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Effect of Mangrove Biochar on Methane Production, Oxidation and Emission of Rice Cultivation in Clay Loam Soil

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Biochar is recommended as soil amendment to CH_4 emissions from rice growing. However, CH_4 mitigation mechanisms are not well known. This study aimed to evaluate the changes of CH_4 production, oxidation and emission of rice cultivation soil that was amended by mangrove biochar to explain the mitigation way. Biochar at rate equivalent to 10 t ha^{-1} was thoroughly mixed with clay loam soil within the 0.32 m^3 bucket on 16th d before rice was sown under greenhouse condition. The redox potential (Eh), dissolved organic carbon (DOC), sulfate (SO_4^{2-}) and nitrate (NO_3^-) of soil and CH_4 emission were measured throughout cultivation season, while abundances of methanogens and methanotrophs and activities of CH_4 production and oxidation were determined from soil that collected in two critical stages (tillering and heading) of rice growth. The results show that biochar enhanced soil oxidation capacity through increasing soil Eh (ranging of 11–26 mV) and major electron acceptors (average of 77.3 % in SO_4^{2-} and 42.1 % in NO_3^-) throughout rice growth period. It also enhanced soil DOC in the most stages of rice cultivation. These effects did not influence the significant change of CH_4 producer (methanogenic archaea) and CH_4 production activity as compared to no amendment soil. A high porosity and surface area of mangrove biochar stimulated CH_4 consumer (methanotrophic bacteria), resulting in enhanced CH_4 oxidation activity by 11.8 % in tillering stage and 34.4 % in heading stage. This greater CH_4 oxidation activity influenced the significant reduction of seasonal CH_4 emission by 24.5 %. A major factor of CH_4 emissions mitigation from rice cultivation by biochar amendment is the increment of CH_4 oxidation particularly during heading stage.

1. Introduction

Rice cultivation is one of agricultural activities that influence the increase of atmospheric greenhouse gas (GHG) particularly methane (CH_4) (Sari et al., 2018). CH_4 is produced by the decomposition of organic matter (OM) by methanogens in anaerobic environment (Conrad, 2002) as a result of continuously flooded soil (Sriphirom et al., 2018). Some technologies are recommended to apply in the rice cultivation to mitigate GHG emissions. A widespread use technology as soil amendment to reduce CH_4 emissions is biochar (Jeffery et al., 2011). Biochar is a by–product of organic material burning through pyrolysis process (Jeffery et al., 2011). Several studies have reported CH_4 mitigation potential from rice cultivation by biochar, but its mitigation mechanisms are not well understood. Feng et al. (2012) has showed the microbial mechanism in reducing CH_4 emissions from rice growing but it is still not clear due to this study only focus on microbial abundance and community. It lacks some essential parameters related to CH_4 production and oxidation in rice cultivation soil. Some studies have reported the conflict result which showed the increase of CH_4 emissions by biochar such as the experiment of Wang et al. (2017) in laboratory soil incubation and the study of Zhang et al. (2012) in the paddy field of China. For the study of CH_4 mitigation pathway, the uncertainty of biochar effect on CH_4 emissions should be eliminated.

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Mangrove biochar according to Sriphirom et al. (2020) that showed the decrease of CH_4 emissions from rice growing in sandy loam soil was utilized in this study. This biochar was applied in another soil type (clay loam soil) for this study what different from the study of Sriphirom et al. (2020). Clay loam soil is a soil type that is much used for rice cultivation in Thailand (Attanandana, 2007). The aim of this study was to evaluate the effect of mangrove biochar on CH_4 activities focused on CH_4 production, oxidation and emission to explain the CH_4 mitigation pathway of rice cultivation in clay loam soil.

2. Materials and methods

2.1 Soil and biochar properties

Paddy soil was collected from Ratchaburi Province, western region of Thailand (13˚31'07"N and 9˚58'43"E) where locates at 6 m above mean sea level with 28.2 °C of average air temperature and 1,210 mm of annual precipitation. This soil is classified as Vertisols according to USDA soil taxonomy with a clay loam texture. It contains 7.00 of pH (H_2O), 1.03 % of OM, 0.85 dS m^{-1} of electrical conductivity (EC), 23.0 cmol kg^{-1} of cation exchange capacity (CEC), 0.60 % of total carbon (C), 0.06 % of total nitrogen (N), 9.47 mg kg^{-1} of available phosphorus (P), 88.0 mg kg^{-1} of available potassium (K) and 1.54 g cm^{-3} of bulk density. Biochar was produced from mangrove (*Rhizophora apiculata*) wood by pyrolysis at approximately 600 °C using a traditional kiln at Yi–san community in Samut Songkarm Province, central region of Thailand (Sriphirom et al., 2020). Biochar was ground by a cutting mill and sieved to size of 0.5–2 mm prior to mixing with soil. Its properties were 7.80 of pH (H_2O), 1.47 dS m^{-1} of EC, 54.4 cmol kg^{-1} of CEC, 58.5 % of total C, 0.28 % of total N, 0.23 % of total P, 0.18 % of total K, 88.5 m^2 g^{-1} of specific surface area and 0.61 cm^3 g^{-1} of specific pore volume.

2.2 Rice cultivation

Fresh paddy soil was directly transferred into the bucket with size of 0.6 m (width), 0.9 m (length) and 0.6 m (depth) to create soil depth level at 0.5 m within the bucket. Biochar at rate equivalent to 10 t ha^{-1} was mixed with soil on 16^{th} d prior to Pathumthani 1 rice (*Oryza sativa* L.) cultivar was sown at rate of 62.5 kg ha^{-1} in the bucket under greenhouse condition. During 1^{st} to 19^{th} d after sowing (DAS), water was kept at the soil surface level. During 20^{th} DAS to complete grain formation stage (approximately 90^{th} DAS), soil was continuously flooded at level of 10 cm above soil surface. After that soil was naturally dried to prepare for harvest at 110^{th} DAS. Soil with no amendment (control: CT) and soil with biochar amendment (BI) were conducted with three replications in a randomized complete block design.

2.3 Soil sampling and analysis

Soil was collected by a polyvinylchloride (PVC) pipe (internal diameter of 2.54 cm) at level of 0–15 cm depth throughout cultivation season to determine dissolved organic carbon (DOC), sulfate (SO_4^{2-}) and nitrate (NO_3^-). Soil for DOC analysis was extracted by 0.5 mol L^{-1} K_2SO_4 and determined using an automated TOC Analyzer (Multi N/C 2100, Jena, Germany) with non–dispersive infrared detection (Wang et al., 2018). For the analysis of SO_4^{2-} and NO_3^-, centrifugation at 8,000 rotations per min (rpm) for 15 min was used to separate the supernatant solution from soil and measured their concentrations by an ion chromatography (IC, Thermo Scientific, Dionex Integrion HPIC system 2016, USA) equipped with Dionex IonPac AS11 and NaOH eluents with suppressed conductivity detection (Morales et al., 1998). Soil redox potential (Eh) was directly measured on the field throughout cultivation period using an ion–selective electrode, pH/ORP combination sensor (YSI Professional Plus, USA). Soil was additionally collected by a sterilised PVC pipe (internal diameter of 3.81 cm) in two critical stages of rice growth to analyse the abundances of methanogens and methanotrophs. It was taken on 29^{th} DAS for tillering stage and 70^{th} DAS for heading stage. 10 g of soil was extracted DNA according to the method of Zhou et al. (1996) by magnetic beads (Agencourt AMPure XP; Beckman, USA) was used to purify DNA. Extracted DNA were determined the concentration by the technique of optical density at 260 nm using a Nanodrop ND–1000 spectrophotometer (Wilmington, USA) and checked the quality by an agarose gel electrophoresis. The primer sets of mcrA–f/mcrA–r (Liu et al., 2016) and A189–f/mb661–r (Kolb et al., 2003) were used to quantify the copy number of methyl coenzyme M reductase (*mcr*A) gene of methanogenic archaea and particulate methane monooxygenase (*pmo*A) gene of methanotrophic bacteria abundances. Real–time PCR was conducted in triplicate using an ABI Prism 7900HT system (Applied Biosystems, CA, USA). Each reaction was performed in 20 µL mixture that contained 10 µL of 2 × SYBR Green PCR Master Mix (TaKaRa, Shiga, Japan), 1.0 µL of template DNA (20 ng), 0.4 µL each of the forward and reverse primers (0.4 µmol L^{-1}) and 8.2 µL of sterile water. The PCR amplification for *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, while *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. Standard curves were generated using 10–fold dilution series of plasmid DNA with the target genes. The amplification efficiencies were 104.5–106.0 % with R^2 values were 0.997–0.999.

The sequenced results in this study were compared with database of the National Center for Biotechnology Information (NCBI) under accession number (GenBank) of KF836867 for methanogenic archaea and AB538965 and U31650 for methanotrophic bacteria.

2.4 CH_4 measurements

2.4.1 CH_4 production and oxidation

10 g of fresh soil that collected from two growth stages was laboratory incubated for 24 h at room temperature (approximately 28–30 °C) to measure the activities of CH_4 production and oxidation. For the study of CH_4 production, soil was mixed with 10 mL of a 0.2 mmol L^{-1} glucose solution in a 100 mL glass serum vial. Headspace of vial was flushed with N_2 (99.999 % purity) gas for 3 min to simulate anaerobic conditions (Dong et al., 2013) and immediately covered the vial with butyl rubber stopper septa and aluminum caps. For the study of CH_4 oxidation, soil was mixed with 2.5 mL of distilled–sterile water under ambient conditions in vial. Vial was covered with butyl rubber stopper septa and aluminum caps prior to injecting 5 mL of CH_4 (99.99 % purity) gas into the vial's headspace (Chan and Parkin, 2001). CH_4 concentration in vial's headspace of both studies were collected in different times and immediately analyzed using a gas chromatograph (GC) (Shimadzu GC–2014, Japan) equipped with a flame ionization detector (FID) and Unibead C Packed column (Shimadzu MTN–1, Japan). High concentration of CH_4 in samples were diluted with air prior to injecting into a GC–FID. Activities of CH_4 production and oxidation were presented in units of micromoles CH_4 per soil mass per unit of time that calculated from the increase and decrease of CH_4 concentration over the sampling time (Dong et al., 2013).

2.4.2 CH_4 emission

The closed rectangular chamber method was used to determine CH_4 emissions from rice cultivation. The details of chamber were described in Sriphirom et al. (2020). Air samples were collected at 0, 5, 10, 15 and 20 min after chamber closure in the area of chamber's headspace by a plastic syringe and transferred into the pre–evacuated vials. All samples were determined CH_4 concentrations using the same GC as described above. The CH_4 emission accumulation in each stage of rice growth was calculated according to the formula and description of Minamikawa et al. (2015).

2.5 Statistical analysis

Data were presented as mean ± standard error and statistically analysed for the significance indication by applying independent samples t–test. The statistical analysis was performed using statistical package for the social sciences (SPSS) version 22 at a confidence level of 95 % and significance was assigned at $p \leq 0.05$.

3. Results and discussion

3.1 Effect of biochar on CH_4 production

CH_4 is produced by the decomposition of organic matter under anaerobic environment (Conrad, 2002) at the last step after any electron acceptors were reduced by their own reducing bacteria at approximately –150 mV of soil Eh (Hou et al., 2000). This study showed that biochar increased soil oxidation capacity through enhancing soil Eh (ranging of 11–26 mV increase) (Figure 1a) and soil electron acceptors (by average of 42.1 % in SO_4^{2-} and 77.3 % in NO_3^-) throughout cultivation season (Figure 1b-c). This change was a result of increasing soil oxygen availability via improving soil aeration and reducing bulk density (Van Zwieten et al., 2009). Larger oxidation capacity particularly the presence of SO_4^{2-} and NO_3^- in biochar addition can inhibit CH_4 formation because sulfate and nitrate reducing bacteria are more capable of capturing the electron donors than CH_4–producing archaea (methanogens) (Conrad, 2002). Concentrations of these electron acceptors in biochar treatment was not high enough to completely inhibit CH_4 formation (Wang et al., 1992). Biochar amendment might be provided more electron donors as a result of higher DOC concentration (Figure 1d), resulting in enough for partial electron donors allocation to CH_4 production by methanogens which has not been changed abundance in biochar treatment (Figure 2a). These effects result in CH_4 can still be produced in biochar treatment and did not see the statistical difference of CH_4 production activity, although biochar reduced this activity by 14.5 % in tillering stage and 18.0 % in heading stage (Figure 2d). Higher electron acceptors and donors (as result of larger DOC) in biochar–mixed soil might result of large surface area and high porosity of biochar that promote the soil absorption capacity (Jing et al., 2018) and potential to bind any ions particularly on biochar surfaces through oxidative reactions (Van Zwieten et al., 2010). It was also observed that providing oxygen of biochar might stimulate oxidizing bacteria (Dangi et al., 2020), resulting in promoting sulfur oxidation and nitrification which lead to the reforming of electron acceptors in anoxic soil. This study indicated that although mangrove biochar increases the reduction processes of electron acceptors, but it did not change CH_4 production in paddy clay loam soil because it provides larger electron donors.

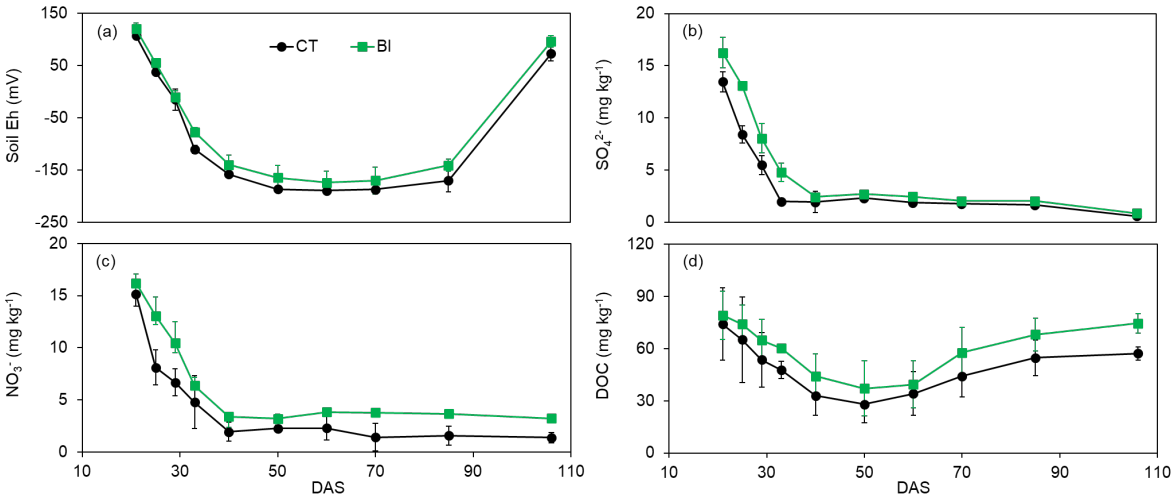

Figure 1: (a)Soil Eh, (b)SO_4^{2-}, (c)NO_3^- and (d)DOC concentrations throughout cultivation season

3.2 Effect of biochar on CH_4 oxidation

Produced CH_4 in anaerobic soil can be oxidized by CH_4–consuming bacteria (methanotrophs) prior to releasing into the atmosphere (Jia et al., 2001). The addition of biochar in this study directly stimulated methanotrophic bacteria abundance particularly in heading stage, accounted for 25.1 % increase (Figure 2b). This stimulation was a result of enhancing soil oxygen availability (Van Zwieten et al., 2009) and providing microbe habitat through biochar porosity (Chen et al., 2017).

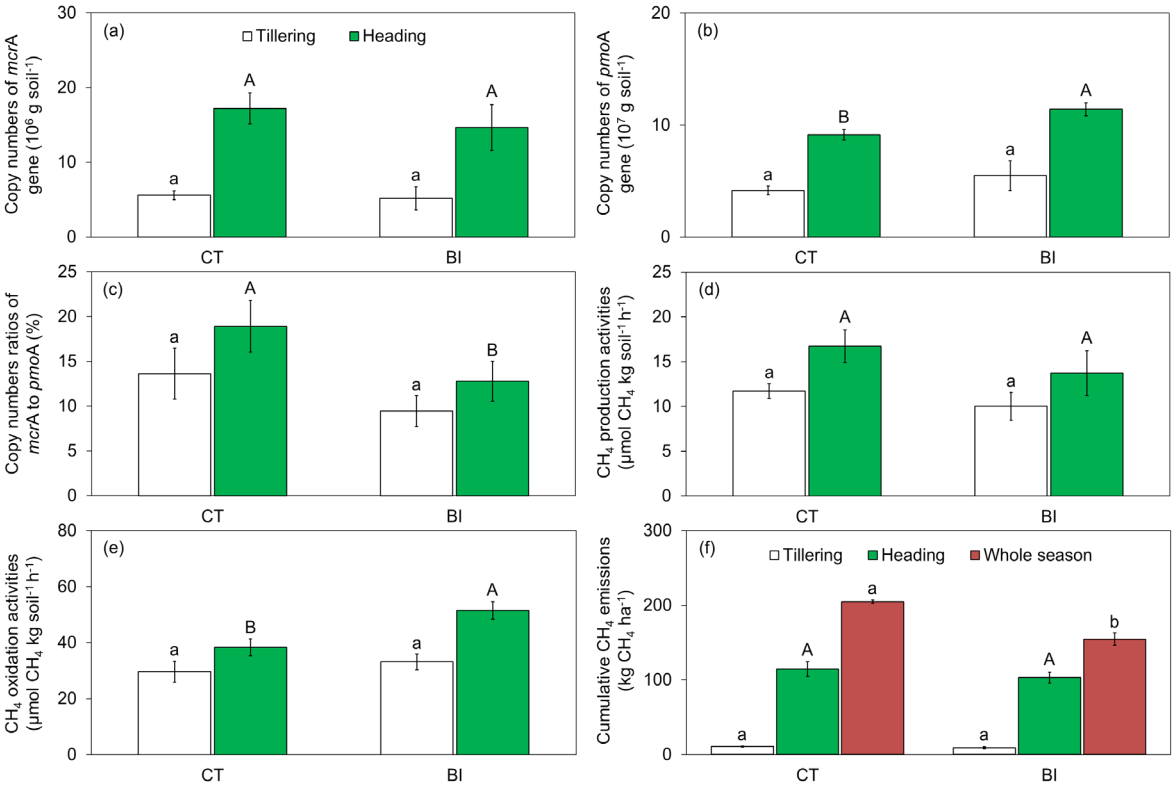

Figure 2: (a)Methanogens (mcrA gene) abundances, (b)methanotrophs (pmoA gene) abundances,(c)ratios of methanogens to methanotrophs,(d) CH_4 production activities and (e)CH_4 oxidation activities at (f)tillering and heading stages of rice growth and cumulative CH_4 emissions at tillering stage, heading stage and whole cultivation season

Im et al. (2011) also reported that facultative methanotrophs growth can be stimulated by DOC utilization that found higher in biochar treatment to produce energy. This finding is consistent with the study of Qin et al. (2016). In this study, methanotrophs stimulation in biochar treatment influenced greater CH_4 oxidation activity by 11.8 % in tillering stage and 34.4 % in heading stage, it only showed the significant increase in heading stage (Figure 2e). The promotion of CH_4 oxidation under biochar amendment contributed the higher opportunity of less atmospheric emissions.

3.3 Effect of biochar on CH_4 emission

The produced CH_4 remaining after oxidation would be emitted into the atmosphere through rice aerenchyma tissue as a major pathway (Conrad, 1993). The reduction of CH_4 production and the increment of CH_4 oxidation as described above resulted the decrease of CH_4 emissions in biochar treatment. This result was confirmed by the reduction of methanogens to methanotrophs ratio by average of 30.7 % in tillering stage and 32.4 % in heading stage (Figure 2c), indicated that CH_4 oxidation outgrow CH_4 production in biochar–mixed soil. Accumulative CH_4 emissions from rice cultivation with biochar was reduced by 15.0 % in tillering stage, 9.96 % in heading stage and 24.5 % for whole growing season (Figure 2f). The result showed the significant decrease of CH_4 emissions for whole season measurement, it was observed that biochar plays the important role on the stimulation of CH_4 oxidation since heading stage onwards. It might be due to greater oxygen releasing to soil as a result of more rice growth development by biochar (Major et al., 2010). This study showed that mangrove biochar can mitigate CH_4 emissions from rice cultivation in clay loam soil that is agreeable with the previous study that applied this biochar into the sandy loam soil of Sriphirom et al. (2020). The application of biochar should be encouraged as mitigation technology for sustainable rice production to reduce atmospheric CH_4.

4. Conclusions

The amendment of mangrove biochar can mitigate seasonal CH_4 emissions from rice cultivation in clay loam soil by 24.5 %. This mitigation was due to biochar increases CH_4 oxidation activity (average of 23.1 %) particularly in the heading stage onwards through stimulating the abundance of methanotrophic bacteria as result of increasing soil oxygen availability and providing habitat via its high surface area and porosity. Biochar did not significantly influence CH_4 production activity although it increases electron acceptors for inhibiting CH_4 formation in anoxic soil. It might be because biochar provides more electron donors as a result of higher DOC which enough to allocation for methanogenic activity. The further study should focus on the change of CH_4 oxidation during the period of heading to harvest in order to increase the knowledge on this topic and suggest the suitable practice of biochar to enhance mitigation potential.

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