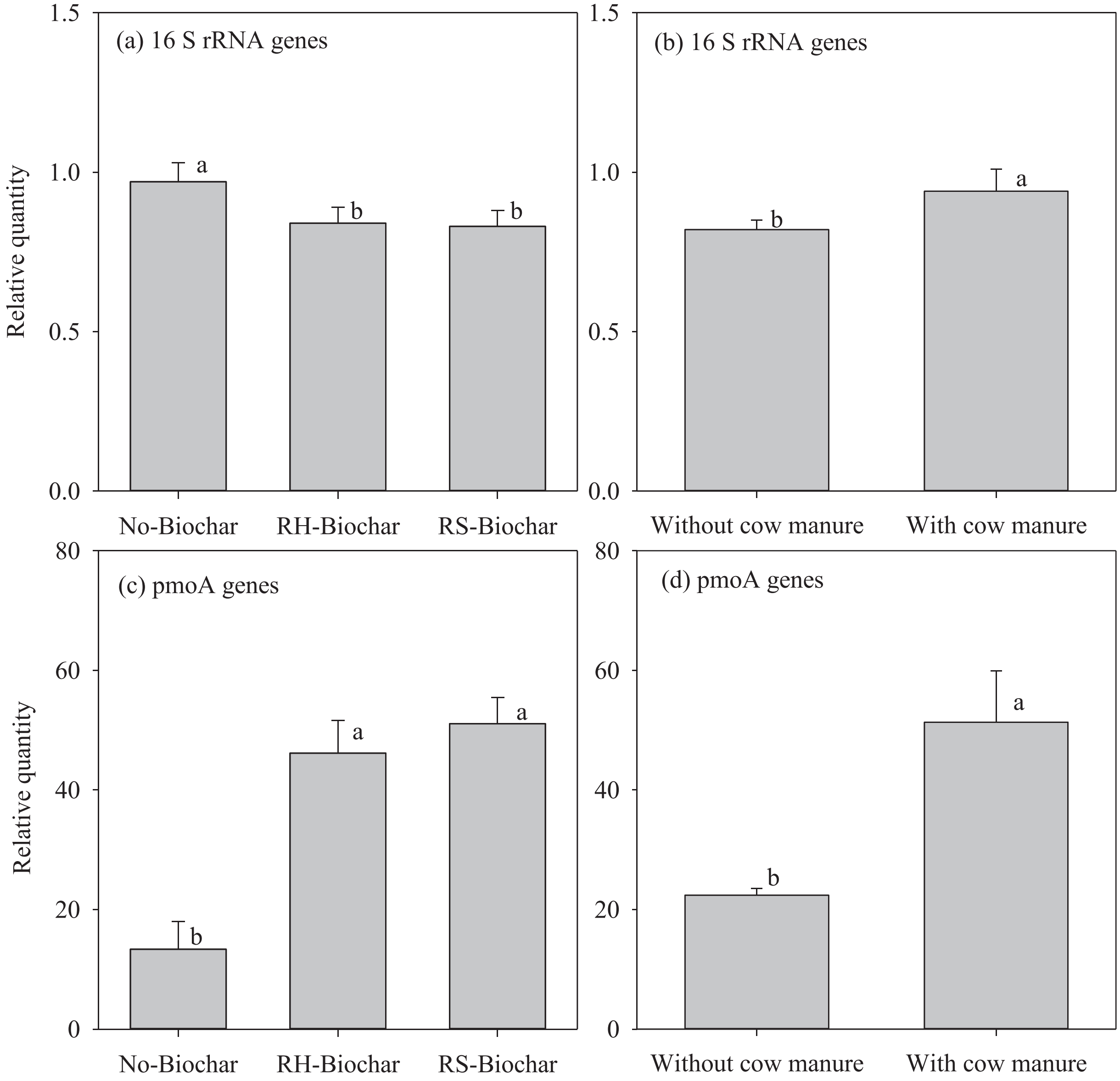

Fig. 2. The relative abundance of the 16S sRNA (a, b) and the pmoA (c, d) genes through relative quantitative real-time PCR as affected by biochar and cow manure addition. Note RH = rice husk, RS = rice straw.

cumulative CH_4 emissions were significantly correlated with the relative abundance of the pmoA genes with a coefficient of determination of 0.53 (Fig. 5b) of the three cow-manure treatments. The same relationship was not observed on the other three treatments applied without cow manure.

Overall, biochar addition to the no cow-manure added soil tended to reduce CH_4 emissions and relative abundance of the 16S rRNA genes but increase soil organic C concentration and relative abundance of the pmoA genes (Fig. 6a). Similar patterns were also found from the soil applied with biochar and with cow manure (Fig. 6b). It was also noticed that biochar applied on the cow-manure added soil reduced methane emissions significantly higher than that applied on the non-cow manure added soil.

4. Discussion

4.1. Effects of cow manure and biochar on methane emissions

Overall, biochar addition reduced methane emissions, relative to the non-biochar addition, but the magnitude of the reduction was much greater on the soil added with cow manure than added without organic manure (Fig. 6a and b). This finding on salt-affected soil was similar to the other studies on non-salt affected soils (Yang et al., 2017; Pratiwi and Shinogi, 2016; Xu et al., 2016). The addition of biochar derived from rice straw to soil significantly decreased CH_4 emissions by up to 30%, compared to the non-biochar addition (Yanan et al., 2018). Similarly, Liu et al. (2014) reported that CH_4 emissions were significantly decreased by up to 40% with biochar addition, relative to the non-biochar addition. The authors explained that an increase in soil pH as a result of biochar addition could be the reason to suppress CH_4 emissions from biochar-added soil. Other studies also attributed the decreased CH_4 emissions to the increased soil pH due to biochar addition (Yang et al., 2017; Qin et al., 2016). Nevertheless, an increase in soil pH following biochar addition could not be the case in the current study, because soil pH was not significantly affected by biochar addition. Main mechanisms responsible for the effects of biochar on methane emissions from the current study would be discussed in more detail in Section 4.3.

Cow manure, containing a high degradable organic C fraction, when added to the soil, could provide substrates as a food source for methanogens and thus increased CH_4 emissions. Consequently, a high

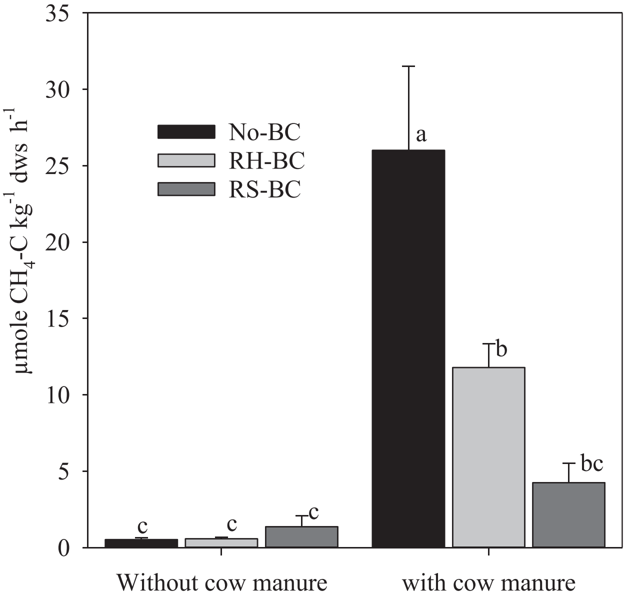

Fig. 3. Methanogenic activities in the soil after the experiment. Error bars are the standard deviation of the mean. Bars attached with the same letters are not significantly different from the other at $P \leq .05$. (Note: CM = Cow Manure; RH-BC = Rice Husk BioChar; RS-BC = Rice Straw BioChar, dws = dry weight of soil).

labile fraction of organic C of the cow manure was attributed to being an influencing factor in determining CH_4 emissions from cow manure-added soils (Kim et al., 2014b). Likewise, Kim et al. (2014a) reported that paddy soils added with cow manure were significantly increased in CH_4 emissions, relative to the chemical-fertilizer addition. The authors also demonstrated that a high fraction of dissolved organic C of, and a high methanogen density of, cattle manure could be the main causes of the enhanced CH_4 emissions from cattle manure-added soil. These results are in agreement with the finding from the current study (Fig. 1b and c). Nevertheless, the current study also found that the effect of cow manure on CH_4 emissions was significantly influenced by biochar addition (Fig. 1b and c). For the no cow-manure treatments, having relatively low CH_4 emissions, biochar played a negligible role in reducing CH_4 emissions, but for the cow-manure treatments, biochar played a significant role. The reasons for the varying roles of biochar could more likely be related to the activities of the methanogenic and methanotrophic communities, which would be discussed in the following Section.

4.2. The activities of methanogenic and methanotrophic bacteria

In general, two bacterial groups could be directly involved in CH_4 emissions, including methanogens generating CH_4 under anaerobic conditions and methanotrophs consuming CH_4. The current study showed that the relative gene abundance of the two groups was significantly affected by biochar and cow manure addition (Fig. 2a, b, c, and d). An increase in the methanotrophic-gene abundance following biochar addition was similar to other studies, such as Han et al. (2016), Wang et al. (2018), and Wang et al. (2019). Meanwhile, a reduction in the methanogenic-gene abundance and activities following biochar addition from the current study was similar to the findings by Han et al. (2016), Liu et al. (2011), and Dong et al. (2013). The authors concluded that biochar addition decreased the activity of methanogens, while increased the abundance of methanotrophs. Nevertheless, the finding from the current study was not in line with other studies. For example, Yuan et al. (2018) and Zhou et al. (2017) found that rice husk-derived biochar stimulated the growth of methanogenic bacteria and the reason accounting the positive effect could be related to the chemically functional groups on the surface of biochar particles that can act as both electron acceptors and donators.

Because of the inconsistent results, we additionally conducted an incubation test to verify our findings. Interestingly, the pattern of CH_4 concentration in the nitrogen fully-filled atmosphere reflecting the activities of methanogenic bacteria (Fig. 3) of six experimental treatments was similar to that of cumulative CH_4 emissions observed from the pot experiment of the same treatments. Several reasons could be responsible for reduced methanogenic activities by biochar. Firstly, biochar addition could suppress the decomposition of native soil organic C through a negative priming effect (Liu et al., 2018; Wang et al., 2016). The authors explained that native-soil C encapsulation and adsorption on the added biochar could protect native soil C from decomposition. Consequently, the adsorbed and protected soil organic C (Kasozi et al., 2010) may reduce the availability of labile C substrate, restricting

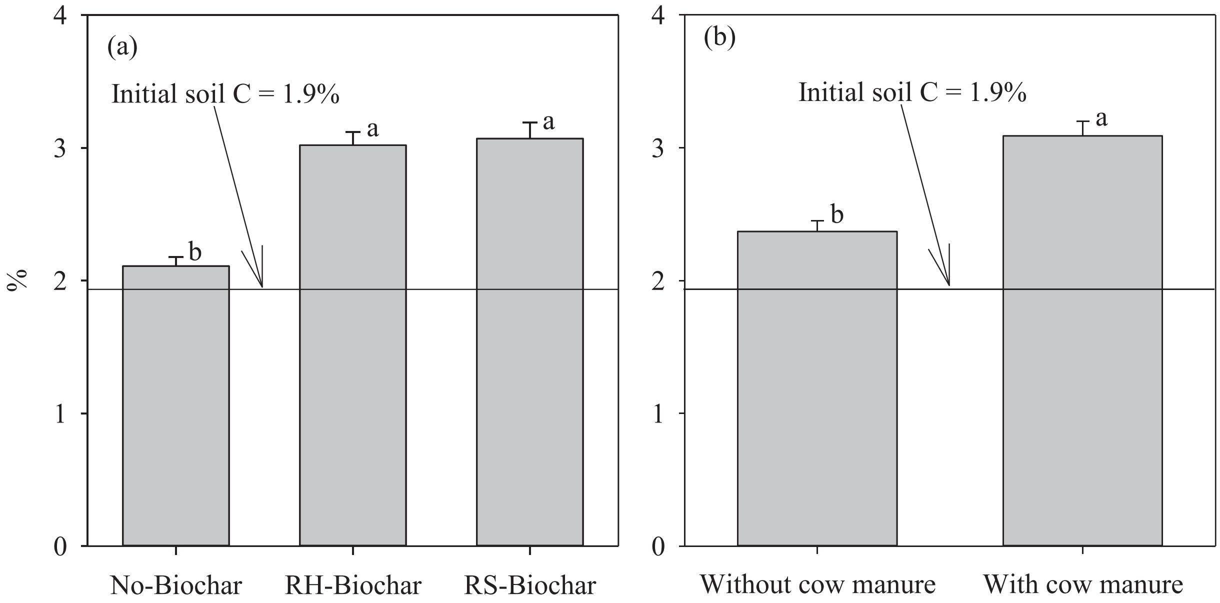

Fig. 4. Soil organic carbon concentration as affected by biochar (a) and manure (b) addition. Error bars are the standard deviation of the mean. Within a panel, bars attached with the same letters are not significantly different from the others at $P \leq .05$. RH = Rice Husk; RS = Rice Straw.

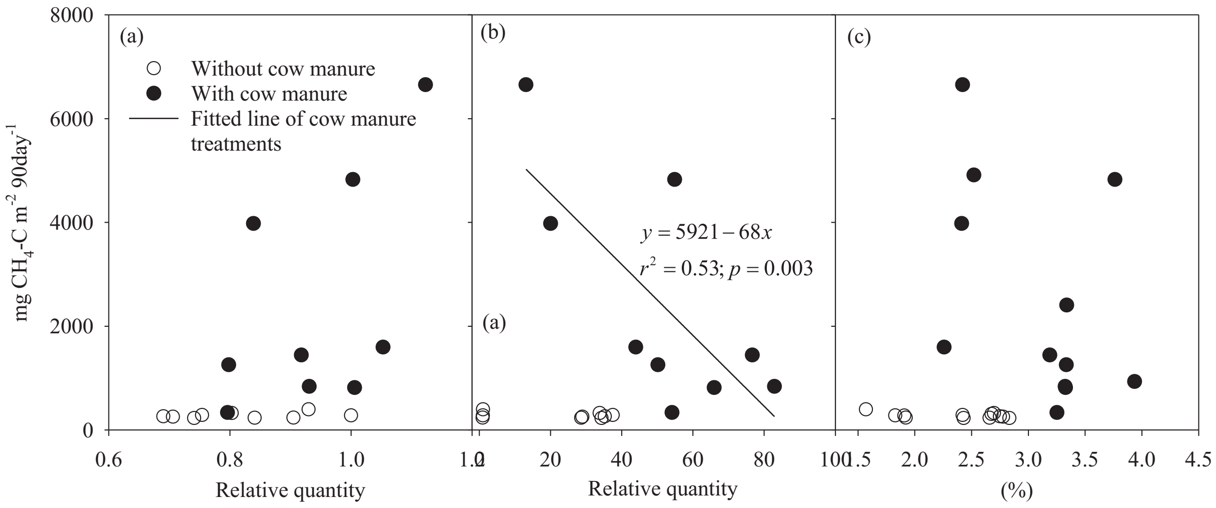

Fig. 5. Relationship between cumulative methane emissions with the relative abundance of the 16S rRNA genes (a), the pmoA genes (b), and with soil organic carbon concentration (c). Only significant relationships were shown with a regression equation, coefficient of determination (r^2), and probability (p).

microbial activities, including methanogens. Secondly, biochar addition significantly improved rice growth (Nguyen et al., 2018; Anak et al., 2018), which could bring more oxygen from the atmosphere to the root system through the aerenchyma channel of the rice plant (Colmer, 2003). This may create relatively aerobic sites within the rhizosphere, additionally restricting methanogenic activities, while stimulating methanotrophic activities.

The enhanced relative quantity of the methanogenic and methanotrophic genes by cow-manure addition (Fig. 2b and d) could be explained with two reasons. The first could more likely be involved in a high density of the two bacterial groups from the added cow manure (Kim et al., 2014a), which was derived from the ruminant animal. The second reason could be related to a high labile fraction of organic C in the added manure, providing available energy sources for methanogens. Consequently, Kim et al. (2014a) attributed the enhanced CH_4 emissions in the cattle-manure treatment to a higher labile C fraction of the cattle manure than the swine manure.

4.3. Methane mitigation by biochar from salt-affected soil

In general, the magnitude of CH_4 emissions is positively correlated with methanogenic activities, while negatively correlated with methanotrophic activities (Dubey et al., 2013; Chen et al., 2017). As many as 80 to 99% of produced CH_4 could be oxidized to reduce its production in paddy soil (Kurniawati et al., 2018). Because CH_4 could be produced by methanogenic bacteria, while oxidized by methanotrophic bacteria, the resultant CH_4 emissions could be a consequent balance in activities of the two bacterial groups. As a result, the reduced CH_4 emissions from paddy soil could finally be a consequence of higher consumptive activities of methanotrophic bacteria than the productive activities of methanogenic bacteria. Similarly, Feng et al. (2012) found that biochar addition increased the abundance of both methanogenic and methanotrophic communities and CH_4 emissions were determined by the ratio of methanogenic to methanotrophic abundance. This was supported by the findings of Yuan et al. (2018) and Zhou et al. (2017), who reported that biochar addition stimulated methane production and abundance of methanogenic communities. Nevertheless, the current study found that relative to the non-biochar addition, biochar addition decreased methanogenic while increased methanotrophic activities (Fig. 2a and c). The current study did not do absolute real-time PCR quantification of the genes associated with the two bacterial groups but did relative quantification. Consequently, it is unable to establish the ratio of methanogenic to methanotrophic abundance in the current study, but it could be expected that the ratio could be decreased in soil applied with biochar.

Another mechanism involved in the reduced CH_4 emissions following biochar addition could be related to the adsorption of newly formed CH_4 on biochar's large surface areas (Dong et al., 2013). The

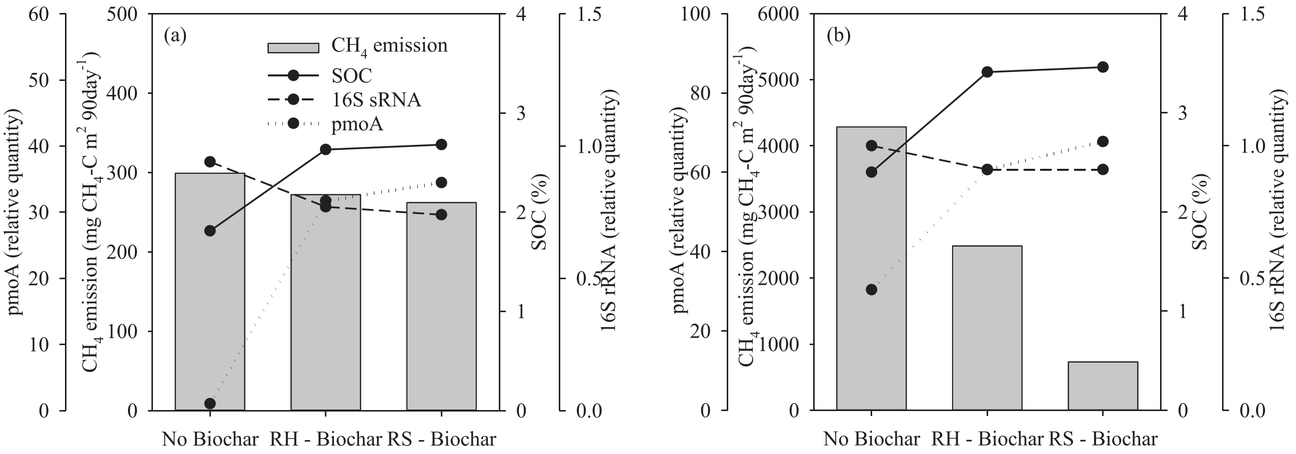

Fig. 6. Overall trends of methane emissions, methanogenic and methanotrophic abundance, and soil organic carbon concentration in the non-cow manure treatments (a) and cow manure treatments (b). Note RH = rice husk, RS = rice straw.

absorbed CH_4 on biochar surface could further stimulate the growth of methanotrophic bacteria, indicated through a significantly higher relative abundance of the pmoA genes in the biochar-added soil than in the non-biochar added soil (Fig. 2c). This may create an additional effect in reducing CH_4 emissions from biochar-added soil.

Methane emissions were reported to be low in salt-affected soil and the related reasons were low soil microbial activities and population (Vo et al., 2018). The authors also attributed the reduced CH_4 emissions to the low available substrate in saline soil, compared to non-saline soil. This mechanism could be in line with the finding from the current study. The salt-affected soil added without cow manure (T1, T2, and T3) had relatively low CH_4 emissions, but that added with cow manure had significant higher CH_4 emissions (Fig. 1b and c). Because soil pH and EC were not significantly improved with biochar and cow manure addition (data not shown), the higher CH_4 emissions in the cow-manure added soil could be more likely involved in the available substrate supplied with the added manure. If this is the case, available C absorption on biochar surface could be a potential indirect reason in lowering CH_4 emissions caused by cow manure.

Organic C concentrations of the tested soil were increased significantly with biochar and cow manure addition (Fig. 4a and b). Nevertheless, the relationship between organic C concentration and methane emissions from the amended soils was not significant (Fig. 5c). Additionally, because of biochar addition, the overall trend of soil organic C was an increase while that of CH_4 emissions was a decrease (Fig. 6). A majority of the added biochar-C could be recalcitrant (Crombie et al., 2012; Nguyen et al., 2014; Wang et al., 2016) and thus the increased soil organic C following biochar addition may be inert and unavailable for methanogenic bacteria. A similar argument was assumed to explain that methanogenic bacteria were not able to use the increased organic C derived from added stable biochar (Yanan et al., 2018).

5. Conclusions and implication

Methane emissions from the salt-affected soil were relatively low and the addition of cow manure significantly increased CH_4 emissions from the same soil. Meanwhile, biochar addition significantly reduced CH_4 emissions from the cow manure - added soil, but did not result in a clear effect on the non-cow manure added soil. The mechanisms related the biochar's effect on methane emissions could be involved in the activities of methanogenic and methanotrophic communities, which were decreased and increased following biochar addition, respectively. The consequent CH_4 emissions could be determined by the balance of methanogenic and methanotrophic activities on salt-affected soil. These findings suggested that improving salt-affected soil should be implemented with animal manure incorporated with biochar to reduce potential emissions of methane, an important greenhouse gas in the context of global warming currently.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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