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Dynamics of methane emission and archaeal microbial community in paddy soil amended with different types of biochar

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ABSTRACT

Biochar, as a valuable and eco-friendly material generated from greenwaste, has a potential to mitigate CH_4 emission in rice paddy soil. However, the response of methanogenesis and associated archaeal community in paddy soil to biochar amendment remains controversial. In this study, we explored the effect of three different biochars (derived from rice straw, orange peel or bamboo powder, respectively) on CH_4 emission and associated archaeal microbial community in paddy soil of southern China within 90 days of anaerobic incubation. Results showed that biochar amendment overall inhibited CH_4 emission in paddy soil. Significant decrease of α-diversity of archaeal community was observed in all samples in the end of incubation as revealed by 16S rRNA gene sequencing, and the addition of biochars mitigated the loss of archaeal biodiversity in paddy soil. Incubation time was found to be the major driver for the succession of archaeal community. Besides, *Methanosaeta*, *Methanocella*, *Methanobacterium* and *Methanosarcina* were mainly responsible for CH_4 production. In addition, biochars had no significant effect on altering relative abundance of methanogens. Overall, our study demonstrated that the addition of three different types of biochar reduced methane emission and total archaeal diversity, while caused no significant change in methanogenic communities in paddy soil.

1. Introduction

Global methane (CH_4) emission has drawn great environmental and social concerns, which has been recognized as the second largest radiating forcing after carbon dioxide (CO_2) (Rao and Riahi, 2006). CH_4 is a powerful greenhouse gas naturally formed in various environments, such as oceans, sediments, wetland, and landfills (Chynoweth, 1996; Conrad, 2009), and rice paddy fields as one of the major sources of worldwide CH_4 emissions, accounting for ~12% (24.4 Tg per year) of the global budget (IPCC, 2013). In flooded paddy soil, organic matters can be degraded to methanogenic precursors (such as CO_2, H_2 and acetate) by diverse functional microbes under anoxic conditions, and the methanogenic archaea are able to further utilize those substrates to produce CH_4 (Watanabe et al., 2007). Moreover, part of CH_4 could be oxidized by methanotrophs before natural emission (Pan et al., 2016). So far, soil amendments including rice straw (Ly et al., 2014), slag (Ali et al., 2008), industrial or agricultural wastes (Kumar et al., 2020; Wang et al., 2017b), as well as biochar (Feng et al., 2012; Liu et al., 2011; Wu et al., 2019) have been applied in rice paddy fields to regulate CH_4 emission.

Biochar can be derived from vast feedstocks, and the reuse of green wastes to produce biochar via pyrolysis adds another environmental benefit (Li et al., 2019). Nowadays, biochar has aroused wide interest in environmental management as a relatively stable and eco-friendly material (Lu et al., 2016; Palansooriya et al., 2019; Zeng et al., 2018). A four-year field experiment found application of biochar decreased annual CH_4 emissions by stimulating the abundances of both methanogens and methanotrophs in the first year, but suppressing the abundance of methanogens in the next three years (Wang et al., 2019a). The inhibition of CH_4 emissions might due to the decreased ratio of methanogens to methanotrophs, in which case biochar addition increased soil pH and methanotrophic community grew faster with the rising soil pH in comparison to methanogens (Han et al., 2016; Jeffery et al., 2016; Le Mer and Roger, 2001). Besides, various organic functional groups (e.g. quinones and hydroquinones) locating on the surface of biochar could facilitate anaerobic oxidation of CH_4 by methanotrophic archaea (Xie

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et al., 2020; Zhang et al., 2019), leading to the reduction of CH_4 emission (Saquing et al., 2016; Wang et al., 2020). Moreover, biochar addition could increase soil aeration due to its high porosity, which might also increase CH_4 oxidation in soil (Karhu et al., 2011). However, some other studies also showed that biochar could stimulate or have no effect on CH_4 emission, and those controversial results largely depended on soil properties and the characterizations of applied biochars (Jeffery et al., 2016; Wang et al., 2017a; Zhang et al., 2012).

Biochars derived from diverse feedstocks are endowed with various chemical or morphology properties (Kloss et al., 2012; Zhao et al., 2013), thus lead to different ability in facilitating or inhibiting the electron transfer in methanogenesis and shaping associated archaeal community. The positive effect of biochar on methanotrophs activity has been widely verified (Han et al., 2016; Reddy et al., 2014; Xu et al., 2016; Zhang et al., 2019), whereas the effect of biochar on methanogenic community remains ambiguous. Positive (Feng et al., 2012), negative (Cai et al., 2018) and neutral (Singla et al., 2014) effect of biochar application on methanogenic archaeal diversity or abundance were reported with different combination of soil and biochar (Anderta and Mumme, 2015). A laboratory research showed that adding biochar derived from rice straw, manure or wood chips to rice paddy soil resulted in significant change in the structure of archaeal community, and a significant enrichment of *Methanobacterium* and *Methanosarcina* was found in manure biochar and rice straw biochar treatments, respectively (Yuan et al., 2018). Biochar addition may introduce more available substrates to soil, resulting in the change of microorganism growth condition and the alteration of community structure (Liu and Whitman, 2008).

Hunan province is one of the major rice-producing provinces in southern China, with over 4.0 Mha rice planting area in 2018 (Wang et al., 2019b). It was reported that CH_4 emissions in Hunan province were among the high emission range in mainland China (Yan et al., 2003). Besides, both application of chemical and organic fertilizer stimulated seasonal cumulative or mean flux of CH_4 emissions in Hunan province (Yang et al., 2010). However, studies on the biochar amendment in rice field soil of southern China were rarely reported. It is essential to monitor the effect of different types of biochar on methanogenesis and associated archaeal microbial community to better evaluate the potential of biochar treatment on regulating CH_4 emissions in paddy soil of southern China.

Therefore, to investigate whether the addition of different types of biochar would result in the regulation of CH_4 emission and succession of archaeal community in paddy soil of southern China, biochars were derived from three local and typical forestry and agricultural residues (i.e. rice straw (RB), orange peel (OB) and bamboo powder (BB)), and incubated anaerobically with local rice paddy soil in laboratory for 90 days. The objectives of this study were: (i) to investigate the influence of biochar amendment on CH_4 emission in paddy field of southern China; (ii) to investigate the diversity and succession of archaeal community during the anaerobic incubation; (iii) to explore the correlation between methanogenic archaeal community and physicochemical parameters.

2. Materials and methods

2.1. Preparation of soil, biochars and incubation samples

The preparation of soil and biochars has been reported previously (Lu et al., 2020). In brief, soil was collected from a rice paddy field at Xiangtan city, Hunan province, southern China (27°53′N, 112°31′E). Biochars were respectively pyrolyzed from three typical forestry and agricultural residues in Hunan province (i.e. rice straw, orange peel and bamboo powder) at 600 °C for 1 h with constant N_2 gas flow. Anaerobic incubation were prepared in 120 mL serum bottles filled with 10 g dry paddy soil and 20 mL N_2-flushed, ultra-pure water. Then, biochar derived from rice straw (RB) or orange peel (OB) or bamboo powder (BB) was added at the ratio of 1% dry soil weight (Rittl et al., 2018; Wang et al., 2019a). After sealing with rubber stopper and aluminum caps, serum bottles were flushed with N_2 for several times. Serum bottles without biochar addition were set as NB treatment. Each treatment was carried out in triplicates and incubated at 30 °C without shaking for 90 days. At Day 0 (incubated for 2 h), Day 1, Day 5, Day 15, Day 40, Day 60, Day 70 and Day 90, 12 bottles (4 treatments × 3 replicates) were sacrificed for further analysis.

2.2. Chemical measurements

Gas samples from headspace of serum bottles were collected by 1 mL syringe at various points during the incubation process, and a gas chromatograph (Shangfen, GC112A, China) with thermal conductivity detector (TCD) and a 3 m × 3 mm column (Zhonghuida Co., GDX-102, China) was used to analyze the concentrations of H_2, CH_4 and CO_2. The temperatures of the injection port, column and detector were controlled at 40, 40 and 80 °C, respectively.

2.3. High-throughput sequencing of archaeal 16S rRNA gene

Fresh slurry from each bottle being destructively sampled were collected and stored at −80 °C. The samples taken at Day 0, 1, 15, 40 and 90 were selected for the molecular analysis. The procedures for genomic DNA extraction have been described previously (Lu et al., 2020). After DNA extraction, PCR amplification of V4-V5 region of archaeal 16S rRNA gene was carried out on a My Cycler thermal cycler (Bio-Rad 580BR, USA), with the primer set: 524f (5′-TGYCAGCCGCCGCGGTAA-3′) and 958r (5′-YCCGGCGTTGAVTCCAATT-3′). The purified amplicons were quantified by Qubit dsDNA assay kit (Life Technologies Q32852, China), pooled in equimolar and sequenced on an Illumina MiSeq platform at Shanghai OE Biotech. Co., Ltd. (Shanghai, China).

Raw sequencing data were preprocessed to remove ambiguous and low-quality sequences by using Trimmomatic software (Bolger et al., 2014). After trimming, paired-end reads were assembled using FLASH software (Reyon et al., 2012), and the sequence data were further quality-filtered to abandon reads with ambiguous, homologous sequences or those with length less than 200 bp. Then, reads with chimera were detected and removed by QIIME software (version 1.8.0) (Caporaso et al., 2010). After the pretreatment described above, clean reads were clustered to generate operational taxonomic units (OTUs) using Vsearch software at 97% similarity level (Edgar et al., 2011). The representative read of each OTU that selected by using QIIME package was annotated and blasted against the SILVA database using RDP classifier with the confidence threshold of 70% (Wang et al., 2007).

2.4. Statistical analysis

OriginPro 8 was used to conduct the data processing and One-Way ANOVA analysis. α-diversity indicated by Chao1, Simpson and Shannon index and β-diversity visualized by non-metric multidimensional scaling (NMDS) based on OTUs abundance were calculated in the R package Vegan and CANOCO 5.0 package, respectively. Permutational multivariate analysis of variance (PERMANOVA) was also performed by R package Vegan to test whether there were significant differences in community composition between different groups based on bray-curtis distance. Spearman's correlation coefficients were calculated to determine the correlation between methanogens and environmental variables and experimental setting conditions including incubation time, three biochar types, CH_4, CO_2, H_2, acetate and pH, and heat maps were created by R software. The concentration of acetate and pH from the same batch cultures was cited from the previous report (Lu et al., 2020) and re-analyzed here to further describe their correlation with methanogenic community.

2.5. Nucleotide sequences accession number

Nucleotide sequence data reported in this study were deposited to the National Center for Biotechnology Information (NCBI) under bioproject number (PRJNA597112) and accession number (SRP238462).

3. Results

3.1. Profiles of CH_4, CO_2 and H_2 concentrations

CH_4 was not detected in all treatments (RB, OB, BB and NB) until Day 4 of anaerobic incubation (Fig. 1a). The patterns of CH_4 emission in four treatments were similar, but those treatments with biochar addition consistently had lower CH_4 concentration than that of NB treatment. The production rates of CH_4 were shown in Fig. S1a. Fast production of CH_4 between Day 10 to 40 was observed, especially for NB treatment, while afterwards CH_4 production rates were fluctuated at low levels. In the end of incubation, CH_4 were 2.59 ± 0.20 mM in NB, 2.15 ± 0.05 mM in OB, 2.18 ± 0.06 mM in BB and 2.05 ± 0.09 mM in RB treatment, respectively (Fig. S1b).

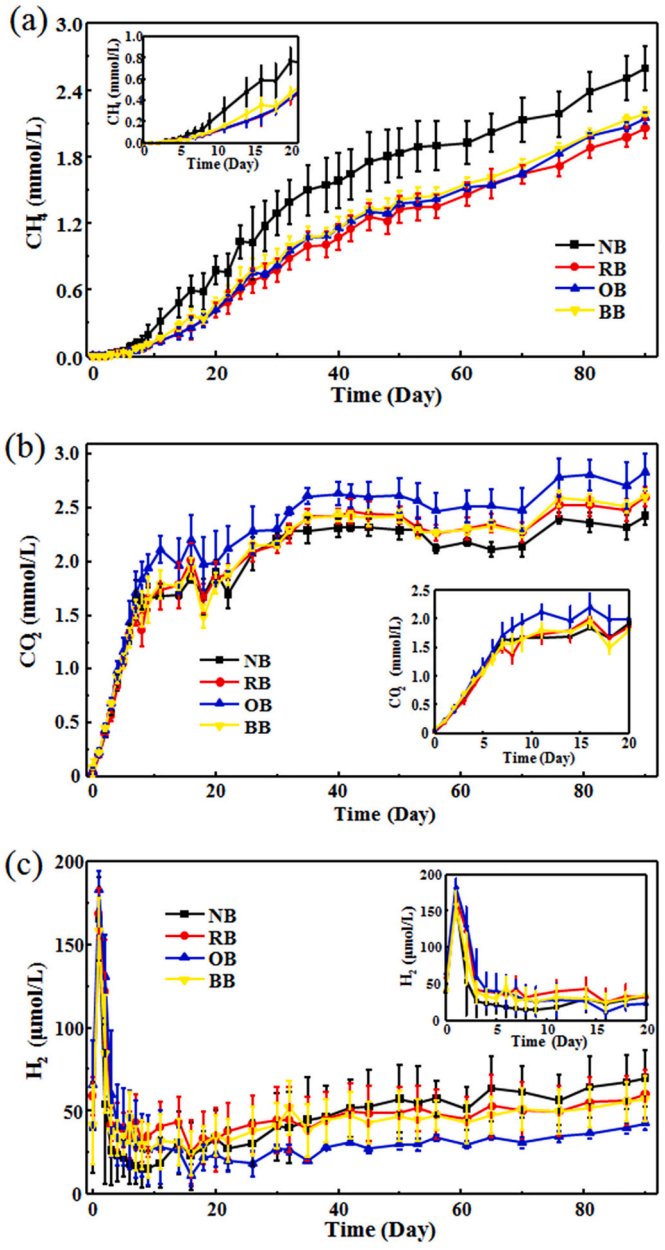

Fig. 1. Variation of (a) CH_4, (b) CO_2 and (c) H_2 production under different biochar treatments over incubation time in paddy soil. Error bars indicate standard deviation, $n = 3$. Different lowercase letters indicate significant differences between treatments at the same sampling date at the 5% level according to one-way ANOVA test.

CO_2 were rapidly accumulated between Day 0 and 10, thereafter CO_2 concentrations underwent a short decrease which followed by the slow increase in all treatments (Figs. 1b and S1c). At the end of incubation, CO_2 had concentration of 2.43 ± 0.09 mM in NB treatment, 2.60 ± 0.09 mM in RB treatment, 2.83 ± 0.18 mM in OB treatment and 2.61 ± 0.06 mM in BB treatment, respectively. Practices of adding biochar enhanced the production of CO_2, especially the accumulation amount of CO_2 in OB treatment being significantly higher than other treatments (Fig. S1d).

The pattern of H_2 production was more complicated than that of CH_4 and CO_2 (Fig. 1c). In all treatments, H_2 production started immediately and reached a peak at Day 1 (range from 157.82 ± 20.16 to 182.43 ± 11.44 μM). Variation of production rate also demonstrated the obvious accumulation of H_2 (Fig. S1e). The maximum concentration of H_2 had no significant difference in four different biochar treatments (Fig. S1g). Thereafter, H_2 concentration decreased rapidly but increased again by a small margin after Day 20. At the beginning, NB treatment had lower concentration of H_2 than other treatments, but at the end of incubation, the concentration of H_2 was 69.71 ± 16.87 μM in NB treatment, which was higher than that of 60.15 ± 14.77 μM in RB treatment, 57.00 ± 13.60 μM in BB treatment and significantly higher than that of 42.49 ± 3.66 μM in OB treatment (Fig. S1f).

3.2. Responses of archaeal community to biochar treatments

In total, 1,622,213 valid tags were generated through quality filtering from 60 soil samples, and classified tags were clustered into 1377 OTUs with 97% sequence identity, with a range from 207 to 368 OTUs for each sample (Fig. 2). The α-diversity of archaeal community including Chao1, Simpson and Shannon indexes in four different treatments at eight individual sampling dates were also showed in Fig. 2. α-diversity indexes of archaeal community all decreased from the beginning of incubation to Day 40 and recovered slightly afterward, and the additions of biochar were favorable to retain the biodiversity of archaeal community. Overall, considerable loss of archaeal species during 90 days incubation was found in all samples, and the loss of archaeal community diversity were hard to recover.

Non-metric multidimensional scaling (NMDS) (Fig. 3) analysis based on 16S rRNA gene sequencing showed the variance of archaeal composition of 60 samples. Those samples were grouped into five envelops classified by incubation time, in detail, samples of Days 0 and 1 were separated from samples of other three date points, and samples belonging to Days 40 and 90 showed high similarities in distribution, suggesting the short period of archaeal composition shifting and the persistently stable period afterwards. Noteworthily, the differences caused by biochar additions were hard to recognize. PERMANOVA analysis (Table S1) further confirmed that biochar addition had less significant ($p < 0.05$) effects on archaeal community compositions in comparison to incubation time.

3.3. Responses of methanogen community structure to biochar treatments

To further investigate the microbial mechanisms of methane production and consumption, putative methanogens were investigated by high throughput sequencing. As shown in Fig. 4, methanogen accounted for 31.12–65.80% in archaeal community, with a significant increase of relative abundance after Day 1 and a slight decrease after Day 15 in all samples. Specifically, *Methanosaeta* (6.20–18.59%), *Methanocella* (9.55–28.58%), *Methanobacterium* (4.44–13.57%), *Methanosarcina* (2.43–13.53%), *Methanomassiliicoccus* (0–5.11%) and *uncultured_methanogenic_archaeon* (1.87–3.58%) were dominant genera in all

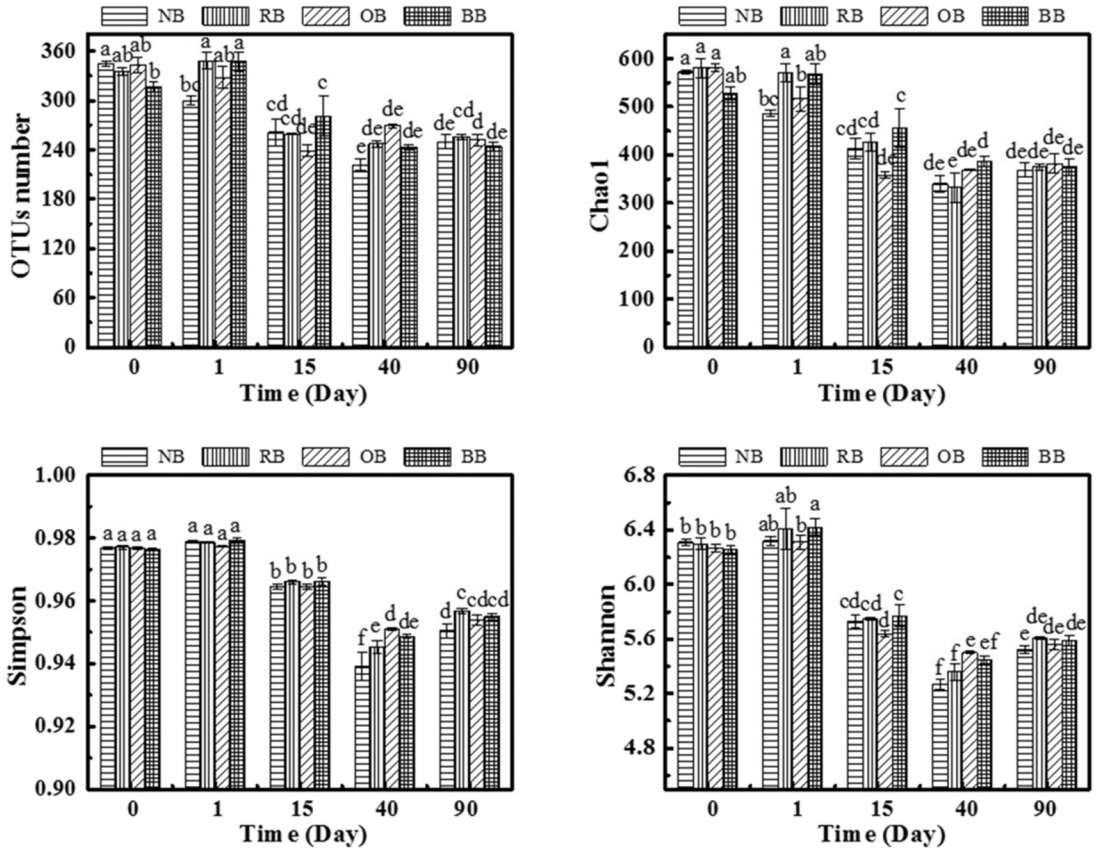

Fig. 2. Biodiversity of archaeal community measured as OTU numbers, Chao1, Simpson and Shannon indexes in four treatments (NB, RB, OB and BB). Different lowercase letters indicate significant differences among treatments at the 5% level according to one-way ANOVA test.

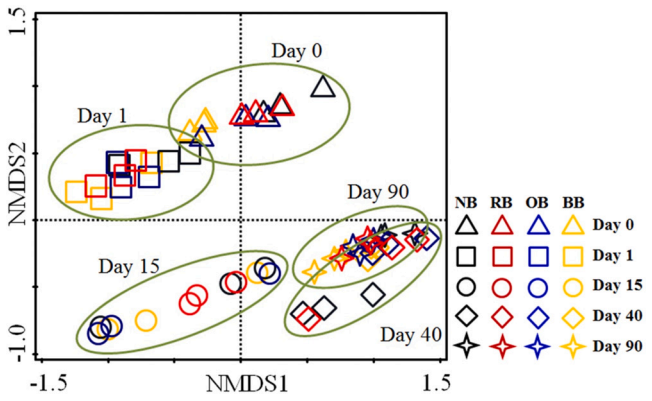

Fig. 3. Non-metric multidimensional scaling (NMDS) shows the difference among samples based on archaeal composition. Different symbols indicate different sampling dates. The color of black, red, blue and yellow denote for NB, RB, OB and BB treatments, respectively.

treatments. *Methanoregula* was detected in all samples along the incubation while its relative abundance was rather low (< 0.62%). Besides, *Methanobrevibacter* (0.02–0.62%), *Methanolinea* (0.02–0.05%) and *Methanospirillum* (0.02–0.05%) were only detected with low relative abundance in several samples. Biochar addition had non-significant difference in the relative abundance of total mathanogens at Days 0, 1 and 90, while compared to NB, OB and RB led to significantly higher relative abundance of total mathanogens at Day 15 and 40, respectively.

3.4. The effect of environmental variables on CH_4 cumulative production and methanogenic community

To summarize the percentage of total variation in CH_4, CO_2, H_2 cumulative production and composition of methanogenic community that can be explained by treatment variables (RB/OB/BB addition and incubation time), relative abundance profiles were further analyzed by redundancy analysis (RDA) (Table S2). All factors together explained 94.9, 96.6, 89.5 and 82.3% of the total variance of CH_4, CO_2, H_2 cumulative production and composition of methanogenic community, respectively. Among which, the last three sampling dates (Day 90, 40 and 15) highly affected CH_4 production, while the first two sampling dates (Day 0 and 1) highly contributed to the total conditional effects of the variation of CO_2 production and methanogenic community structure. Besides, the factors Day 1 and 15 as explanatory variables contributed for 84.3% and 4.3% of the total variation of H_2 production, respectively. Whereas, RB and OB merely significantly ($p < 0.05$) varied CH_4 production with 0.9 and 0.6% of the total variance, and only 2.9% variation of CO_2 production were significantly explained by OB addition.

Furthermore, spearman's correlation analysis revealed the relationship between microbial parameters (relative abundance of methanogens and α-diversity indexes of archaeal community) and physicochemical parameters (CH_4, CO_2, H_2, acetate, and pH value) as well as treatment variables (including incubation time and three biochar types) (Fig. 5). Results showed that *Methanosaeta*, *Methanocella*, *Methanosarcina* and *Methanomassiliicoccus* were significantly positively correlated with CH_4, CO_2 and pH value ($p < 0.005$), while negatively associated with H_2 concentration. On the contrary, *Methanoregula* and *Methanospirillum* showed significantly negative association with the accumulation of CH_4 and CO_2 ($p < 0.05$) and significantly positive correlation with the

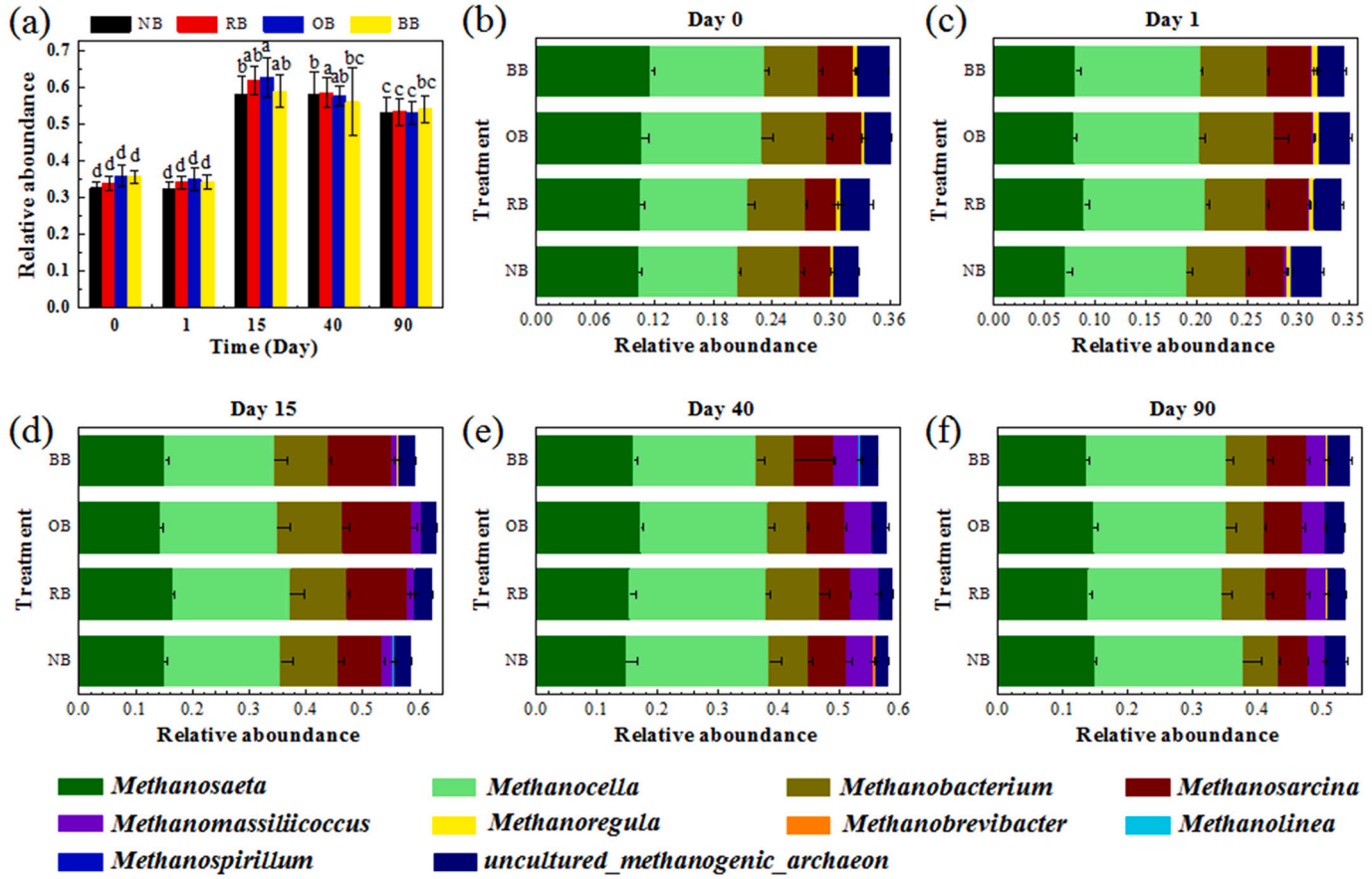

Fig. 4. (a) The total relative abundance of methanogens in different biochar treatments (NB, RB OB, and BB); The distribution of methanogenic archaeal genera in paddy soil amended with different types of biochar at (b) Day 0, (c) Day 1, (d) Day 15, (e) Day 40 and (f) Day 90, respectively. Error bars indicate standard deviation, n = 3.

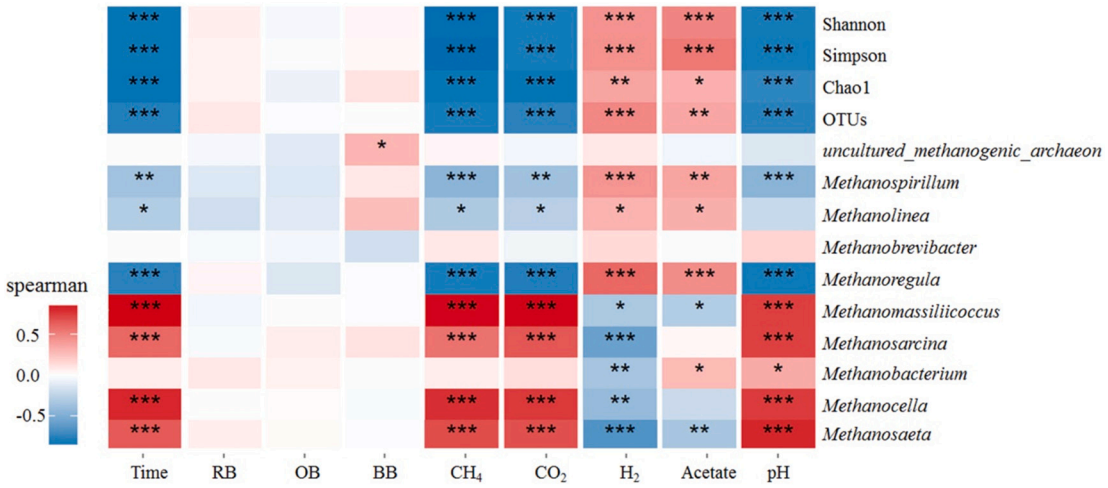

Fig. 5. Heatmap of the spearman's correlation coefficient between environmental variables and methanogenic community. *Adjusted *p* value <0.5; **adjusted p value <0.05; ***adjusted p value <0.005.

concentration of H_2 and acetate ($p < 0.05$). In addition, OTUs, Chao1, Simpson and Shannon indexes of archaeal community had significant negative correlation with CH_4 and CO_2 concentration ($p < 0.005$) but positive correlation with H_2, acetate and pH value. Besides, most genera showed positive or negative association with incubation time, while the influences of biochar amendment on the shift of methanogen and total archaeal diversity were almost non-significant.

4. Discussion

In the present study, we systematically investigate the effect of different types of biochar on CH_4 emission and the succession of associated archaeal community in rice paddy soil of southern China. Biochars were respectively derived from three local and typical forestry and agricultural residues (i.e. rice straw, orange peel and bamboo powder).

The addition of three biochars exerted different degrees of inhibition on CH_4 emission in paddy soil. RB and OB were significantly contributed to the variation of CH_4 production according to RDA result. The decreased CH_4 emission was consistent with the previous studies using biochar derived from corn stalk, bamboo, wood, straw (Chen et al., 2017; Feng et al., 2012; Liu et al., 2011; Wang et al., 2019a), in which the reduction of CH_4 emissions with biochar amendment in paddy soil may result from the inhibition on the abundance of associated methanogens or a stimulation effect on the methanotrophs (Dong et al., 2013; Han et al., 2016; Qin et al., 2016). Furthermore, the negative effect of biochar on the growth/activity of methanogens may be explained by the poor adaptability of methanogens to the increase of soil pH and aeration that caused by biochar amendment (Pietikäinen et al., 2000; Williams and Crawford, 1985).

In order to understand the effects of biochar on archaeal community during the anaerobic incubation, we systematically analyzed the diversity and dynamics of community composition. Results showed that there were considerable losses of archaeal species since Day 1 in all samples, although α-diversity recovered transiently and slightly in the later period. The significant decline of α-diversity during incubation may be attributed to the increases of soil pH (from 5.55 ± 0.02–5.78 ± 0.09 at Day 1 to 6.58 ± 0.03–6.74 ± 0.02 at Day 90 in four treatments as previously measured in the same batch cultures) (Lu et al., 2020). Previous reports also showed that pH was significantly negatively correlated with archaeal diversity (Tripathi et al., 2013), which was consistent with the result of spearman's correlation between α-diversity indexes and pH value in our study. In addition, biochars amendment tended to reduce the loss of archaeal diversity, which could possibly be explained by the lower pH value observed in three biochar-amended treatments than NB treatment as reported previously (Lu et al., 2020). However, Jia et al. (2018) observed an increase in pH following the addition of unmodified biochar to paddy soil, attributing to the dissolution of alkaline functional groups in biochars. Previous study showed that pyrolysis temperature and feedstock as important factor affected the species and quantity of biologically available carbon (eg. water-soluble carbon) in biochar (Luo et al., 2013). Besides, some volatile organic compounds could be absorbed to biochar, which all could serve as nutrients to sustain microbial fermentation (Sun et al., 2014). Biochars generated in our study tended to have higher carbon content comparing to those reported in Jia et al. (2018), thus more organic acids were produced during anaerobic decomposition of organic matter in biochar treatments to reduce alkalinity, which might explain the observation of lower pH value comparing to NB treatment. Also, the presence of high porosity in biochar provided better colonization of microorganisms (Thies and Rillig, 2012). With the analysis on the structure of archaeal community, NMDS results indicated an obvious succession of archaeal community with the process of incubation, including a short period of shifting and the persistently stable period afterwards. Moreover, PERMANOVA and RDA results both demonstrated that the effect of incubation time on the succession of archaeal community surpassed that part of variation caused by different biochar amendment.

Furthermore, biochar amendment in our study showed no significant effect on methanogenic community compositions, which was in accordance with previous reports that indicated the strong resistance of methanogenic community structure to biochar application (Feng et al., 2012; Singla et al., 2014). While in other incubation experiment, straw biochar pyrolyzed at 700 °C was found to have little effect on the community compositions but decrease methanogen abundance and thus decrease CH_4 emission (Cai et al., 2018). Another study also found significant decrease of CH_4 emission under biochar amendment, whereas it was not resulted from the inhibition of methanogenic archaeal growth but from significantly increased abundance of methanotrophic proteobacterial groups (Feng et al., 2012). Besides, RDA results showed that the early stage significantly varied the composition of methanogenic community, which demonstrated that whether biochar was added or not, the compositions of methanogens were shaped during a short period and then tended to be stable. As it was reported that pH was the most influential factor on shaping the archaeal community (Zhou et al., 2017), and in the batch cultures of our study, pH varied in the early stage and became stable since Day 15 in all samples (Lu et al., 2020), which might partly explain the succession of methanogenic community.

Depending on the substrates and metabolic pathways, methanogenic archaea are able to couple methane production and energy generation via the hydrogenotrophic, acetoclastic or methylotrophic methanogenesis, and those methanogens utilizing H_2/CO_2 or acetate are predominant in paddy soil (Bao et al., 2016; Lu and Conrad, 2005; Lu et al., 2015). In our study, *Methanosaeta* and *Methanosarcina* were found to be the dominant acetoclastic methanogen, but *Methanosarcina* was less active than *Methanosaeta* when acetate decreased to 0–0.4 mM after Day 15 (Lu et al., 2020), mainly due to its higher threshold of acetate (0.2–1.2 mM) comparing to *Methanosaeta* (7–70 μM) (Jetten et al., 1992; Peng et al., 2008). Excepting for *Methanosarcina*, *Methanocella* and *Methanobacterium* were suggested to significantly contribute to hydrogenotrophic methanogenesis, in which *Methanobacterium* were active in the early stages with an increased relative abundance, while *Methanocella* potentially dominated this pathway due to its superior living ability to low H_2 threshold (Yuan and Lu, 2009).

Methane emission was inhibited during the anaerobic incubation of paddy soil with three different biochars, which is beneficial for the large-scale application of biochar in the field. Besides, biochar treatments also enable the alleviation of archaeal biodiversity loss and favor to the archaeal community stability. Moreover, non-significant differences were observed among the biochars derived from three different agricultural and forestry residue, which extend the practical scope of those green wastes.

5. Conclusions

In this study, the amendment of three different biochars (RB, OB, BB) all inhibited CH_4 emission in rice paddy soil of southern China within 90 days of anaerobic incubation. The biodiversity of soil archaea transiently decreased due to the change of pH during incubation, while three different biochars all mitigated the loss of archaeal diversity. Incubation time was the driving force for the shift of archaeal community structure rather than biochar addition, compared to NB treatment, only OB and RB addition led to significantly higher relative abundance of total mathanogens at Day 15 and 40, respectively. *Methanosaeta*, *Methanocella*, *Methanobacterium* and *Methanosarcina* as major methanogens were responsible for the production of CH_4 during incubation. Overall, amendment of different types of biochar decreased CH_4 emission, led to the change of total archaeal diversity, but almost had no significant effect on methanogenic community in rice paddy soil. This study reveals the ability of biochar to reduce CH_4 emission and alter associated microbial community, which expands our knowledge to overall assess the influence of biochar amendment on the greenhouse gas emission in paddy soil. Also, it provides fundamental information for developing future strategies of biochar generation from various types of greenwaste and biochar amendment in rice cropping ecosystem.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2021.103892.

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