

MECHANISM ON METHANE OXIDATION OF LANDFILL COVER SOIL AMENDED BY BIOCHAR: A SIMULATED COLUMN EXPERIMENT

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Abstract: Application to landfill cover soils (LCS) of biochar (BC) has been widely suggested as a tool for reducing methane (CH₄) emissions from landfill. However, it is not clear whether biochar promotes methane oxidation through porous structure or nutrients of BC in BC-amended landfill cover soil (BLCS). To explore this question, a BLCS and a ceramic-amended landfill cover soil (CLCS) was prepared. A hydrophobic BC-amended landfill cover soil (HLCS) was also prepared to avoid a contradiction between the waterproofness of the ameliorated soil cover and the promotion of CH₄ oxidation. Three simulated columns were used and named RB, RH and RC based on BC, hydrophobic BC and ceramic. With the CH₄ concentration from 25% to 35%, the relative abundance of *Proteobacteria* in RB and RH was higher than that of RC. And the relative abundance of *Methylobacter* and MOB in RB was higher 10% than that of RC. The difference abundance of MOB in RC was greater between 1#, 5# and 9# sampling ports. The result suggested that CH₄ oxidation is caused by the nutrients of BC.

INTRODUCTION

Landfills become one of the main sources of CH₄ emissions (Bian et al., 2021). It is now well established from a variety of studies, that LCS can be used to reduce CH₄ emissions from landfills (Huang et al., 2020; Wang et al., 2011). MOB in the LCS can oxidize CH₄ (Huang et al. 2021; Laet al., 2018), which mainly produces CO₂, H₂O and microbial energy (Majdinasab et al., 2017), thereby decreasing it emissions from landfills. However, common landfill covering materials have shortcomings (Huang et al., 2020; Scheutz et al., 2011). For example, composting which may contribute to excessive nitrate concentration in soils can pollute the environment (Naggar et al., 2019). Therefore, better replacement materials need to be explored. BC is a product of waste biomass in the absence of oxygen and at high temperatures, and is a low-cost, environmentally friendly carbon-rich material (Campos et al., 2020; M. Zhang et al., 2022). Some researchers have found that adding BC to the LCS can improve CH₄ emissions, proving that is a kind of good

alternative materials (Sadasivam et al., 2014).

BC can enhance the activity of MOB in soil, thereby improving the oxidation performance of CH₄ (Chetri et al., & Green, 2022; Weber et al., 2018). In addition, it also can help the soil retain nutrients (Xia et al., 2022). The nutrients in the modified soil were released at a relatively low rate (Mukherjee et al., 2013), which was conducive to the growth of microorganisms, especially in the newly established landfill soil with low nutrient content. BC has porous structure and high surface area (Sun et al., 2018). It is an ideal habitat and shelter for microorganisms (Huang et al., 2021; Wang et al., 2017) because they can live in pores or attach to the surface of BC (Quilliam et al., 2013). Studies have found that the addition of BC can improve the cation exchange capacity (CEC) of soil (Yao et al., 2022). This can help the growth of microorganisms because soil microorganisms absorb nutrients mainly in the form of ions (Huang et al., 2019; Weber et al., 2018). BC has also been shown to have a strong adsorption capacity and is evident in the effect of CH₄, which helps reduce CH₄ emissions (Issaka et al., 2022; Oliveira et al.,

2017;Windeatt et al., 2014).

The mechanism of BC promoting CH₄ oxidation is controversial. At present, researchers have generally maintained that both porous structure and nutrients have been influential. But some people think contribution to promoting CH₄ oxidation by porous structure (Reddy et al., 2014). (Wu et al., 2020). analysis that the porous structure of BC played an important part in capacity of CH₄ oxidation during researching the BC-amended landfill cover soil (BLCS) in compared with the conventional cover soil. Others think nutrients more responsibilities than the porous structure (Bing et al., 2015). And this study puts forward certain thought that find a substitute similar to of BC with porous structure and unable to provide nutrients. The porous ceramic material has the characteristics of high porosity, large specific surface area and low permeability resistance (Liu et al., 2016; Moritz et al., 2007). It is used to replace BC as LCS amendment in this experiment.

However, in the BLCS, investigators have examined that the permeability coefficient of LCS increased with the content of BC, which leads to the entry of rainwater and affect the CH₄ oxidation. Chen's experiment proposed that water resistance of composites by silane coupling agent (KH-570 was used) amended had been effectively improved (Chen et al., 2021). Sun et al. used this material as a hydrophobic amended for BC and it can act as a waterproof agent and change the hydrophilic soil was confirmed. (Sun et al, 2019). Therefore, CH₄ oxidation was studied by a hydrophobic BC-amended landfill cover soil (HLCS).

The comparative tests of CH₄ oxidation are conducted for the BLCS, HLCS and CLCS. The changes of microbial community structure and spatial distribution of functional microorganisms at different

depths in the simulated columns of the three materials was observed. Two hypotheses are proposed: If the methane oxidation effect of BLCS is better than that of CLCS, it proves that the methane oxidation of BLCS is the result of the action of nutrients of biochar. If the methane oxidation effect of BLCS is the same or basically the same as that of CLCS, it indicates that the porous structure of biochar coating is the main reason for promoting methane oxidation. The mechanism of promoting methane oxidation by biochar can be revealed according to the similarities and differences of methane oxidation effects.

MATERIALS AND METHOD

Experimental materials

The study was conducted as a follow-up to previous study and used the same materials which BC was prepared from waste rice straw at 500°C without oxygen and modified it with hydrophobic modifier silane coupling agent (CH₂=C (CH₃)COOC₃H₆Si (OCH₃)) employed in (Zhang et al., 2022).

The soil exposed to CH₄ for the landfill experiments was obtained from landfill film overlay of Mountain pass site in Guilin, Guangxi, China. It was dried and then passed through 30 mesh sieve to remove rubbish and big stones. Silicon nitride inorganic ceramic material was also used in this experiment, which an atomic crystal prepared by the solid state reaction method and was purchased from Kurt New Material Technology Co., LTD. The properties were similar to BC of the test, with highly porous structure and high surface area (113.24 m²/g). Besides, Total pore volume of adsorption was 0.09 cm³/g and the average diam

TABLE 1

Physical and chemical properties of three covering materials

Proterties	BC-amended soil	Hydrophobic BC-amended soil	Silicon nitride landfill cover soil
Organic matter content/g·kg ⁻¹	59.20	63.300	32.200
Porosity/g·kg ⁻¹	46.12	46.310	25.860
pH	7.640	7.7400	7.5200
P/g·kg ⁻¹	1.500	1.2600	1.2600
K/g·kg ⁻¹	16.30	15.200	13.300
N/g·kg ⁻¹	2.710	2.1600	11.600
Maximum water-holding capacity/g·kg ⁻¹	530.41	410.96	344.06

Experimental setup

Three simulated columns of different cover materials (BC-amended, hydrophobic BC-amended and silicon nitride landfill cover soils) were used in this study. Those columns were named RB, RH and RC accordingly, which made of polyvinyl chloride (PVC) with 100 cm height and 15 cm diameter. The structure was constituted by permeable (15 cm), gravel (10 cm), cover (60 cm), and air (10 cm) layer from bottom to top. The gravel layer facilitated to support the cover layer and drainage. A rainfall device was in the cover layer and drainage. A rainfall device was introduced above the simulated column to mimic precipitation. When excess moisture was in the cover layer, it could be discharged through the permeable layer. Water bath cycle temperature control device was installed outside the simulated column to control the ambient temperature. No. 0 sampling port was set at the top left of the simulation column, and nine sampling ports which numbered 1 to 9 and an interval of 5 cm from top to bottom were also set in front of the cover layer.

Operation method

The simulated landfill gas consisted of CO₂, CH₄ and N₂, which passed through a pipe with holes of uniform size and through an air inlet at the bottom of the column (Yargicoglu et al., 2017), then at a certain rate through the rotameter and humidifier evenly into the gravel layer as a buffer area, and eventually arrived the cover layer. The air was blown by a blower and entered the two instruments described above, then was sent to the simulated column through the air inlet.

The flow rates of air and simulated landfill gas controlled by the rotameter were 50 mL/min and 10-20 mL/min, respectively. The initial temperature of water bath temperature control device and moisture content was 25 °C and 10%. There were four stages to the experiment: stage I (the rate of 15 mL/min) with CH₄ content of simulated landfill gas was 25%, stage II (the rate of 10 mL/min, 15 mL/min and 20 mL/min, respectively) with CH₄ content of simulated landfill gas was 35%, stage III (the rate of 20 mL/min) with CH₄ content was the same as in the stage II, and stage IV (the temperature was maintained at 25°C) with conditions were the same as the previous stage.

For RB, RH and RC, the samples (which were named B1.1, B1.5 and B1.9, H1.1, H1.5 and H1.9, C1.1, C1.5 and C1.9, respectively) obtained by the 1#, 5# and

9# sampling ports at the beginning of operation. At the same time, the end-stage samples were named B2.1, B2.5 and B2.9, H2.1, H2.5 and H2.9, C2.1, C2.5 and C2.9, respectively.

RESULTS AND DISCUSSION

Alpha Diversity

Variations in alpha-diversity of community obtained for analyzing at 97 % identity across different

samples at the end of simulation column operation (Table 2). Alpha Diversity was estimated with the community richness indices (the Chaol and ACE index) and diversity indices (Shannon, Simpson and Good’s coverage index) (Sun, et al., 2022; Zhang, Yi, & Lu, 2022). Good's coverage index represents sample library coverage and reflects whether the sequencing results present the true picture of the sample. Table 2 shows that the values Good's coverage index for all samples exceeded 0.995.

TABLE 2
Alpha Indices Statistics for different covering materials

Sample name	shannon	simpson	chao1	ACE	goods_coverage
B1.1	2.180	0.420	514.84	537.615	0.998
B1.5	1.732	0.316	430.75	445.208	0.998
B1.9	2.161	0.382	466.628	477.666	0.999
B2.1	4.639	0.861	725.082	682.229	0.997
B2.5	3.096	0.557	547.111	556.638	0.999
B2.9	2.992	0.574	512.511	532.057	0.998
H1.1	3.602	0.617	706.211	739.512	0.997
H1.5	3.304	0.601	643.554	651.088	0.997
H1.9	3.952	0.665	779.364	805.204	0.997
H2.1	4.379	0.773	770.507	811.905	0.997
H2.5	4.513	0.774	950.85	1000.6	0.995
H2.9	3.561	0.622	702.078	732.664	0.997
C1.1	4.699	0.890	568.333	565.124	0.998
C1.5	3.140	0.543	660.374	669.01	0.998
C1.9	5.611	0.899	702.202	711.96	0.998
C2.1	3.954	0.800	684.288	707.336	0.998
C2.5	2.988	0.561	568.481	576.494	0.998
C2.9	4.615	0.767	743.01	750.131	0.998

The analysis of Shannon and Simpson

The diversity of microbial community was strongly and positively correlated with the value of Shannon and Simpson, the higher value of Shannon and Simpson indicating that the the diversity was higher and the structure was more complex(Liu et al., 2021). Compared to B2.1, B2.5 and B2.9, the value of

Shannon and Simpson of was increasing than that of B1.1, B1.5 and B1.9. The rising trend explained that the number of microbial species increased with the concentration of CH₄ increased from 25% to 35% in BLCS, and the adaptability of microorganisms to the environment is enhanced by adding BC. The value of 1# and 5# sampling ports of RH were also elevated but 9# were gradually decreased. However, the value of

Shannon and Simpson was almost always higher than that of RB from the beginning to the end of the simulated column operation. The circumstance suggested that hydrophobic BC contributed to diffuse smoothly for CH₄ and oxygen in HLCS, which might be favoring growth of the MOB and acclimated constantly. And other creatures were eliminated because of unable to adapt to the environment with increasing CH₄ concentration. Whereas the value of Shannon and Simpson in RC almost declined, indicating that the microbial diversity was significantly lower. It implies that CLCS could not provide optimum nutrients for advantageous bacteria growth and feasible environment for bacteria activities, which led to fail to acclimate by it.

The changes of Chaol and ACE

The value of Chaol and ACE is proportional to the distribution abundance of the community (Liu et al., 2021). From the beginning to the end of the simulated column operation, the changes of value of Chaol and ACE of 1#, 5# and 9# sampling ports progressively increased in RB. The value of 1# and 5# sampling ports also increased in RH but 9# sampling ports was reduced. The data was the same as the result of Shannon and Simpson. The decline was caused by the hydrophobic BC provided suitable moisture content and gas permeability in HLCS, which was helpful in accelerate upward diffusion of CH₄. And then caused that microorganisms made more active in the middle and upper of HLCS when CH₄ diffused upward with CH₄ concentration from 25% to 35%. The value Chaol and ACE of 1# and 9# sampling ports increased and the value of 5# sampling port decreased in RC. The data showed that the changes of value Chaol and ACE was irregularly at three sampling ports. It might be the complicated environment in CLCS and no nutrients were provided to the microbes. Eventually leading to the microbial communities was unadaptable and not evenly distributed.

COMPOSITION CHANGES OF MICROBIAL COMMUNITY STRUCTURE

The relative abundance of Phylum level

As shown in Fig. 4, *Proteobacteria* were the dominant phyla in the bacterial community occupying >50% of the total abundance (Kubaczyński et al., 2022). Because the MOB belongs to the *Proteobacteria* (Chetri et al., 2022), which speculated that had greater methanotrophic abundance in the three simulated columns. The abundance of *Proteobacteria* of the 1#, 5#, 9# sampling part in RB goes down from 92%, 88% and 89% to 77%, 84% and 78%, respectively, with increasing CH₄ concentration (from 25% to 35%). The relative abundance of *Proteobacteria* of the 1#, 5#, 9# sampling part in RH goes down from 83%, 81% and 79% to 82%, 77% and 79%, respectively. The relative abundance of *Proteobacteria* of the 1#, 5#, 9# sampling part in RC change from 54%, 82% and 61% to 52%, 82% and 71%, respectively. The relative abundance of *Proteobacteria* of the beginning and end simulated column operation in RB and RH was greater than that of RC, suggesting that *Proteobacteria* obtained a good living environment by adding BC and nutrients provided for *Proteobacteria* by BC. And the downward trend in RB and RH might be caused by the organic matter significantly decreased was due to constantly consumed in the limited column environment decreasing of the organic matter which can provide sufficient nutrients for the growth of *Proteobacteria*. Comparing the relative abundance of *Proteobacteria* of initial #1, #5 and #9 sampling parts with that of terminal #1, #5 and #9 sampling parts in RB, RH and RC, the abundance of *Proteobacteria* decreased the least in RH, illustrated that microorganisms were relatively unaffected by elevated methane concentrations. The conclusion was previously confirmed (Kubaczyński et al., 2022).

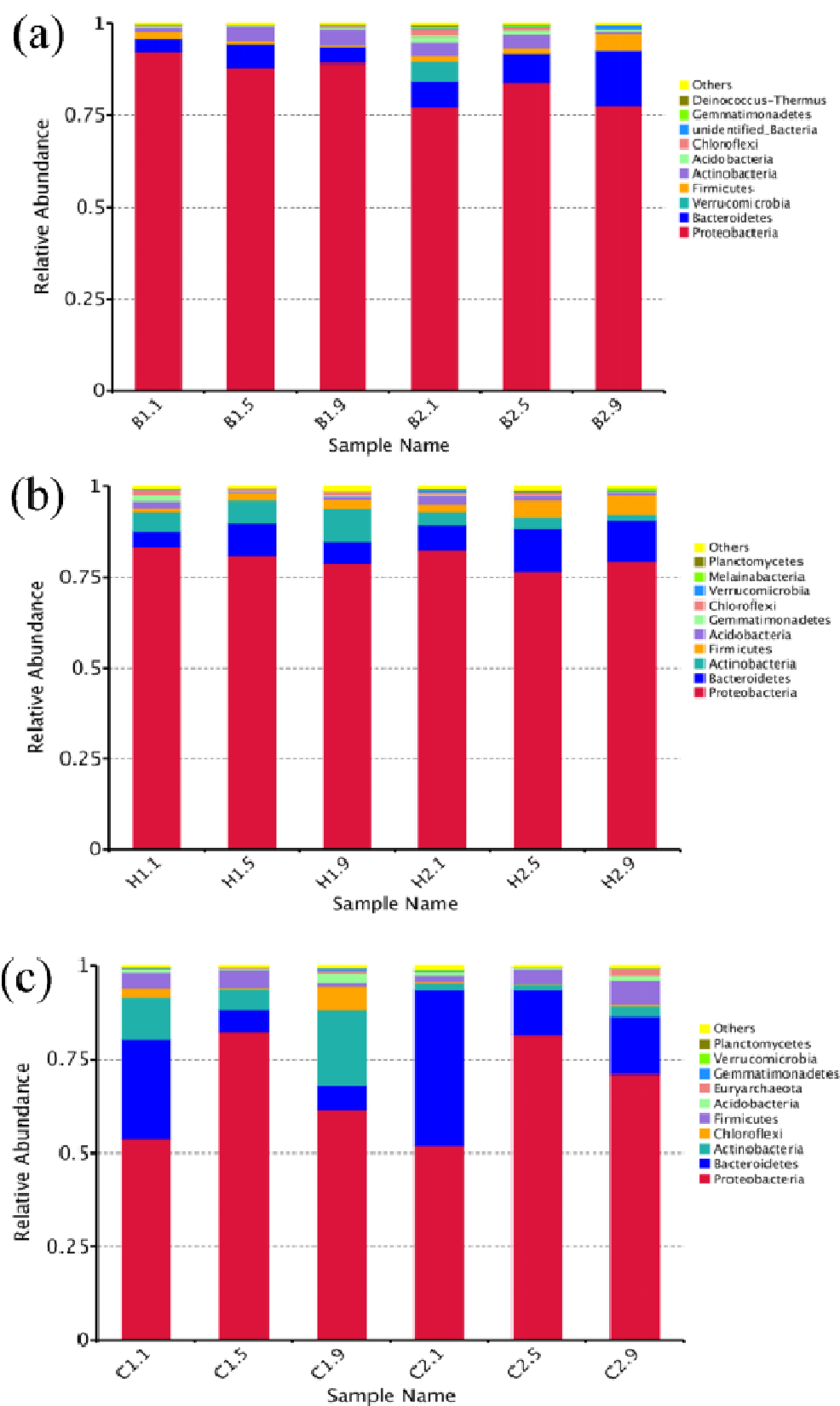


FIG. 1 Spectral relative abundance histogram at the phylum level of RB (a), RH (b) and RC (c)

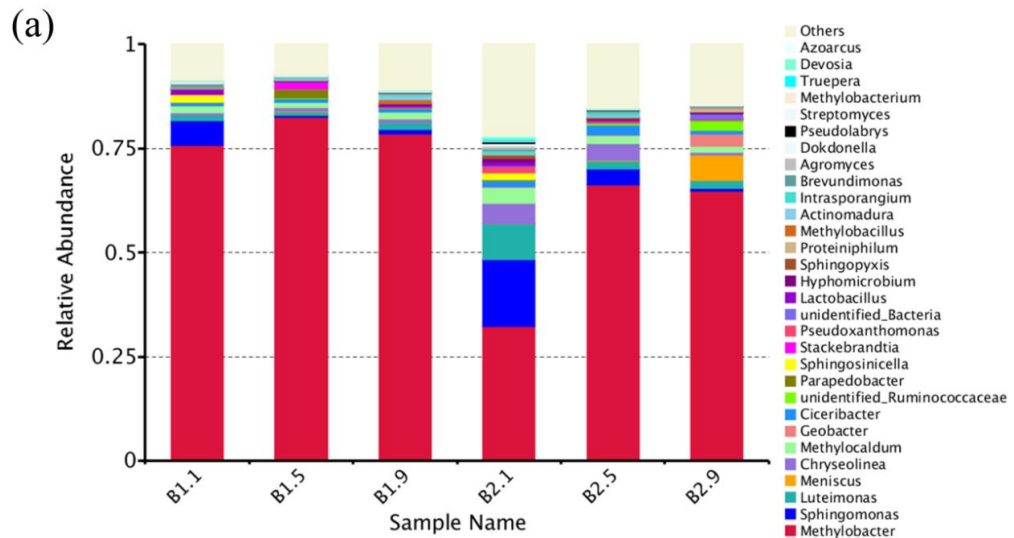
The relative abundance of genus level

The bacterial community structure in simulated

columns has been relatively stable with CH₄ concentration increasing from 25% to 35%. The relative abundance of samples was dominated by *Methyobacter* in the RB, RH and RC (Fig. 6). However, the relative abundance of *Methyobacter* of terminal #1, #5 and #9 sampling parts decreased than that of initial #1, #5 and #9 sampling parts in the RB, RH and RC. The relative abundance of *Methyobacter* decreased in the end because the reduction of organic matter which provided growth and reproduction of microorganism. Compared to RB and RH, the relative abundance of #1, #5 and #9 sampling parts in RC was lower and showed that have significant difference at the same concentration of CH₄. This phenomenon argued that BC addition was beneficial to the growth of *Methyobacter*.

The relative abundance of *Methyobacter* of B2.1, B2.5 and B2.9 were 32.40%, 66.23% and 64.82%, respectively. The relative abundance of *Methyobacter* of H2.1, H2.5 and H2.9 sampling ports were 46.00%, 47.24% and 61.30%, respectively. Meanwhile, the proportion of C2.1, C2.5 and C2.9 were 24.92%, 66.16% and 48.13%, respectively. The relative abundance difference of #1, #5 and #9 sampling ports

in the same simulated column showed that bacterial community distribution of the upper, middle and bottom in the RH was more evenly distributed than that of RB and RC. It indicated that *Methyobacter* was less affected by the change of CH₄ concentration than RB and RC. The average relative abundance of *Methyobacter* of RB, RH and RC was about 54.48%, 51.51% and 46.40%. By contrasting the data between RB and RC, the abundance of *Methyobacter* in #1, #5 and #9 sampling part of RB was greater 7.48%, 0.07% and 16.69% than that of RC (Figs.6 [a] and 6[c]). The data showed that the abundance of adding BC was significantly higher than that of not adding BC. The results demonstrate that the relative abundance of *Methyobacter* was significantly higher caused by BC's nutrients in RB and RH. Furthermore, the relative abundance *Methyobacter* was the most stable in RH. Meanwhile, *Sphingomonas* and *Chryseolinea* were also found more active in the upper layer of RB, RH and RC, probably because that belonged to aerobic bacteria, the results consistent with (Zhang et al., 2022; Zhao et al., 2008).



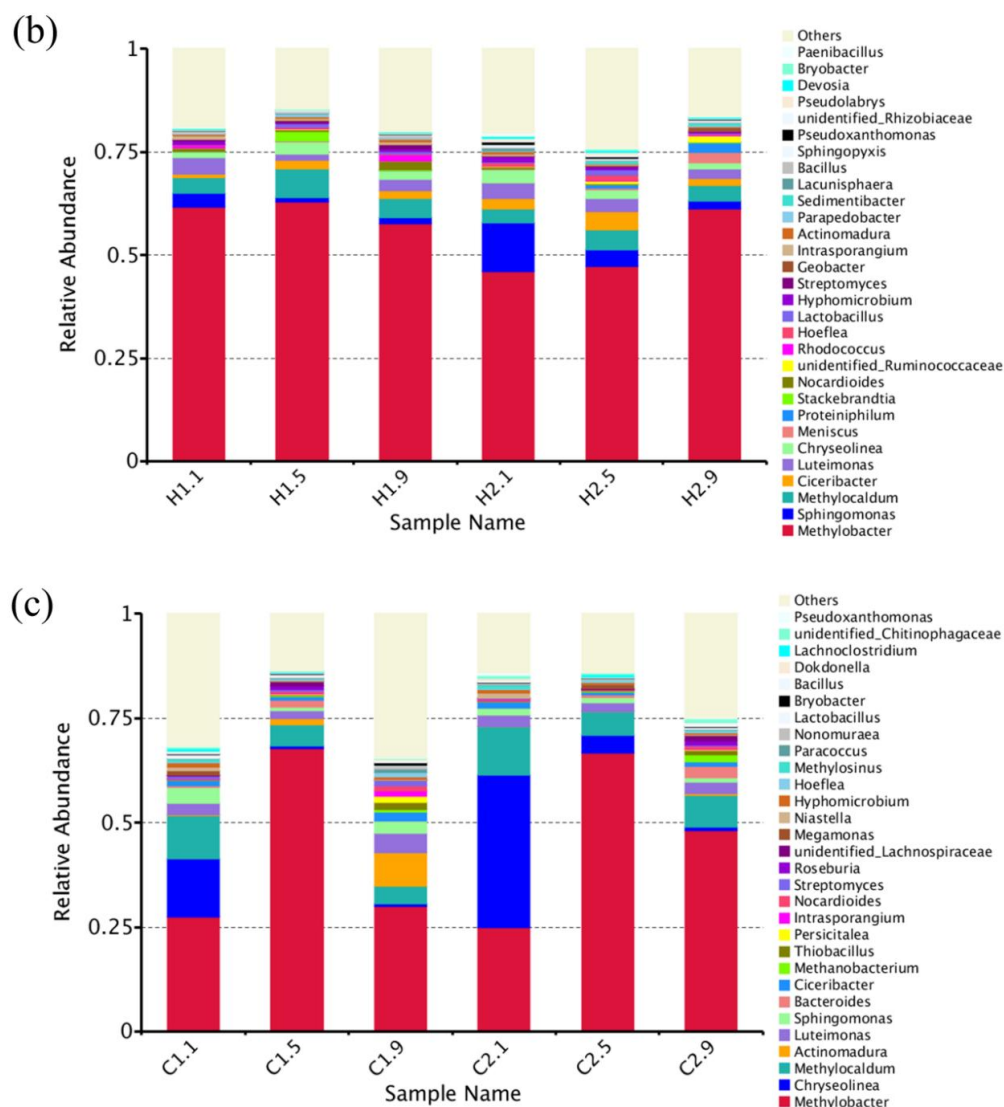


FIG. 2 Spectral relative abundance histogram at the genus level of RB (a), RH (b) and RC (c)

Conclusion

The research verified that the effect of the higher CH_4 oxidation is mainly due to nutrients of BC. With the increase of CH_4 concentration from 25% to 35% in the simulate column, the analysis of Alpha and Beta diversity exemplifies that the diversity of bacteria influenced by CH_4 concentration ranked as: $\text{RC} > \text{RB} > \text{RH}$. The analysis of bacterial community structure and composition of MOB showed that the largest relative abundance of bacteria categories was *Proteobacteria* and the dominant MOB was *Methylobacter* in RB, RH and RC. And the MOB

distribution in RH was more evenly than RB.

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