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Use of methanotrophically activated biochar in novel biogeochemical cover system for carbon sequestration: Microbial characterization

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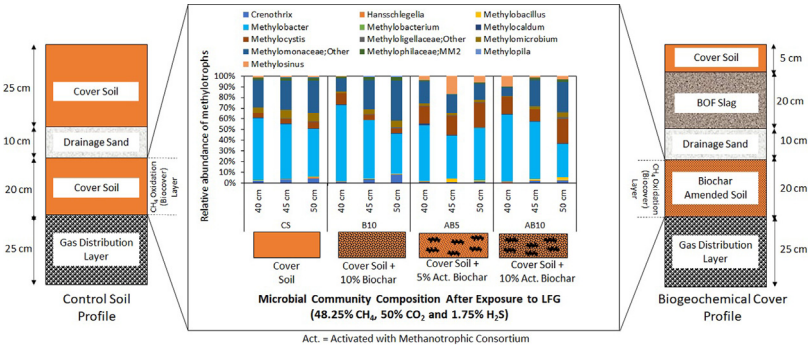
HIGHLIGHTS

- Introduces a biogeochemical landfill cover system
- Utilizes BOF slag and biochar amended soil layers for CH_4, CO_2 and H_2S mitigation
- Proposes methanotrophically activated biochar to enhance microbial activity
- Demonstrates microbial community response to biogeochemical cover configuration
- Evaluates effect of BOF slag layer on methanotrophic communities in biologic layer

GRAPHICAL ABSTRACT



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ABSTRACT

Biochar-amended soils have been explored to enhance microbial methane (CH_4) oxidation in landfill cover systems. Recently, research priorities have expanded to include the mitigation of other components of landfill gas such as carbon dioxide (CO_2) and hydrogen sulfide (H_2S) along with CH_4. In this study, column tests were performed to simulate the newly proposed biogeochemical cover systems, which incorporate biochar-amended soil for CH_4 oxidation and basic oxygen furnace (BOF) slag for CO_2 and H_2S mitigation, to evaluate the effect of cover configuration on microbial CH_4 oxidation and community composition. Biogeochemical covers included a biochar-amended soil (10% *w*/w), and methanotroph-enriched activated biochar amended soil (5% or 10% w/w) as a biocover layer or CH_4 oxidation layer. The primary outcome measures of interest were CH_4 oxidation rates and the structure and abundance of methane-oxidation bacteria in the covers. All column reactors were active in CH_4 oxidation, but columns containing activated biochar-amended soils had higher CH_4 oxidation rates (133 to 143 μg CH_4 g^{-1} day^{-1}) than those containing non-activated biochar-amended soil (50 μg CH_4 g^{-1} day^{-1}) and no-biochar soil or control soil (43 μg CH_4 g^{-1} day^{-1}). All treatments showed significant increases in the relative abundance of methanotrophs from an average relative abundance of 5.6% before incubation to a maximum of 45% following incubation. In activated biochar, the abundance of Type II methanotrophs, primarily *Methylocystis* and *Methylosinus*, was greater than that of Type I methanotrophs (*Methylobacter*) due to which activated biochar-amended soils also showed higher abundance of Type II methanotrophs. Overall, biogeochemical cover profiles showed promising potential for CH_4 oxidation without any adverse effect on microbial community composition and methane oxidation. Biochar activation led to an alteration of the dominant methanotrophic communities and increased CH_4 oxidation.

1. Introduction

Methane (CH_4) emission from municipal solid waste (MSW) landfills has been a growing concern due to its high global warming potential (28

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to 36 times that of CO_2 in 100 years, USEPA, 2021) and its highly explosive nature. Novel landfill cover systems, primarily organics-based systems, have been explored extensively to mitigate landfill CH_4 emissions. Microbially mediated CH_4 oxidation has been identified as a cost-effective technology to manage emissions from MSW landfills (Scheutz et al., 2009; Scheutz and Kjeldsen, 2011; Sadasivam and Reddy, 2014). Recently, focus has shifted to mitigation of other landfill gas (LFG) components such as carbon dioxide (CO_2) and hydrogen sulfide (H_2S) which are also emitted from waste along with CH_4 in the amounts significant enough to cause harmful effects to human health and the environment. In this regard, a novel biogeochemical cover system consisting of biochar-amended soil layer overlain by basic oxygen furnace (BOF) steel slag layer is proposed to mitigate CH_4, CO_2 and H_2S simultaneously (Reddy et al., 2018a, 2018b; Chetri et al., 2019, 2020). Biochar-amended soil can mitigate CH_4 emissions by microbially-mediated CH_4 oxidation (Yargicoglu and Reddy, 2018) and BOF slag can mitigate CO_2 and H_2S by geochemical reactions induced by the mineralogical composition of the slag, mainly calcium and iron containing minerals (Chetri et al., 2019, 2020).

Biochar is a solid carbonaceous product derived from waste biomass through thermochemical processes such as pyrolysis and gasification. Biochar has been used in wide range of environmental applications such as remediation of organic and inorganic soil contaminants, dissolved contaminants in groundwater, and sorption of gases (Xie et al., 2015). The environmental applicability of biochar is governed by its physicochemical properties such as pore size, porosity, specific surface area, pH, functional groups, electrical conductivity, cation exchange capacity, and elemental composition (Kumar et al., 2021). Biochar is characterized by high internal porosity, larger micropores, moisture retention and gas adsorption properties which are suitable for microbial growth (Kumar et al., 2021) and thus microbial gas treatment systems such as landfill covers (Yargicoglu et al., 2015). BOF slag is a type of steel making slag which is characterized with high alkalinity (pH ~12) and rich in metal oxides such as calcium oxides, magnesium oxides and iron oxides (Chetri et al., 2020). Calcium oxides have high propensity to react with CO_2 and form calcium carbonate. Similarly, iron oxides have potential to bind H_2S forming stable iron sulfides (Chetri et al., 2020; Sarperi et al., 2014). The biogeochemical cover leverages these beneficial properties of biochar and BOF slag to simultaneously remove CH_4, CO_2 and H_2S from LFG.

Methane oxidizing bacteria (MOB) play a crucial role in global CH_4 budget. Soils serve as a sink for atmospheric CH_4 due to the activity of MOB (IPCC, 2013), and in the landfills, a portion of the produced CH_4 is oxidized by the MOB before escaping into the atmosphere through landfill cover. Methanotrophs are a group of MOB which have highly specialized metabolic systems capable of utilizing CH_4 as the sole source of carbon and energy, though some can utilize other C1 compounds like methanol (Hanson and Hanson, 1996; Bowman, 2006). The unique ability of methanotrophs to metabolize CH_4 is catalyzed by an enzyme called methane monooxygenase (MMO) (Bowman, 2006). Methanotrophs are part of bigger group of bacteria called methylotrophs which can utilize wide range of C1 compounds other than CH_4 such as methanol, methylated amines, halomethanes, and methylated compounds containing sulfur (Hanson and Hanson, 1996; Bowman, 2006; Jiang et al., 2010). Landfill cover soils have shown wide diversity of methanotrophs and methylotrophs. Both Type I (e.g., *Methylobacter*, *Methylomicrobium*, *Methylococcus*, and *Methylocaldum*) and Type II methanotrophs (e.g., *Methylocystis*, and *Methylosinus*) have been detected in landfill cover soils (Su et al., 2014; Yargicoglu and Reddy, 2017a, 2017b; Reddy et al., 2019). However, there has been mixed reports on the dominant methanotrophic groups. Some studies observed dominance of Type II methanotrophs, such as *Methylocystis* and *Methylosinus* (Gebert et al., 2008), whereas others have observed dominance of Type I methanotrophs mainly *Methylobacter* (Reddy et al., 2019). The structure of methanotrophic communities is affected by a wide range of environmental factors such as pH, temperature, moisture content, and oxygen and CH_4 mixing ratios (Su et al., 2014; Reddy et al., 2019, 2020a).

Biochar-amended soils have shown enhanced potential for CH_4 oxidation with habitation of a broad diversity of methanotrophic and methylotrophic communities including both Type I and Type II methanotrophs (Yargicoglu and Reddy, 2017a, 2018). In prior column-based studies, CH_4 oxidation rates have been positively correlated with the relative abundance of methanotrophs as part of the total microbial community as well as CH_4 exposure history in landfill cover (Yargicoglu and Reddy, 2017a). Exposure history here represents the CH_4 exposure conditions in the landfill cover prior to obtaining the soil sample for the laboratory column tests. Yargicoglu and Reddy (2017a) observed that the locations which were exposed to higher CH_4 fluxes had greater methanotrophic abundance. Similarly, CH_4 and oxygen mixing ratios can play important role in the methanotrophic community composition. Gebert and Perner (2015) reported that Type I methanotrophs were dominant in the upper layers of a cover system with high oxygen and low CH_4 concentrations, while Type II methanotrophs dominated in lower layers with low oxygen and high CH_4 concentrations. Likewise, Wilshusen et al. (2004) observed dominance of Type II methanotrophs over Type I in compost under microaerophilic and nitrogen limiting conditions (lower oxygen concentrations) which were also capable of oxidation CH_4 at superior rates. Similarly, Henckel et al. (2000) also reported greater abundance of Type II methanotrophs at high CH_4 mixing ratios and Type I at low CH_4 mixing ratios in rice field soil.

In the biogeochemical cover, the biochar-amended soil layer is overlain by the BOF slag and the effect of this configuration on oxygen intrusion and microbial community structure and activity is unknown. It is hypothesized that the presence of highly alkaline BOF slag would change the microbial community composition and CH_4 oxidation potential of the underlying biochar-amended soil layer. Column experiments have been commonly used to simulate LFG flow conditions and microbial CH_4 oxidation activities (e.g., De Visscher et al., 1999, 2004; Rachor et al., 2011; Yargicoglu and Reddy, 2017a, etc.). Hence, column experiments were performed with different biogeochemical cover profiles to investigate the potential of biogeochemical cover profiles to remove CH_4, CO_2 and H_2S simultaneously from LFG and to identify the cover profile with the highest LFG mitigation potential which is summarized in Chetri et al. (2022a). This study focuses mainly on the CH_4 oxidation potential and microbial community structure in the biologic layers in the novel biogeochemical cover profiles. The prime objectives of this study were to: 1) Assess the effect of BOF slag layer on the biological CH_4 oxidation potential of underlying biochar amended soil layer in the biogeochemical cover system; 2) Evaluate the effect of biochar and activated biochar amendment to soil on biological CH_4 oxidation in experimental landfill columns; and 3) Evaluate microbial community structure in the experimental columns at different soil depths in relation to CH_4 oxidation.

2. Methods

2.1. Column tests

Three biogeochemical cover profiles along with a control soil were tested in column reactors which are shown in Fig. 1. The column set up is explained in detail in Chetri et al. (2022a). Briefly, Plexiglas columns, each of internal diameter 18.40 cm and height 100 cm, were used for the tests. The inlet LFG was fed from the bottom of the column and humidified air was fed from the top of the column. Gas sampling ports were provided at an interval of 5–10 cm along the depth of the column. The columns had flanged top and bottom and were sealed with rubber O-rings. The flow of LFG into the columns were controlled and the outlet gas flow rates were monitored through flowmeters.

Column 1, served as control, had a 20 cm thick cover soil layer (CS) as CH_4 oxidation (biocover) layer with an overlying 10 cm thick sand layer and a 25 cm thick cover soil layer on top of sand as shown in Fig. 1. The cover soil layer above sand serves as erosion control or vegetative layer rather than supporting microbial activity like biocover. Column 2 had a 20 cm thick 10% (*w/w*) of biochar-amended cover soil (B10) as a CH_4 oxidation (biocover) layer overlain by a 10 cm thick sand, a 20 cm thick BOF slag and a 5 cm thick cover soil layer (Fig. 1). Column 3 had similar configuration as Column 2 except for 5% (*w/w*) of methanotrophically activated

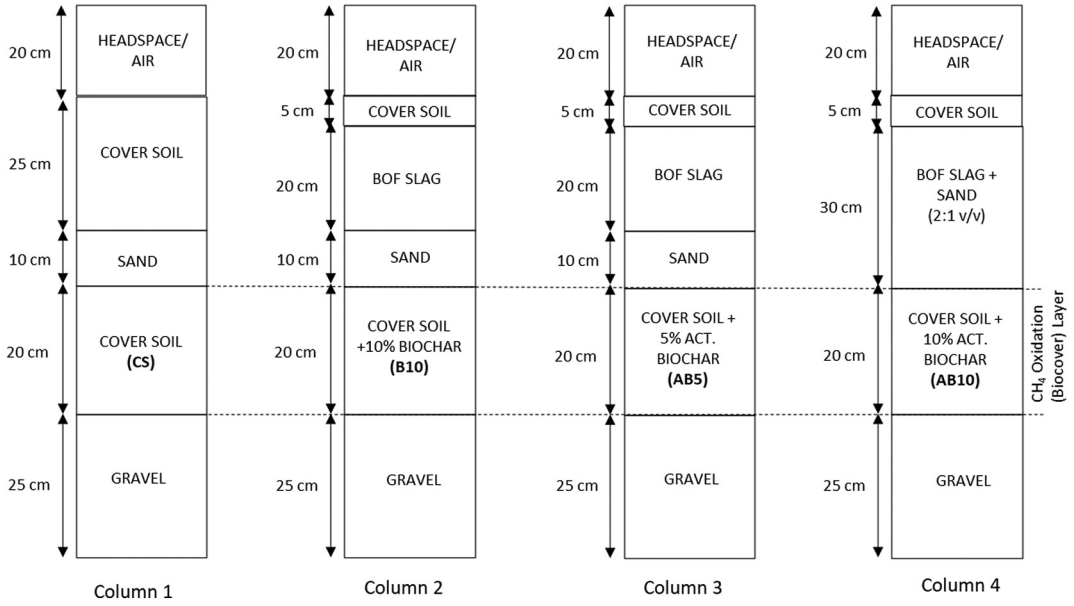

Fig. 1. Schematic of biogeochemical cover profiles tested in the column reactors. Note: CS, B10, AB5 and AB10 layers represent CH_4 oxidation (biocover) layer in each column.

biochar-amended cover soil (AB5) as CH_4 oxidation (biocover) layer, and Column 4 had 10% (*w/w*) methanotrophically activated biochar amended cover soil (AB10) as CH_4 oxidation (biocover) layer overlain by a 30 cm thick mixture of sand and BOF slag (1:2 *v/v*) layer and a 5 cm thick cover soil layer (Fig. 1).

Biochar used in this study was pinewood derived biochar obtained from Chip Energy, Goodfield, IL, USA. The average internal porosity of the biochar was 42%. The high internal porosity of the biochar is suitable for gas adsorption and moisture retention (Yargicoglu et al., 2015). The biochar used in this study had negligible metal contents and leachability of toxic constituents such as polycyclic aromatic hydrocarbons (PAHs) and toxic metals (Yargicoglu et al., 2015) making it suitable for environmental application in landfill cover.

Biochar was methanotrophically activated to introduce methanotrophic communities in the biochar amended soil and reduce lag phase and improve CH_4 oxidation. Biochar activation process is explained in detail by Chetri et al. (2022b). Briefly, a methanotrophic culture consortium was prepared by enriching landfill cover soil in Nitrate Mineral Salts (NMS) medium. Nearly, 5 g of cover soil was mixed with 100 mL of the NMS solution in a 500 mL glass bottle and sealed with long sleeve rubber septa. 80 mL of LFG containing 50% CH_4 and 50% CO_2 was added to the headspace of the bottle after removing equal volume of air. The enrichment of the culture was ensured by monitoring the headspace concentration of CH_4 and CO_2. After enrichment, the supernatant was transferred to acrylic columns for biochar activation. The enrichment cultures were prepared in volumes such that the required amount of biochars were completely soaked in the cultures for activation.

The CH_4 oxidation (biocover) layers were first added to the column and incubated under different LFG flow conditions through Phase I to III (see Table 1) to investigate the microbial community response to changing LFG conditions which is summarized in Chetri et al. (2022b). The layers above CH_4 oxidation (biocover) layer were added in Phase IV to simulate the biogeochemical cover profiles and their LFG mitigation potential. The main reason for exposing biocover layers to changing LFG conditions prior to adding BOF slag layer was to understand the development of microbial communities and their response to changing LFG conditions that may prevail in a landfill cover. The microbial characterization of the samples was performed during each LFG exposure phase outlined in Table 1. Adding BOF slag layer on top would have hindered the sampling process and microbial characterization would not have been possible during Phases I to III, hence BOF slag was added in Phase IV. The columns were exposed to LFG in each phase until a quasi-steady state gas flow conditions were established.

This study focuses on microbial response during Phase IV incubation. LFG was supplied at the base of the column and flushed with atmospheric air from the headspace. Following column tests, the cover soil and CH_4 oxidation (biocover) layer samples (10–15 g) were extracted from various depths of the column (5 cm (cover soil layer), and 40 cm, 45 cm, and 50 cm (biocover layer)). Three replicates were sampled at each depth and analyzed for microbial characterization. Batch incubations were also performed on the samples obtained from these depths and CH_4 oxidation rates were evaluated.

2.2. Batch incubation experiments

The samples exhumed from the columns were stored at 4 °C before performing batch incubation testing. Batch testing was performed following procedure detailed in Reddy et al. (2019). Briefly, approximately 10 g of each sample was taken in a 125 mL sterilized glass serum vial (Wheaton Glass, Milville, NJ, USA). The vial was then sealed with rubber septa and aluminum crimps. Approximately 20 mL of air was removed from the headspace and replaced with an equal volume of 50% CH_4 and 50% CO_2 to achieve headspace concentrations of ~6% CH_4 and 6% CO_2. The headspace concentrations of CH_4 and CO_2 were monitored at regular intervals until CH_4 dropped below 1% or became invariant. Monitoring was performed using a gas chromatograph (SRI Instruments, Torrance, CA, USA) equipped with TCD and FPD/FID detectors for simultaneous measurement of CH_4, CO_2 and H_2S. Each sample was tested in triplicate. Blank vials, containing only headspace, were similarly tested to evaluate CH_4 loss by non-biological processes such as leakage. Methane oxidation rates were calculated

Table 1
Column testing phases.

Testing phase	Inlet gas composition (% *v/v*)	Avg. methane influx (g CH_4 m^{-2} day^{-1})	Duration (days)
I[a]	50% CH_4, 50% CO_2	50	90
II[a]	48.25% CH_4, 50% CO_2, 1.75 H_2S	138	55
III[a]	99% CH_4	100	59
IV	48.25% CH_4, 50% CO_2, 1.75 H_2S	127	36

[a] Results presented in Chetri et al. (2022b).

from concentration vs time plots following zero order kinetics (Yargicoglu and Reddy, 2017b).

2.3. Microbial characterization

Microbial characterization was performed following similar procedure outlined in Chetri et al. (2022b) Soil samples (~1 g) were obtained from various depths of cover soil and CH_4 oxidation (biocover) layers in column reactors after column termination to characterize the structure of microbial communities in response to experimental conditions. Genomic DNA was extracted from each sample using a DNeasy PowerSoil Pro Kit (Qiagen) implemented on a Qiagen instrument. Each sample was weighed before DNA extraction (0.2 to 0.5 g). Bead-beating was performed off-instrument prior to automated extraction using MP FastPrep-24 5G homogenizer (MP Biomedicals, CA) at 6 m/s for 40 s. Microbial 16S rRNA gene abundance was quantified using quantitative real-time PCR, as described previously (Nadkarni et al., 2002). Primers, probes, and a double-stranded synthetic DNA standard (gBLOCKs) were synthesized by Integrated DNA Technologies. Analysis was performed using a ViiA7 real-time PCR instrument (Thermo Fisher), with an 8-order of magnitude dilution series for absolute quantification. Genomic DNA was also used as template for amplification of microbial 16S rRNA gene amplicons using a two-stage PCR protocol as described previously (Naqib et al., 2018). The primer set 515F-806R was employed (Parada et al., 2016; Apprill et al., 2015), and libraries were sequenced on an Illumina MiniSeq instrument, employing paired-end 2 × 153 base reads. Nucleic acid extraction, quantitative PCR, library preparation and sequencing were performed by the Genome Research Core (GRC) at the University of Illinois at Chicago (UIC).

Raw sequence data were processed through a standard bioinformatic pipeline. Briefly, forward, and reverse reads were merged using the software package PEAR (Zhang et al., 2014). Merged reads were trimmed to remove ambiguous nucleotides, primer sequences, and trimmed based on quality threshold of $p = 0.01$. Reads that lacked either primer sequence or any sequences shorter than 225 bases were discarded. Chimeric sequences were identified and removed using the USEARCH algorithm with a comparison to Silva v132 reference sequence (Glöckner et al., 2017; Edgar, 2010). Amplicon sequence variants (ASVs) were identified using DADA2 (Callahan et al., 2016). Representative sequences for each ASVs were then annotated using the Naïve Bayesian classifier included in DADA2 with the Silva v132 reference sequence database (Quast et al., 2012). Basic annotation pipelines were performed by the Research Informatics Core (RIC) at the UIC.

2.4. Terminal material characterization

The CH_4 oxidation (biocover) layer samples exhumed from columns after termination were analyzed for moisture content, pH, and organic content as per ASTM D2216, D4972, and D2974, respectively which are summarized in Table 2 in addition to the microbial community characterization. The main aim was to assess the effect of soil properties on the microbial communities, as studies in the past have pointed out profound effect of these parameters on the microbial communities and their activities (Huber-Humer et al., 2008; Albanna and Fernandes, 2009).

2.5. Statistical analysis

Statistical analysis of batch test results was tested using one-way ANOVA and *t*-tests (equivalency of sample means) using Microsoft Excel-2019. An alpha = 0.05 was used to assess statistical significance in all tests. Microbial community sequence data were analyzed within the software package Primer7 (Clarke and Gorley, 2015) to calculate alpha-diversity indices and generate multidimensional scaling (MDS) plots. Significant differences in community structure between biocover samples were assessed using analysis of similarity (ANOSIM).

3. Results and discussion

3.1. Methane oxidation rates

Methane oxidation rates were determined for each column samples obtained from various depths of cover soil and CH_4 oxidation (biocover) layers, summarized in Table 2. Methane oxidation rates were significantly different among the amendment groups ($p = 0.01$, ANOVA). The maximum CH_4 oxidation rates of 143.2 μg CH_4 g^{-1} day^{-1} and 133.9 μg CH_4 g^{-1} day^{-1} were observed in AB5 and AB10 at 50 cm and 45 cm depth, respectively (Table 2). Methane oxidation rates in CS and B10 were significantly lower than that of activated biochar amended groups across all depths within the biocover layer and ranged from 38.7 to 43.3 μg CH_4 g^{-1} day^{-1} in CS and 27.9 to 50.3 μg CH_4 g^{-1} day^{-1} in B10 (Table 2). The oxidation rates observed in this study are within the range reported in the previous studies conducted on landfill cover soils (Whalen et al., 1990; Reddy et al., 2019; Yargicoglu and Reddy, 2017a). The biocover layers showed significant CH_4 oxidation potential despite being located deeper in the cover system as studies in the past have observed occurrence of methanotrophic activity close to the surface of the cover (Barlaz et al., 2004; Yargicoglu and Reddy, 2017a). One of the plausible reasons for ability of the biocover layer to oxidize significant CH_4 could be sufficient

Table 2
Properties of terminal samples, methane oxidation rates, and relative abundance of methylotrophs at various depths of the tested cover profiles.

Column	Layer description	Depth	MC (%)	OC (%)	pH	Methane oxidation rate (μg CH_4 g^{-1} day^{-1})	Relative abundance of methylotrophs (%)
Column 1	Cover soil	5 cm	16.1	3.2	7.9	59.1 ± 3.6	18.8 ± 0.6
		10 cm	15.9	3.5	7.9	63.1 ± 1.6	15.2 ± 1.6
		15 cm	15.7	3.9	7.8	64.0 ± 1.6	20.5 ± 1.7
		20 cm	16.0	3.2	7.8	57.6 ± 0.1	14.5 ± 0.8
	Cover soil (CS)	40 cm	7.8	5.1	7.6	42.3 ± 3.1	25.5 ± 0.6
		45 cm	8.4	4.9	7.6	38.7 ± 2.1	22.1 ± 0.6
		50 cm	8.8	5.4	7.7	43.3 ± 2.1	17.1 ± 0.9
Column 2	Cover soil	5 cm	19.5	3.3	8.4	21.0 ± 0.6	15.1 ± 1.9
	Soil + 10% biochar (B10)	40 cm	10.4	12.8	7.7	50.3 ± 2.5	30.0 ± 1.6
		45 cm	8.3	12.5	7.8	36.7 ± 0.7	14.4 ± 0
		50 cm	10.4	13.1	7.5	27.9 ± 1.1	13.7 ± 1.0
Column 3	Cover soil	5 cm	18.6	3.5	8.0	24.3 ± 0.4	13.7 ± 0.2
	Soil + 5% activated biochar (AB5)	40 cm	12.5	9.5	7.5	130.6 ± 2.9	33.8 ± 1.4
		45 cm	12.7	10.1	7.4	127. 8 ± 2.3	34.2 ± 0.5
		50 cm	12.8	9.7	7.5	143.2 ± 0.7	26.6 ± 1.6
Column 4	Cover soil	5 cm	17.6	3.8	8.3	48.7 ± 11.7	15.7 ± 0.6
	Soil + 10% activated biochar (AB10)	40 cm	11.8	16.9	7.9	108.6 ± 1.0	48.7 ± 2.3
		45 cm	11.9	12.6	7.9	133.9 ± 1.5	23.4 ± 1.5
		50 cm	11.7	11.1	8.0	80.6 ± 0.4	14.5 ± 0.5

Note: MC = moisture content; OC = organic content.

aeration owing to the high porosity of the overlying layers (sand, BOF slag and soil) as porosity plays important role in gas flow. In addition, methanotrophic communities can oxidize CH_4 even at lower oxygen mixing ratios as Wang et al. (2011) found that oxygen as low as 5% (*v/v*) was sufficient for CH_4 oxidation activity. Studies have also highlighted that aerobic methanotrophs have ability to function under micro-oxic conditions by altering their metabolic activities (Guerrero-Cruz et al., 2021).

3.2. Microbial community composition in the biocover layers

The total bacterial 16S rRNA gene abundance in the CH_4 oxidation (biocover) layers ranged from 1.5×10^9 to 3.31×10^9 (Fig. 2a). The total bacterial 16S rRNA gene abundance was not significantly different across the biocover groups in general and a particular trend with depth could not be established. However, significant differences between non-activated groups and activated biochar amended groups were observed mainly at 40 cm depth (Fig. 3a). The estimated normalized methylotrophic bacterial abundance, calculated by multiplying relative abundance of methylotrophs with total bacterial gene abundance, ranged from 5.63×10^8 to 2.39×10^9 (Fig. 3b). Like total bacterial abundance, a particular trend with amendment groups were not observed in methylotrophic abundance. However, at 40 cm depth, the activated biochar amended biocover groups showed slightly higher methylotrophic abundance than non-activated groups (Fig. 3b). It is likely that the top 10 cm of the biocover layer (40 cm from top surface) had better conditions for methylotrophic growth relative to the deeper parts of the layer.

Soil bacterial communities were comprised mostly of bacteria from 5 phyla (Fig. 4a). *Proteobacteria*, which comprised 30–46% of all sequences, were the most abundant phylum. Methanotrophs were 34–92% of all Proteobacterial sequences. Apart from *Proteobacteria*, samples were inhabited with non-methane oxidation bacterial phyla such as *Actinobacteria* (29 to 38%), *Chloroflexi* (8.5 to 12%), *Firmicutes* (4 to 6%), *Bacteroidetes* (2.5 to 4%), and *Gemmatimonadetes* (2 to 3.3%) which are known to be commonly present in soil and play crucial role in its nutrient cycling (Yargicoglu and Reddy, 2017a, 2017b; Long et al., 2016).

The biocover samples were primarily inhabited with methanotrophic families such as *Methylomonaceae* (which houses Type I methanotrophs) and *Beijerinckiaceae* (which houses Type II methanotrophs) attributing to 9–32% and 1–12% of total bacterial sequences, respectively (Fig. 4b). The relative abundance of methanotrophic family *Methylomonaceae* was not significantly different among the treatment groups while *Beijerinckiaceae* was significantly different among the groups ($p = 0.014$, ANOVA) with activated biochar (AB5 and AB10) showing significantly higher abundance (7–10%) in comparison to the non-activated groups (B10 and CS, 1.5–1.8%).

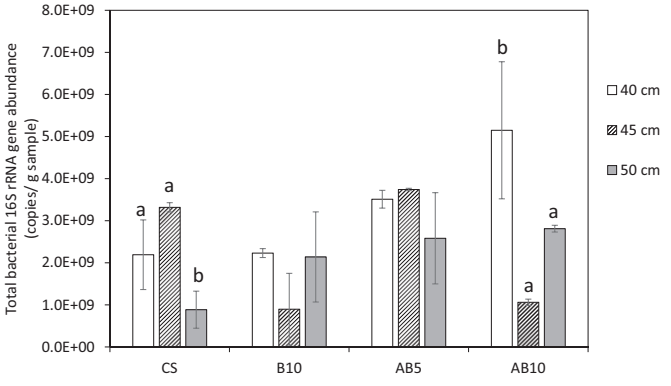

Fig. 2. Total bacterial 16S rRNA gene abundance along the depths of CH_4 oxidation (biocover) layer in each column. Note: The alphabets on top of the bar refer to significant differences at the 5% level based on the *t*-test (MS Excel 2019).

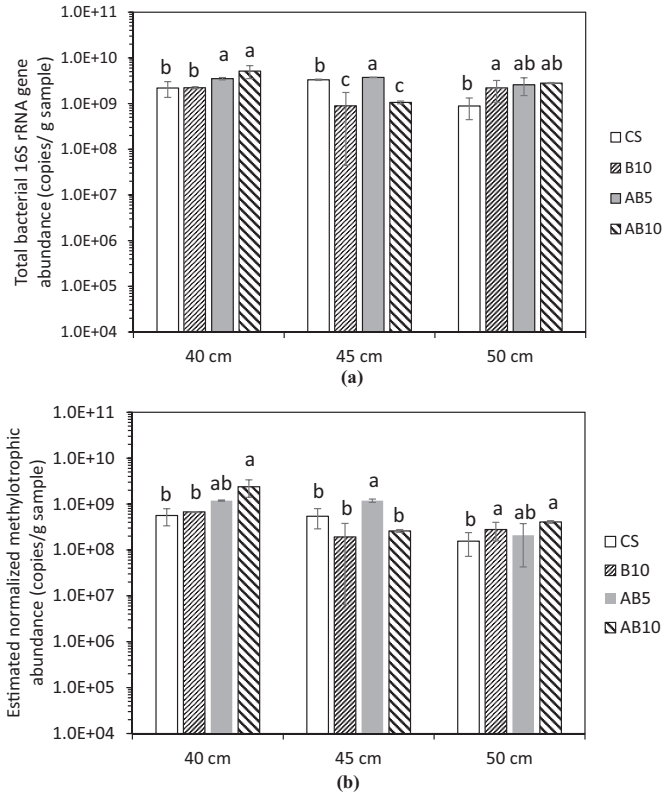

Fig. 3. Abundance of: a) Total bacterial 16S rRNA gene copies; and b) Estimated normalized methylotrophic abundance obtained by multiplying total bacterial copies with relative abundance of methylotrophs in the CH_4 oxidation (biocover) layer in each column. Note: Different letters within the graph refer to significant differences within the soil groups at the 5% level based on the *t*-test (MS Excel 2019).

3.3. Effect of biochar amendment on methanotrophic community composition

Each of the biocover sample was analyzed for microbial diversity prior to incubation as well as after column termination to track changes in the community composition due to exposure to synthetic LFG in column reactors. The cover soil used in this study was exposed to CH_4 in the landfill prior to sampling and hence showed presence of methanotrophs before incubation in column reactors as methanotrophs utilize CH_4 as the sole source of carbon and grow in a CH_4 rich environment. During incubation in column reactors, the methanotrophic abundance increased as the injected LFG had 48.25% CH_4 (*v/v*) which acted as the main source of carbon for the methanotrophic growth. Although the injected LFG also comprised 50% CO_2 (v/v), a minimal effect on microbial communities due to CO_2 was anticipated. Studies in the past have also reported negligible effect of CO_2 on microbial CH_4 oxidation (Spokas and Bogner, 2011).

The cover soil (CS) in the biocover layer in Column 1 had average methanotrophic abundance of 5.8% before incubation in the column reactor (Table 3). Amendment of cover soil with non-activated biochar (B10) resulted in reduction of methanotrophic abundance (4.1%) before column incubation as biochar itself is not microbially active and thus replaces the microbially active soil, reducing the overall microbial load (Rai et al., 2019). Therefore, biochar was infused with MOB consortium through activation process to offset the impact of adding non-activated biochar to cover soil. The activated biochar alone had 23.6% of methylotrophic abundance out of which 38% were methanotrophs (Fig. S1a). As a result, biocovers with activated biochar had greater relative abundance of methylotrophs prior to column incubation (AB10, 7.4% and AB5, 5.3%) than B10 (Fig. S1b).

After incubation in column reactors, methylotrophic bacteria grew rapidly across all the biocover groups leading to 270–490% increase in the

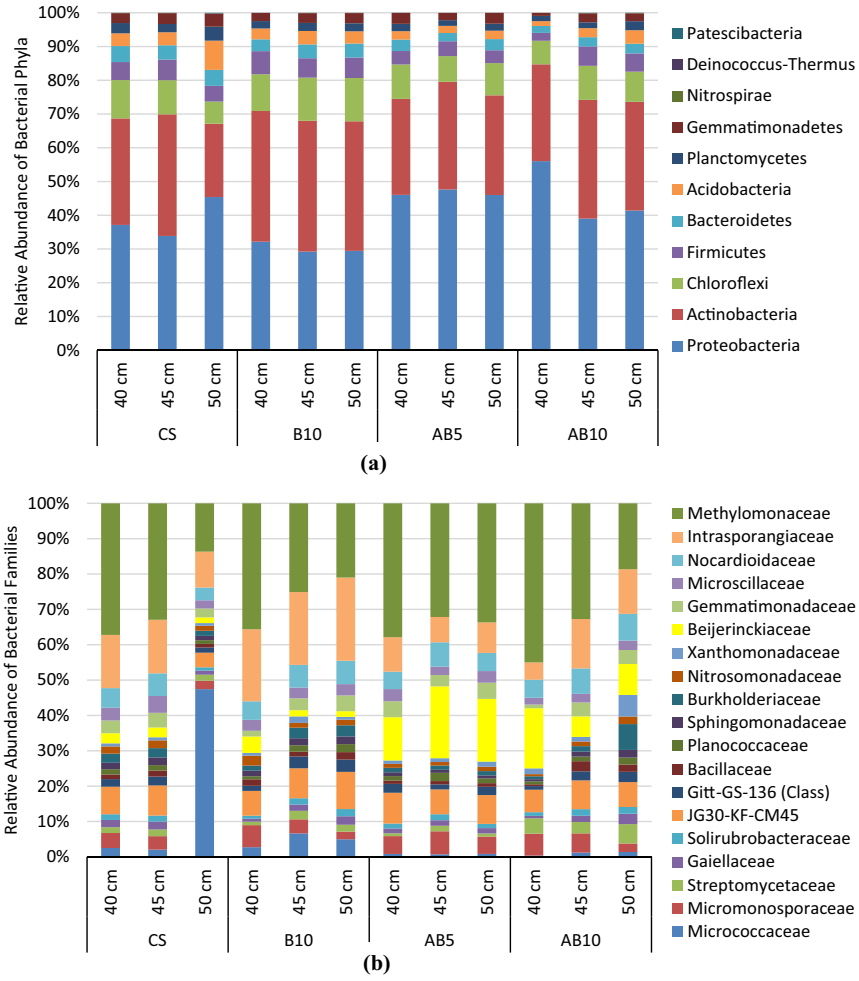

Fig. 4. Relative abundance of: a) Dominant bacterial phyla; and b) Dominant bacterial families in the CH_4 oxidation (biocover) layer in each column.

average methylotrophic relative abundance (Table 3). About 97 to 98% of the methylotrophs sequenced were methanotrophs. Biochar amended biocovers demonstrated greater increase in methylotrophic abundance in general than the CS which could be attributed to the physicochemical changes in soil properties induced by biochar addition, that likely created conducive environment for microbial proliferation. Similarly, the maximum methylotrophic abundance was observed at 40 cm below ground surface (bgs) or top 5 cm of the biocover layer in each column and ranged from 26 to 48% of the total sequences. Moreover, activated biochar amended biocovers (AB5 and AB10) had significantly higher methanotrophic abundance in comparison to B10 and CS combined ($p = 0.0077$, ANOVA). This could be attributed to the collective advantage of biochar activation and physicochemical properties of biochar which promoted microbial growth and proliferation in the column reactors.

Table 3
Comparison of relative abundance of methylotrophs in CH_4 oxidation (biocover) layers before incubation and after column termination.

Methane oxidation layer (biocover)	Relative abundance of methylotrophs				
	Before column incubation (avg.)	After termination (avg.)	After termination (max.)[a]	% Increase (avg.)	% Increase (max.)
CS	5.8	21.6	25.5	272	340
B10	4.1	20.1	30	390	632
AB5	5.3	31.3	32.9	491	521
AB10	7.4	33.6	48.8	354	559

[a] Maximum abundance of methanotrophs were observed in the top 5 cm (40 cm bgs) of the CH_4 oxidation (biocover) layer in each column.

The major methanotrophic genera identified were *Methylobacter*, *Methylocaldum*, *Methylomicrobium*, and *Crenothrix*, grouped as Type I methanotrophs and *Methylocystis*, and *Methylosinus* grouped as Type II methanotrophs (Fig. 5). The samples were dominated by Type I methanotrophs mainly genus *Methylobacter* accounting for 60 to 73% of the total methanotrophic abundance (Fig. 5) which is consistent with the past studies on landfill cover soil (Cébron et al., 2007; Jugnia et al., 2009; Reddy et al., 2019, 2020a, 2020b). However, activated biochar amended biocovers (AB5 and AB10) had significantly higher ($p = 0.01$, ANOVA) abundance of Type II methanotrophs (*Methylocystis* and *Methylosinus*) accounting for 28 to 30% of the total methanotrophic abundance in comparison to CS and B10 (7.6 to 11%) (Fig. 5). This is because activated biochar alone had Type II methanotrophic dominance over Type I, accounting for 84% of total methanotrophic abundance (Fig. S1b). The selection of Type II over Type I methanotrophs could be attributed to the biochar activation process. Since large quantity of biochar was soaked in the MOB consortium in the process of biochar activation, there may have been competition for the nutrients and oxygen among the heterotrophs leading to nutrient and oxygen deficient environment. Studies suggest that Type II methanotrophs survive better in the nutrient/nitrogen depleted or scarce environments (Hanson and Hanson, 1996; Wise et al., 1999) as well as in the environments with low oxygen and high CH_4 concentration (Cébron et al., 2007; Gebert et al., 2008, 2009). In addition, some studies showed the growth of Type II methanotrophs over Type I in

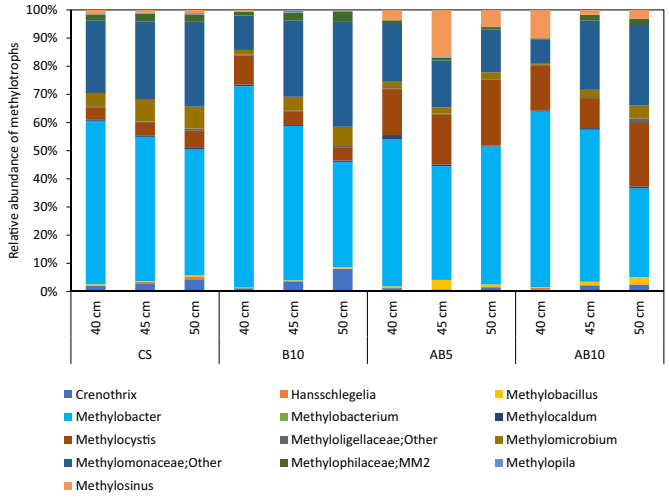

Fig. 5. Relative abundance of methylotrophs present in the CH_4 oxidation (biocover) layer in each column post termination.

water saturated soil with lower CH_4 mixing ratios (Knief et al., 2006). Since biochar was completely soaked in the MOB culture during activation process, it may have created hydromorphic conditions for methanotrophs leading to selection of Type II methanotrophs over Type I.

A slight shift in the community composition of Type I methanotrophs was observed before and after column incubation. Before incubation in column reactor, CS sample had greater abundance of Type I methanotrophs such as *Crenothrix*, *Methylovulum*, and *Methylomicrobium* accounting for 8.3%, 4.6% and 16.22% of the total methanotrophic bacterial abundance, respectively (Fig. S1b). However, in the column incubated CS samples, the abundance of *Crenothrix* had reduced to 2.1% and *Methylomicrobium* to 4.8% out of total methanotrophic bacterial abundance (Fig. 5). *Methylovulum* was not detected in the column incubated CS samples. This shift could be attributed to differences in oxygen and CH_4 mixing ratios in the landfill cover and column reactor. The cover soil used in the column test was sampled from near the surface (0–30 cm) of the intermediate landfill cover. However, in the column reactor, the biocover layer was placed 35 cm bgs and extended up to 55 cm bgs with layers of sand and cover soil lying on top. Some of these methanotrophs such as *Crenothrix* are commonly present in upland soils, oxidizing atmospheric CH_4 and therefore, likely thrive well near the surface with abundant oxygen supply (Kolb, 2009; Knief, 2015; Yargicoglu and Reddy, 2017a).

Along with methanotrophs, the samples were inhabited with non-methane oxidizing methylotrophs such as *Methylobacillus*, *Hansschlegelia*, *Methyloversatilis*, *Methylopila*, and *Methylibium*. Landfill cover soils have shown such wide diversity in microbial communities in the past (Yargicoglu and Reddy, 2017a; Reddy et al., 2019, 2020a, 2020b). The co-existence of these methylotrophs with methanotrophs suggest denitrification as well as assimilation of intermediate products of methanotrophic CH_4 oxidation (do Carmo Linhares et al., 2021).

3.4. Effect of depth on methanotrophic distribution within biocover layer

Methanotrophic abundance varied along the depth in CH_4 oxidation (biocover) layer mainly in Column 1, Column 2 and Column 4 ($p = 0.019$, ANOVA). A decreasing trend was observed with depth in each column (Fig. 6a). Fig. 6b shows relative abundance of four of the most abundant methanotrophic genera in each biocover layer at various depths. *Methylobacter* (Type I methanotroph), one of the most abundantly present methanotrophic bacteria differed significantly along the depth in each biocover, whereas other abundant methanotrophic genera such as *Methylocystis* and *Methylosinus* (Type II methanotrophs) were not significantly different along the depth. It is indicative of the sensitivity of the *Methylobacter* genus towards gas transport, oxygen and CH_4 mixing ratios as Type I methanotrophs are known to react more sensitively towards changing environmental conditions including soil gas transport and oxygen availability (Henneberger et al., 2012; Gebert et al., 2009; Henckel et al., 2000). In contrary, Type II methanotrophs showed mostly stable distribution along the depth which is consistent with the observations of Henckel et al. (2000) where Type II methanotrophs remained fairly stable under different gas mixtures. Overall, prevalence of Type I methanotrophs in the biocover layer in all the columns suggests sufficient aeration as Type I methanotrophs are selective towards high oxygen concentrations (Cébron et al., 2007; Gebert et al., 2008, 2009). Sufficient availability of oxygen in the biocover layer also indicates that the overlying BOF slag did not impede oxygen flow into the underlying biocover layer which was anticipated to be a concern in the beginning.

3.5. Relation between methane oxidation rates and methanotrophic distribution

The average methanotrophic abundance was significantly higher in activated biochar amended biocovers (AB5 and AB10) in comparison to non-activated groups (CS and B10, $p = 0.014$, ANOVA) (Fig. S2a). However, the average relative abundances of Type I methanotrophs were not significantly different among the treatment groups (Fig. S2b). On the other hand, Type II methanotrophs were significantly different among the groups with activated biochar having significantly higher relative abundance than non-activated groups ($p = 0.013$, ANOVA) (Fig. 7). Activated biochar amended biocovers also showed greater CH_4 oxidation rates (Table 3), hence it suggests a likely link between Type II methanotrophs and CH_4 turnover rates. Furthermore, a positive correlation was observed between relative abundance of Type II methanotrophs (*Methylocystis* and *Methylosinus*) and CH_4 oxidation rates ($R^2 = 0.75$, $p < 0.01$, Regression, Excel 2021) (Fig. 8a). Similarly, ratio of Type II/Type I methanotrophs also showed positive correlation with the CH_4 oxidation rates ($R^2 = 0.95$, $p < 0.01$, Regression, Excel 2021) (Fig. 8b). Type II methanotrophs are considered "low affinity" methanotrophs which initiate CH_4 oxidation only at higher CH_4 levels (0.8–66 μmol L^{-1}) but have higher kinetic parameters resulting in higher CH_4 turnover rates (Bender and Conrad, 1992; Henckel et al., 2000; Huber-Humer et al., 2008; Yargicoglu and Reddy, 2017a). Hence, activated biochar amended soils had greater CH_4 oxidation rates likely due to the greater abundance of Type II methanotrophs. Although the trends observed in this study indicate likely link between Type II methanotrophs and CH_4 oxidation rates (Fig. 8), the quality of data may not be enough to reach conclusive remarks. Hence, further studies are warranted to confirm these findings.

3.6. Diversity of microbial communities in cover soil

The biogeochemical cover profiles tested had 5 cm thick cover soil layer overlying BOF slag layer in each column. The control column (Column 1) had 20 cm thick cover soil layer above sand layer instead of BOF slag layer making a total cover soil layer thickness of 25 cm (Fig. 1). The layers above CH4 oxidation (biocover) layer were added in Phase IV and hence were incubated for lesser duration than the biocover layers (Table 1). Cover soil samples from Columns 2, 3 and 4 taken from a depth of 5 cm bgs and from Column 1 taken from depths of 5 cm, 10 cm, 15 cm, and 20 cm bgs were analyzed for microbial distribution. The total bacterial communities and methanotrophic communities were significantly different between cover soil and biocover layers ($R = 0.297$ and $R = 0.337$, respectively at $p = 0.001$, ANOSIM) as shown in Fig. 9. Similarly, relative abundances of the methylotrophic bacterial communities in the cover soil were relatively lower than that of the biocover layers which were incubated for longer duration (Table 2). This suggests a possible link between CH_4 exposure time and methanotrophic abundance rather than oxygen mixing ratio since the cover soil had greater oxygen availability in comparison to the biocover layer. Wang et al. (2011) reported that the 5% (*v*/*v*) oxygen was enough to sustain methanotrophic activity in landfill cover soil and they observed that oxygen concentrations greater than 5% did not increase CH_4 oxidation. Since oxygen penetrated deeper into the biocover layers throughout the incubation period, there was sufficient oxygen mixing

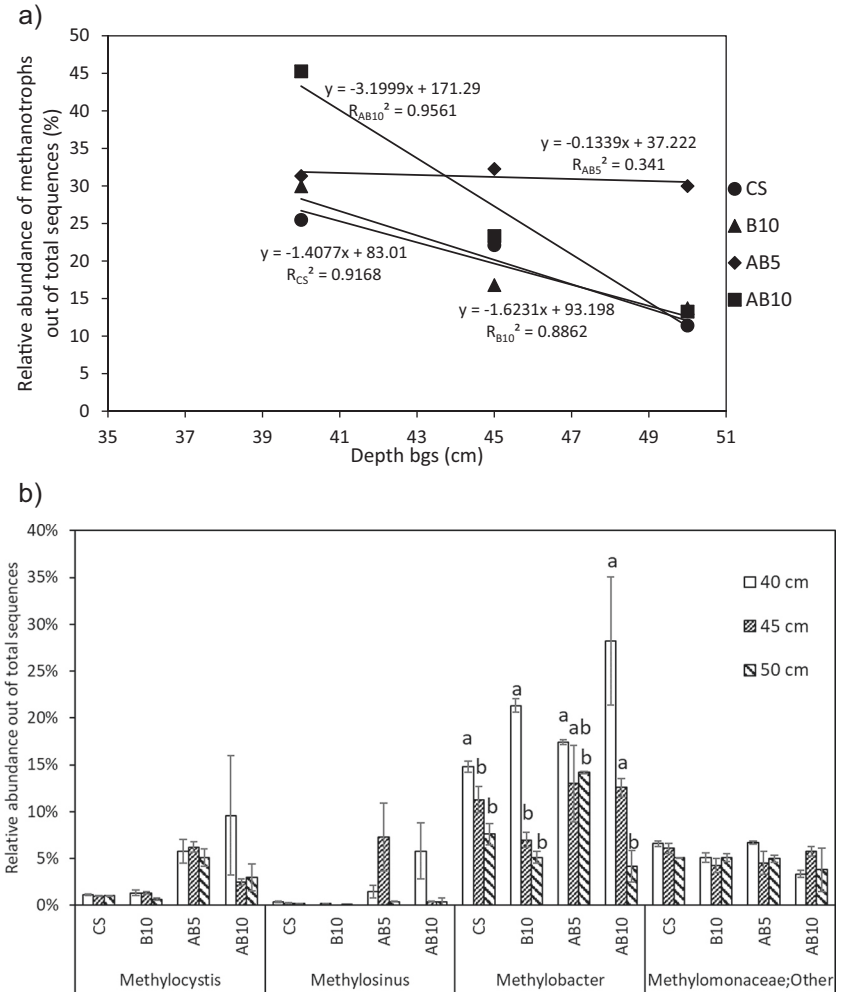

Fig. 6. a) Correlation between relative abundance of methanotrophs and depth within CH_4 oxidation (biocover) layer; and b) Relative abundance of most abundant methanotrophs out of total sequences along the depths of biocover layer in each column. Note: The alphabets on top of the bar refer to significant differences at the 5% level based on the *t*-test (MS Excel 2019).

ratio to sustain methanotrophic population. Although the cover soil in Column 1 had lower methanotrophic abundance, it had greater CH_4 oxidation rates than the CS layer (Table 2). This could be likely due to variation in moisture contents. The CS layer had undergone significant drying due to long-term incubation and moisture content dropped to 7.8–8.8% from initial 15% while cover soil maintained the moisture content of approximately 15% (Table 2) which is an optimum moisture content for CH_4 oxidation in landfill cover soils (Boeckx and Van Cleemput, 1996; Visvanathan et al., 1999).

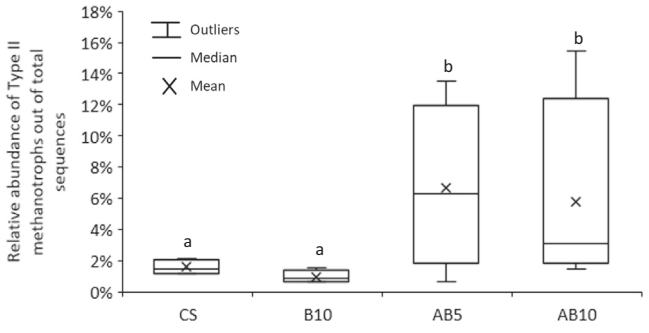

Fig. 7. Box plots showing average relative abundances of Type II methanotrophs out of total sequences in the CH_4 (biocover) layers. Note: The alphabets on top of the bar refer to significant differences at the 5% level based on the *t*-test (MS Excel 2019). The activated biochar amended soils had significantly higher relative abundance of Type II methanotrophs than the non-activated groups ($p = 0.004$, ANOVA).

Interestingly, no bacterial cells were detected in the sand layer overlying the biocover layer in all the columns. The reason could be the negligible organic content (0.6%) of the sand and the moisture content as the sand was placed dry with zero moisture content. Moisture is an important parameter for microbial CH_4 oxidation. Studies have shown inhibited CH_4 oxidation rates in dry state of soil (Wang et al., 2011; Saari et al., 1998). In addition, Rachor et al. (2011) showed lowest CH_4 removal efficiency in sandy soil with lowest organic matter (0.7%).

3.7. Effect of H_2S on microbial community composition

The columns were exposed to LFG mixture containing 1.75% (*v/v*) of H_2S along with 48.25% CH_4 and 50% CO_2 during two of the incubation phases (Table 1). In an oxic environment, some bacteria can consume sulfide ions and oxidize them to elemental sulfur or sulfur species (Burgess et al., 2001). Biological H_2S oxidation results in production of H^+ ions resulting in reduction of soil pH which may negatively affect the methanotrophic growth. In this study, a notable effect was not observed on the CH_4 oxidation and microbial community structure due to injection of H_2S. Sulfur oxidizing bacteria such as *Halothiobacillus*, *Thiobacillus*, *Thiovirga* and *Bradyrhizobium* and sulfur-metabolizing bacteria such as *Comamonas* and *Acinetobacter* previously reported in landfill cover soils and different biocover soils (Xia et al., 2014, 2015) were not detected in the column samples in this study. This

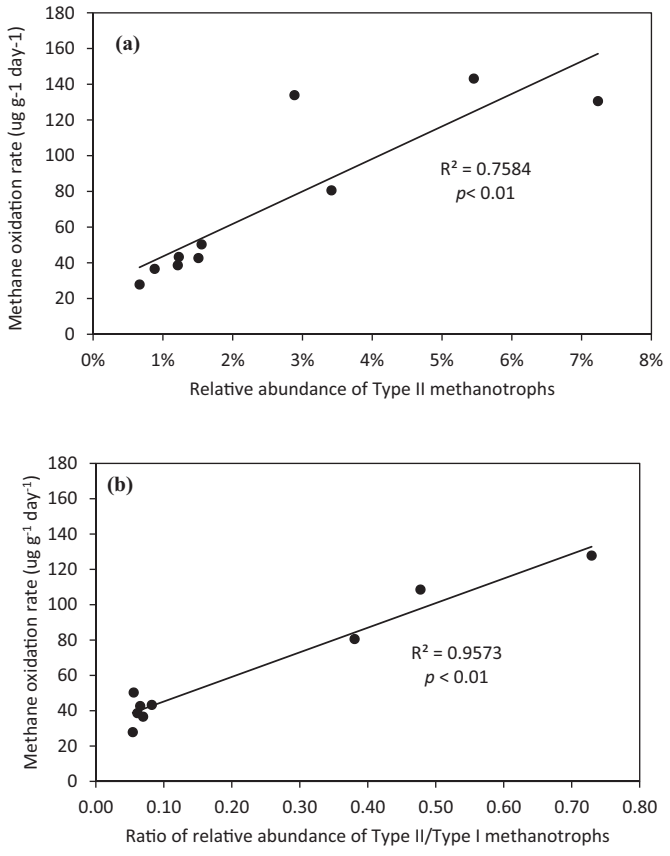

Fig. 8. Relation between methane oxidation rate and: a) Relative abundance of Type II methanotrophs (*Methylocystis* and *Methylosinus*); and b) Ratio of relative abundance of Type II/Type I methanotrophs in the CH_4 oxidation (biocover) layer in each column.

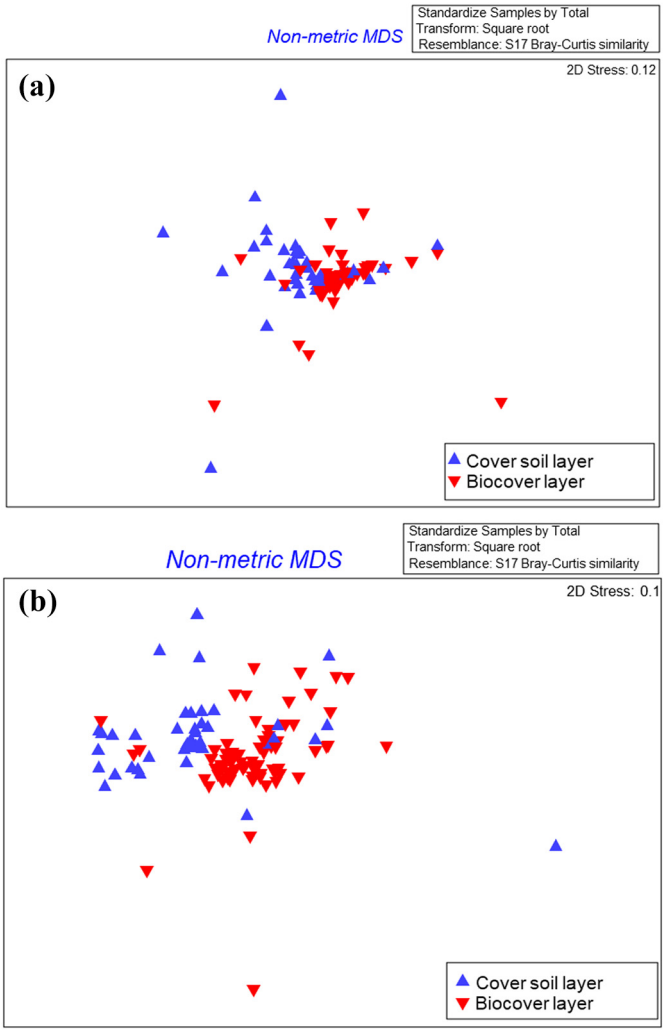

Fig. 9. Non-metric multidimensional scaling plot of: a) Total bacterial communities (ANOSIM, $R = 0.297, p = 0.001$, 999 permutations); and b) Total methanotrophic communities (ANOSIM, $R = 0.337$, p = 0.001, 999 permutations) in cover soil and CH_4 oxidation (biocover) samples from the columns.

indicates either microbial oxidation of H_2S did not take place in the biocover layers or H_2S was adsorbed in the underlying gravel layer before entering the biocover layers. This observation was backed by the evidence of darkening of the gravel (Fig. S3) which could be due to precipitation of metal sulfides from reaction of H_2S with the metal salts (such as iron salts) present in the gravel (Wohlers and Feldstein, 1966). Similar darkening was not observed in the overlying layers and H_2S was never detected in the gas sampling ports within the soil layers which further affirms the hypothesis that H_2S was absorbed in the underlying gravel layer preventing overlying soil layers from exposure to H_2S. The sulfur content of the gravel layer was also elevated which are presented in Chetri et al. (2021a). Studies have reported inhibitory effect of H_2S on the methanotrophic oxidation (Lee et al., 2011) however, the inhibitory effects were prominent only at lower concentrations of CH_4 due to the competitive inhibition (Long et al., 2013). Such inhibitory effects were not observed in this study which could also be attributed to the high inlet CH_4 concentrations (~50%). In addition, a drop in soil pH was not observed which is also an indication of absence of microbial H_2S oxidation in biologic layers.

3.8. Effect of soil properties on the microbial communities

Microbial CH_4 oxidation and community composition are dependent on various environmental factors such as pH, temperature, moisture, oxygen, and CH_4 mixing ratios, nutrient conditions in soils, etc. (Visvanathan et al., 1999; Huber-Humer et al., 2008; Albanna and Fernandes, 2009; Yargicoglu and Reddy, 2017b). Studies have shown optimum pH for CH_4 oxidation in landfill cover soil to be in the range of 6.5–7.5 (Reddy et al., 2020a; Scheutz and Kjeldsen, 2004). The pH of the terminal column exhumed biocover samples ranged from 7.4 to 8 which is close to the optimum pH range for CH_4 oxidation. Presence of H_2S in LFG did not result in reduction of pH which is an indication that microbial H_2S oxidation was not prevalent. Reddy et al. (2020a) observed prevalence of methanotrophs such as *Methylobacter* (Type I) and *Methylocystis* (Type II) at pH 7.6 in the landfill cover soil which is consistent with this study. Similarly, the columns were incubated at room temperature (23 ± 2 °C) which is in the range of optimum temperature of 20–30 °C for CH_4 oxidation as reported by various studies (Reddy et al., 2019; Scheutz and Kjeldsen, 2004; Park et al., 2005). Reddy et al. (2019) also found dominance of *Methylobacter* and *Methylocystis* in landfill cover soil incubated at 23 °C consistent with this study. Hence, pH and temperature conditions in the column reactors were favorable for the growth of methanotrophs and CH_4 oxidation.

Moisture content is another crucial parameter which affects microbial CH_4 oxidation. Many studies have reported reduced microbial activity and CH_4 oxidation at lower moisture content (5% *w/w*) due to increased water stress (Boeckx and Van Cleemput, 1996; Visvanathan et al., 1999) and at high moisture content due to interference of soil moisture in gas exchange (Albanna and Fernandes, 2009). Optimum moisture content for microbial CH_4 oxidation has been reported to be in the range of 10–20% (Boeckx and Van Cleemput, 1996; Visvanathan et al., 1999; Yargicoglu and Reddy, 2017a). In this study, the initial moisture content during column set up was maintained at 15% (*w/w*) which falls within the optimum moisture content range for CH_4 oxidation. However, due to prolonged exposure to LFG, the biocover layers lost considerable amount of moisture, mainly CS in Column 1 (Table 2). Nonetheless, biochar amended biocovers

had relatively lesser loss in moisture than CS and terminal moisture contents remained above 10%. This could be attributed to the high moisture retention property of biochar. The higher terminal CH_4 oxidation rates in biochar amended biocover samples (Table 2) could be associated with the higher moisture contents, supporting greater abundances of microbial population.

3.9. Future prospects

Methane and carbon dioxide which are prime components of LFG are also major greenhouse gases. With the increase in the frequency and intensity of extreme climatic events associated with global warming, every step towards curbing greenhouse gases could prove instrumental in reducing the effects of global warming. The proposed biogeochemical cover shows promising potential to mitigate CH_4 and CO_2 by using sustainable materials such as waste wood derived biochar and steel slag. Steel slags are produced in huge quantities every year and have limited applications, hence are often stockpiled, or landfilled. The use of steel slag in the proposed biogeochemical cover could be instrumental in valorizing the steel slag and reducing landfilling of the same. In addition, landfill odor is one of the primary concerns of the landfill operators and H_2S is one of the prime odor causing components of LFG (Chetri and Reddy, 2021). The potential of biogeochemical cover to remove H_2S from LFG provides added advantage of odor management in the landfills. However, these findings need to be verified with field scale studies. Hence, future studies will include performing field scale pilot tests with the proposed biogeochemical cover under real landfill conditions and understand the performance of the cover under dynamic meteorological conditions.

4. Conclusion

Three biogeochemical cover profiles along with a control soil profile were tested in column reactors under landfill conditions. The CH_4 oxidation (biocover) layer in each column was inhabited by methylotrophs with dominance of CH_4 oxidizing methanotrophs (<95% of methylotrophs were methanotrophs). In general, the biocover samples were dominated by Type I methanotrophs mainly *Methylobacter* genus. However, activated biochar amended biocovers (AB5 and AB10) showed significantly higher abundance of Type II methanotrophs (mainly *Methylocystis* and *Methylosinus*) in comparison to the CS and B10. The methylotrophic abundance was higher in the biocover layer in all the columns which was incubated for longer duration than the cover soil showing the significance of CH_4 exposure time on methylotrophic communities. Activated biochar amended biocovers (AB5 and AB10) had higher CH_4 oxidation rates and methanotrophic abundance than B10 and CS which was attributed to the combined effect of MOB infusion and physicochemical properties of biochar. Methane oxidation rates were positively correlated with relative abundance of Type II methanotrophs ($R^2 = 0.75$, $p < 0.01$) and ratio of Type II/Type I methanotrophic abundance ($R^2 = 0.95$, $p < 0.01$). Although Type II methanotrophs were in lower abundance in comparison to Type I, their positive correlation with CH_4 oxidation rates indicates their potential for greater CH_4 turnover rates which has also been supported by some studies in the past. Similarly, having highly alkaline BOF slag layer on top of the biochar amended soil layer did not affect the gas transport and microbial CH_4 oxidation. Hence, biogeochemical cover profile could be a sustainable alternative cover system for mitigating CH_4, CO_2 and H_2S without compromising the CH_4 oxidation capacity of the biologic soil layers.

Data archive

Raw sequence data (FASTQ files) were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA), under the BioProject identified PRJNA750081.

CRediT authorship contribution statement

Jyoti K Chetri: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing.

Krishna R Reddy: Conceptualization, Methodology, Formal analysis, Resources, Supervision, Project administration, Funding acquisition, Writing - Original Draft, Writing - Review & Editing.

Stefan Green: Conceptualization, Methodology, Formal analysis, Resources, Supervision, Writing - Original Draft, Writing - Review & Editing.

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.scitotenv.2022.153429.

References

Albanna, M., Fernandes, L., 2009. Effects of temperature, moisture content, and fertilizer addition on biological methane oxidation in landfill cover soils. Practice Periodical of Hazard Toxic Radioact Waste. Manage 13 (3), 187–195.

Apprill, A., McNally, S., Parsons, R., Weber, L., 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat. Microb. Ecol. 75 (2), 129–137.

Barlaz, M.A., Green, R.B., Chanton, J.P., Goldsmith, C.D., Hater, G.R., 2004. Evaluation of a biologically active cover for mitigation of landfill gas emissions. Environ. Sci. Technol. 38 (18), 4891–4899.

Bender, M., Conrad, R., 1992. Kinetics of CH4 oxidation in oxic soils exposed to ambient air or high CH4 mixing ratios. FEMS Microbiol. Lett. 101 (4), 261–270.

Boeckx, P., Van Cleemput, O., 1996. Methane oxidation in a neutral landfill cover soil: influence of moisture content, temperature, and nitrogen-turnover. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America 25 (1), 178–183.

Bowman, J., 2006. The methanotrophs—the families methylococcaceae and methylocystaceae. Prokaryotes 5, 266–289.

Burgess, Parsons, S.A., Stuetz, R.M., 2001. Developments in odour control and waste gas treatment biotechnology: a review. Biotechnol. Adv. 19 (1), 35–63.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from illumina amplicon data. Nat. Methods 13 (7), 581–583.

Cébron, A., Bodrossy, L., Chen, Y., Singer, A.C., Thompson, I.P., Prosser, J.I., Murrell, J.C., 2007. Identity of active methanotrophs in landfill cover soil as revealed by DNA-stable isotope probing. FEMS Microbiol. Ecol. 62 (1), 12–23.

Chetri, J.K., Reddy, K.R., 2021. Advancements in municipal solid waste landfill cover system: a review. J. Indian Inst. Sci. 1–32.

Chetri, J.K., Reddy, K.R., Grubb, D.G., 2019. Innovative biogeochemical cover to mitigate landfill gas emissions: investigation of controlling parameters based on batch and column experiments. Environ. Process. 6 (4), 935–949.

Chetri, J.K., Reddy, K.R., Grubb, D.G., 2020. Carbon-dioxide and hydrogen-sulfide removal from simulated landfill gas using steel slag. J. Environ. Eng. 146 (12), 04020139.

Chetri, J.K., Reddy, K.R., Grubb, D.G., 2021a. Investigation of different biogeochemical cover configurations for mitigation of landfill gas emissions: large scale column experiments. Acta Geotechnica submitted.

Chetri, J.K., Reddy, K.R., Greem, J.S., 2022b. Methane oxidation and microbial community dynamics in activated biochar amended soil cover. J. Environ. Eng. 148 (2). https://doi.org/10.1061/(ASCE)20EE.1943-7870.0001984.

Clarke, K.R., Gorley, R.N., 2015. Getting started with PRIMER v7. PRIMER-E: Plymouth, Plymouth Marine Laboratory http://updates.primer-e.com/primer7/manuals/Getting_started_with_PRIMER_7.pdf.

De Visscher, A., Thomas, D., Boeckx, P., Van Cleemput, O., 1999. Methane oxidation in simulated landfill cover soil environments. Environ. Sci. Technol. 33 (11), 1854–1859.

De Visscher, A., De Pourcq, I., Chanton, J., 2004. Isotope fractionation effects by diffusion and methane oxidation in landfill cover soils. J. Geophys. Res. Atmos. 109, D18111.

do Carmo Linhares, D., Saia, F.T., Duarte, R.T.D., Nakayama, C.R., de Melo, I.S., Pellizari, V.H., 2021. Methanotrophic community detected by DNA-SIP at Bertioga's Mangrove Area, Southeast Brazil. Microb. Ecol. 81 (4), 954–964.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26 (19), 2460–2461.

Gebert, J., Perner, M., 2015. Impact of preferential methane flow through soil on microbial community composition. Eur. J. Soil Biol. 69, 8–16.

Gebert, J., Stralis-Pavese, N., Alawi, M., Bodrossy, L., 2008. Analysis of methanotrophic communities in landfill biofilters using diagnostic microarray. Environ. Microbiol. 10 (5), 1175–1188.

Gebert, J., Singh, B.K., Pan, Y., Bodrossy, L., 2009. Activity and structure of methanotrophic communities in landfill cover soils. Environ. Microbiol. Rep. 1 (5), 414–423.

Glöckner, F.O., Yilmaz, P., Quast, C., Gerken, J., Beccati, A., Ciuprina, A., Bruns, G., Yarza, P., Peplies, J., Westram, R., Ludwig, W., 2017. 25 years of serving the community with ribosomal RNA gene reference databases and tools. J. Biotechnol. 261, 169–176.

Guerrero-Cruz, S., Vaksmaa, A., Horn, M.A., Niemann, H., Pijuan, M., Ho, A., 2021. Methanotrophs: discoveries, environmental relevance, and a perspective on current and future applications. Front. Microbiol. 12, 1057.

Hanson, R.S., Hanson, T.E., 1996. Methanotrophic bacteria. Microbiol. Rev. 60 (2), 439–471.

Henckel, T., Roslev, P., Conrad, R., 2000. Effects of O2 and CH4 on presence and activity of the indigenous methanotrophic community in rice field soil. Environ. Microbiol. 2 (6), 666–679.

Henneberger, R., Lüke, C., Mosberger, L., Schroth, M.H., 2012. Structure and function of methanotrophic communities in a landfill-cover soil. FEMS Microbiol. Ecol. 81 (1), 52–65.

Huber-Humer, M., Gebert, J., Hilger, H., 2008. Biotic systems to mitigate landfill methane emissions. Waste Manag. Res. 26 (1), 33–46.

IPCC, 2013. Climatechange2013: the physical science basis. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., et al. (Eds.), Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge; NewYork, NY, pp. 1–1535.

Jiang, H., Chen, Y., Jiang, P., Zhang, C., Smith, T.J., Murrell, J.C., Xing, X.H., 2010. Methanotrophs: multifunctional bacteria with promising applications in environmental bioengineering. Biochem. Eng. J. 49 (3), 277–288.

Jugnia, L.B., Aït-Benichou, S., Fortin, N., Cabral, A.R., Greer, C.W., 2009. Diversity and dynamics of methanotrophs within an experimental landfill cover soil. Soil Sci. Soc. Am. J. 73 (5), 1479–1487.

Knief, C., 2015. Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on pmoA as molecular marker. Front. Microbiol. 6, 1346.

Knief, C., Kolb, S., Bodelier, P.L., Lipski, A., Dunfield, P.F., 2006. The active methanotrophic community in hydromorphic soils changes in response to changing methane concentration. Environ. Microbiol. 8 (2), 321–333.

Kolb, S., 2009. The quest for atmospheric methane oxidizers in forest soils. Environ. Microbiol. Rep. 1 (5), 336–346.

Kumar, M., Dutta, S., You, S., Luo, G., Zhang, S., Show, P.L., Sawarkar, A.D., Singh, L., Tsang, D.C., 2021. A critical review on biochar for enhancing biogas production from anaerobic digestion of food waste and sludge. J. Clean. Prod. 127143.

Lee, E.H., Yi, T.W., Moon, K.E., Park, H.J., Ryu, H.W., Cho, K.S., 2011. Characterization of methane oxidation by a methanotroph isolated from a landfill cover soil, South Korea. J. Microbiol. Biotechnol. 21 (7), 753–756.

Long, Y.Y., Liao, Y., Zhang, K., Hu, L.F., Fang, C.R., Shen, D.S., 2013. Can H2S affect the methane oxidation in a landfill? Ecol. Eng. 60, 438–444.

Long, X.E., Wang, J., Huang, Y., Yao, H., 2016. Microbial community structures and metabolic profiles response differently to physiochemical properties between three landfill cover soils. Environ. Sci. Pollut. Res. 23 (15), 15483–15494.

Nadkarni, M.A., Martin, F.E., Jacques, N.A., Hunter, N., 2002. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology 148 (1), 257–266.

Naqib, A., Poggi, S., Wang, W., Hyde, M., Kunstman, K., Green, S.J., 2018. Making and sequencing heavily multiplexed, high-throughput 16S ribosomal RNA gene amplicon libraries using a flexible, two-stage PCR protocol. Gene Expression Analysis. Humana Press, New York, NY, pp. 149–169.

Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ. Microbiol. 18 (5), 1403–1414.

Park, J.R., Moon, S., Ahn, Y.M., Kim, J.Y., Nam, K., 2005. Determination of environmental factors influencing methane oxidation in a sandy landfill cover soil. Environ. Technol. 26 (1), 93–102.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41 (D1), D590–D596.

Rachor, I., Gebert, J., Gröngröft, A., Pfeiffer, E.M., 2011. Assessment of the methane oxidation capacity of compacted soils intended for use as landfill cover materials. Waste Manag. 31 (5), 833–842.

Rai, R.K., Chetri, J.K., Reddy, K.R., 2019. Effect of methanotrophic-activated biochar-amended soil in mitigating CH4 emissions from landfills. Proc. 4th International Conference on Civil and Environmental Geology and Mining Engineering, Trabzon, Turkey.

Reddy, K.R., Grubb, D.G., Kumar, G., 2018. Innovative biogeochemical soil cover to mitigate landfill gas emissions. International Conference on Protection and Restoration of the Environment XIV, Thessaloniki, Greece. 2018.

Reddy, K.R., Kumar, G., Gopakumar, A., Rai, R.K., Grubb, D.G., 2018. CO2 Sequestration using BOF slag: application in landfill cover. International Conference on Protection and Restoration of the Environment XIV, Thessaloniki, Greece. 2018.

Reddy, K.R., Rai, R.K., Green, S.J., Chetri, J.K., 2019. Effect of temperature on methane oxidation and community composition in landfill cover soil. J. Ind. Microbiol. Biotechnol. 46 (9–10), 1283–1295.

Reddy, K.R., Rai, R.K., Green, S.J., Chetri, J.K., 2020a. Effect of pH on methane oxidation and community composition in landfill cover soil. J. Environ. Eng. 146 (6), 04020037.

Reddy, K.R., Rai, R.K., Green, S.J., Chetri, J.K., 2020b. Effect of basic oxygen furnace slag-infiltrated water on methane oxidation and community composition in biogeochemical landfill cover system. J. Hazard. Toxic, Radioact. Waste 24 (2), 04020001.

Saari, A., Heiskanen, J., Martikainen, P.J., 1998. Effect of the organic horizon on methane oxidation and uptake in soil of a boreal scots pine forest. FEMS Microbiol. Ecol. 26 (3), 245–255.

Sadasivam, B.Y., Reddy, K.R., 2014. Landfill methane oxidation in soil and bio-based cover systems: a review. Rev. Environ. Sci. Biotechnol. 13 (1), 79–107.

Sarperi, L., Surbrenat, A., Kerihuel, A., Chazarenc, F., 2014. The use of an industrial by-product as a sorbent to remove CO2 and H2S from biogas. J. Environ. Chem. Eng. 2 (2), 1207–1213.

Scheutz, C., Kjeldsen, P., 2004. Environmental factors influencing attenuation of methane and hydrochlorofluorocarbons in landfill cover soils. J. Environ. Qual. 33 (1), 72–79.

Scheutz, C., Kjeldsen, P., 2011. Landfill top covers. Solid Waste Technology and Management. Wiley, pp. 830–840.

Scheutz, C., Kjeldsen, P., Bogner, J.E., De Visscher, A., Gebert, J., Hilger, H.A., Huber-Humer, M., Spokas, K., 2009. Microbial methane oxidation processes and technologies for mitigation of landfill gas emissions. Waste Manag. Res. 27 (5), 409–455.

Spokas, K.A., Bogner, J.E., 2011. Limits and dynamics of methane oxidation in landfill cover soils. Waste Manag. 31 (5), 823–832.

Su, Y., Zhang, X., Xia, F.F., Zhang, Q.Q., Kong, J.Y., Wang, J., He, R., 2014. Diversity and activity of methanotrophs in landfill cover soils with and without landfill gas recovery systems. Syst. Appl. Microbiol. 37 (3), 200–207.

USEPA, 2021A. Basic Information about Landfill Gas. Landfill Methane Outreach Program (LMOP). Available atUnited States Environmental Protection Agency. https://www.epa.gov/lmop/basic-information-about-landfill-gas. (Accessed 18 June 2021).

Visvanathan, C., Pokhrel, D., Cheimchaisri, W., Hettiaratchi, J.P.A., Wu, J.S., 1999. Methanotrophic activities in tropical landfill cover soils: effects of temperature, moisture content and methane concentration. Waste Manag. Res. 17 (4), 313–323.

Wang, J., Xia, F.F., Bai, Y., Fang, C.R., Shen, D.S., He, R., 2011. Methane oxidation in landfill waste biocover soil: kinetics and sensitivity to ambient conditions. Waste Manag. 31 (5), 864–870.

Whalen, S.C., Reeburgh, W.S., Sandbeck, K., 1990. Rapid methane oxidation in a landfill cover soil. Appl. Environ. Microbiol. 56 (11), 3405–3411.

Wilshusen, J.H., Hettiaratchi, J.P.A., De Visscher, A., Saint-Fort, R., 2004. Methane oxidation and formation of EPS in compost: effect of oxygen concentration. Environ. Pollut. 129 (2), 305–314.

Wise, M.G., McArthur, J.V., Shimkets, L.J., 1999. Methanotroph diversity in landfill soil: isolation of novel type I and type II methanotrophs whose presence was suggested by culture-independent 16S ribosomal DNA analysis. Appl. Environ. Microbiol. 65 (11), 4887–4897.

Wohlers, H.C., Feldstein, M., 1966. Hydrogen sulfide darkening of exterior paint. J Air Pollut. Control Assoc. 16 (1), 19–21.

Xia, F.F., Su, Y., Wei, X.M., He, Y.H., Wu, Z.C., Ghulam, A., He, R., 2014. Diversity and activity of Sulphur-oxidation bacteria and sulphate-reducing bacteria in landfill cover soils. Lett. Appl. Microbiol. 59 (1), 26–34.

Xia, F.F., Zhang, H.T., Wei, X.M., Su, Y., He, R., 2015. Characterization of H2S removal and microbial community in landfill cover soils. Environ. Sci. Pollut. Res. 22 (23), 18906–18917.

Xie, T., Reddy, K.R., Wang, C., Yargicoglu, E., Spokas, K., 2015. Characteristics and applications of biochar for environmental remediation: a review. Crit. Rev. Environ. Sci. Technol. 45 (9), 939–969.

Yargicoglu, E.N., Reddy, K.R., 2017a. Microbial abundance and activity in biochar-amended landfill cover soils: evidence from large-scale column and field experiments. J. Environ. Eng. 143 (9), 04017058.

Yargicoglu, E.N., Reddy, K.R., 2017b. Effects of biochar and wood pellets amendments added to landfill cover soil on microbial methane oxidation: a laboratory column study. J. Environ. Manag. 193, 19–31.

Yargicoglu, E.N., Reddy, K.R., 2018. Biochar-amended soil cover for microbial methane oxidation: Effect of biochar amendment ratio and cover profile. J. Geotech. Geoenviron. 144 (3), 04017123.

Yargicoglu, E.N., Sadasivam, B.Y., Reddy, K.R., Spokas, K., 2015. Physical and chemical characterization of waste wood derived biochars. Waste Manag. 36, 256–268.

Zhang, J., Kobert, K., Flouri, T., Stamatakis, A., 2014. PEAR: a fast and accurate illumina paired-end reAd mergeR. Bioinformatics 30 (5), 614–620.