ASCE

Methane Oxidation and Microbial Community Dynamics in Activated Biochar-Amended Landfill Soil Cover

Jyoti K. Chetri, S.M.ASCE[1]; Krishna R. Reddy, F.ASCE[2]; and Stefan J. Green[3]

Abstract: In recent years, biochar-amended soil cover has shown promise for enhancing microbial methane (CH_4) oxidation and mitigate fugitive CH_4 emissions at municipal solid waste landfills. However, addition of biochar in landfill cover soil has an initial lag phase due to microbial acclimation and colonization, resulting in lower CH_4 oxidation rates relative to CH_4-exposed landfill cover soil. Therefore, this study explored amendment of landfill cover soil with biochar infused with methane-oxidizing bacterial (MOB) consortium (termed activated biochar) to reduce acclimation time and enhance the CH_4 oxidation activity. Experimental long-term incubation tests were performed on soil columns containing one of four different biocovers: soil control (CS), soil with 10% by weight of biochar (B10), soil with 5% MOB-activated biochar (AB5), and soil with 10% MOB-activated biochar (AB10), exposed to continuous flow of simulated landfill gas (LFG). The AB10 soil column had a reduced lag phase with notable CH_4 oxidation efficiency (ranging from 13% to 50%) during the initial exposure phase compared with all other biocover columns (0.4%–36%). In addition, the activated biochar–amended soil biocovers had higher CH_4 oxidation rates (69–74.3 $\mu g\, CH_4\, g^{-1}\, day^{-1}$) than the nonactivated biochar–amended soil (42 $\mu g\, CH_4\, g^{-1}\, day^{-1}$) and soil control (36 $\mu g\, CH_4\, g^{-1}\, day^{-1}$). The activated biochar–amended columns had higher relative abundances of Type II methanotrophs, mainly *Methylocystis* and *Methylosinus* (relative abundance ~10%) than did nonactivated biochar–amended soil and control columns (relative abundance 3.0%–3.6%). A positive correlation was observed between CH_4 oxidation rate and the ratio of Type II/Type I abundance ($R^2 = 0.84$, $p < 0.01$), further suggesting an important role for the biochar activation in the biological CH_4 mitigation process. Overall, biochar activation appears to be a promising mechanism to reduce microbial lag phase and enhance CH_4 oxidation rates in biochar-amended landfill cover soils. **DOI: 10.1061/(ASCE)EE.1943-7870.0001984.** © *2022 American Society of Civil Engineers.*

Author keywords: Activated biochar; Biochar; Landfill gas; Methane (CH4) oxidation; Methanotrophs.

Introduction

In 2018, 292.4 million tons of municipal solid waste (MSW) was generated in the US, of which nearly 146.1 million tons (~50%) was landfilled (USEPA 2021). Landfilling is a common practice in many parts of the world because landfills are an environmentally accepted waste management strategy that is among the least expensive options (Bogner et al. 1995; Sharma and Reddy 2004). About 37% of waste ends up in some category of landfill globally (Kaza et al. 2018). At landfills, waste undergoes anaerobic decomposition soon after the depletion of free oxygen (O_2) and generates significant amount of methane (CH_4) and carbon dioxide (CO_2), which are major greenhouse gases (GHGs). MSW landfills have been identified as the third largest source of anthropogenic CH_4 emissions in the US (USEPA 2020) and second largest source in Europe (Kjeldsen and Scheutz 2018). Landfill CH_4 emissions are controlled by (1) gas recovery or control systems, (2) thickness of the landfill cover materials, and (3) CH_4 oxidation by methanotrophic bacteria present in the daily, intermediate, or final cover soil (Bogner et al. 2011). Landfill cover soils generally are enriched with CH_4-oxidizing microbes called methanotrophs by the continuous exposure to CH_4 emanating from underlying waste (Sadasivam and Reddy 2014; Kjeldsen and Scheutz 2018). Methanotrophic bacteria have the unique ability to oxidize CH_4 in the presence of O_2 and release CO_2 and water as the products of oxidation (Hanson and Hanson 1996; Huber-Humer et al. 2008). The CH_4 oxidation potential of the indigenous landfill soil methanotrophic microbial communities has been explored extensively (Huber-Humer et al. 2008). Various biocovers have been developed to mitigate landfill CH_4 emissions through the activity of methanotrophic bacteria (Powelson et al. 2006; Rachor et al. 2011; La et al. 2018). Biochar amendment has gained prominence for enhancing CH_4 oxidation in landfill cover soil due to the favorable physical properties of the biochar for microbial colonization (Reddy et al. 2020a, b; Yargicoglu and Reddy 2018).

Biochar is a solid porous carbonaceous product generated from waste biomass by thermochemical treatment such as pyrolysis or gasification in oxygen-deficient conditions (Shackley et al. 2013; Xie et al. 2016). Biochar has been used increasingly in agricultural soil and environmental contaminants remediation due to its unique properties such as high internal porosity and surface area, and high adsorption potential for various chemical compounds, including gases (Yargicoglu et al. 2015). Biochar also has been used in carbon-sequestration applications (Lehmann et al. 2006; Shackley et al. 2013). Recently, the use of biochar has been explored in landfill cover soil for mitigation of CH_4 emissions by utilizing the favorable properties of biochar such as high moisture

[1]Graduate Research Assistant, Dept. of Civil, Materials, and Environmental Engineering, Univ. of Illinois at Chicago, 842 West Taylor St., Chicago, IL 60607. ORCID: https://orcid.org/0000-0003-4842-1317. Email: jkc4@uic.edu

[2]Professor, Dept of Civil, Materials, and Environmental Engineering, Univ. of Illinois at Chigago, 842 West Taylor St., Chicago, IL 60607 (corresponding author). ORCID: https://orcid.org/0000-0002-6577-1151. Email: kreddy@uic.edu

[3]Director, Genomics and Microbiome Core Facility, Rush Univ. Medical Center, 1653 W. Congress Parkway, Jelke Building, Room 444, Chicago, IL 60612. Email: Stefan_Green@rush.edu

Note. This manuscript was submitted on June 7, 2021; approved on November 30, 2021; published online on February 9, 2022. Discussion period open until July 9, 2022; separate discussions must be submitted for individual papers. This paper is part of the ***Journal of Environmental Engineering***, © ASCE, ISSN 0733-9372.

retention and high internal porosity to enhance the microbial activity of indigenous methanotrophic bacteria (Yargicoglu and Reddy 2018; Reddy et al. 2020a, b).

Biochar itself does not react with CH_4, but it promotes adsorption of CH_4 (Sadasivam and Reddy 2015a, b). Moreover, biochar promotes microbial colonization and enhances CH_4 oxidation when mixed with landfill cover soil. However, biochar-amended soils require time for acclimation and microbial colonization; the lag time associated with these stages leads to lower CH_4 oxidation rates at the beginning of experimental incubation, particularly compared with rates associated with acclimated landfill cover soil (Rai et al. 2019). Rai et al. (2019) prepared activated biochar by soaking biochar in a high-density mixed methanotrophic culture. The activated biochar then was added to landfill cover soil as part of laboratory microcosm tests to compare the CH_4 oxidation performance of activated biochar and nonactivated biochar–amended soils. Rai et al. observed that soil amended with 10% and 2% activated biochar by weight (w/w) had significantly higher CH_4 oxidation rates than did soil amended with nonactivated biochar-amended soil or soil without amendment. This phenomenon occurred at the beginning of incubation, without showing any lag phase. In addition, Rai et al. also observed that the CH_4 oxidation rate of nonactivated biochar-amended soil was lower than that of soil alone in the beginning, and started to overtake soil alone only after 50 days of incubation. Similarly, Huang et al. (2019) performed batch incubations with biochar-amended soil by inoculating 15% (w/w) and ~2.5% (w/w) biochar-amended soil. They inoculated biochar-amended soil with a methane-oxidizing bacterial (MOB) consortium by applying the consortium in media directly to the soil. Huang et al. observed a higher CH_4 oxidation efficiency (~46%) in MOB-inoculated biochar–amended soil than in landfill cover soil alone (30%) without MOB inoculation.

Thus, biochar activation with MOB consortia has the potential to expedite CH_4 oxidation in landfill cover soils. However, such findings have been verified only in batch-scale incubations which were closed systems with a limited amount of substrates. No studies have assessed methanotrophy in large-scale column experiments under dynamic gas flow conditions that represent near-field conditions. This study evaluated the CH_4 oxidation performance of landfill cover soil alone, soil amended with 10% (w/w) nonactivated biochar, and soils amended with 5% and 10% (w/w) activated biochar in soil columns under continuous flow of changing landfill gas (LFG). In previous studies (e.g., Yargicoglu and Reddy 2018), 10% biochar amendment performed better in terms of CH_4 oxidation. Hence, a 10% amendment ratio was chosen in the present study. Similarly, a 5% amendment ratio was chosen to investigate whether the biochar use can be economized. The specific objectives of this study were to (1) evaluate the effect of methanotrophic activation of biochar on microbial acclimation and growth in biochar-amended soil under near field conditions; (2) compare the CH_4 oxidation rates of MOB-activated biochar–amended soils, nonactivated biochar–amended soil, and landfill cover soil alone; and (3) evaluate the effect of various LFG gas compositions on soil's CH_4 oxidation efficiency and methanotrophic community structure. To achieve these objectives, column tests were performed by exposing the test cover substrates to continuous flows of synthetic LFG from the bottom and air from the top, and analyzing the gas concentrations along the depth of the cover substrates over time. The testing was performed in four phases, each corresponding to different simulated LFGs to evaluate the resilience of microbial communities to the changing gas conditions.

Materials and Methods

Soil and Biochar Characterization

Soil was obtained from the interim cover of the Zion landfill, located in Zion, Illinois, from a depth of ~15–30 cm and was stored at room temperature (23°C ± 2°C). Soil samples were air dried, pulverized, and screened through a 4.75-mm sieve prior to conducting the experiments in order to remove very large particles and maintain homogeneity in the biocovers. Biochar was supplied by Chip Energy, Goodfield, Illinois. Biochar used in this study was pinewood-derived biochar obtained in the form of pellets. The soil and biochar were characterized for their physical, chemical, and geotechnical properties as per ASTM standards (Table 1). Specific gravity, grainsize analysis, Atterberg limits, organic content and water holding capacity were evaluated as per ASTM D854 (ASTM 2014), ASTM D6913/D6913M-17 (ASTM 2017d), ASTM D4318-17e1 (ASTM 2017c), ASTM D2974-20e1 (ASTM 2020) and ASTM D2980-17e1 (ASTM 2017b), respectively. Soil and biochar were classified as per USCS classification [ASTM D2487-17e1 (ASTM 2017a)]. The hydraulic conductivity of soil was measured using flexible wall permeameter in a triaxial cell as per ASTM D5084-16a (ASTM 2016) whereas for biochar, rigid wall permeameter was used (ASTM D2434-19 ASTM 2019a). The pH was measured as per ASTM D4972-19 (ASTM 2019b) using an Orion 720A

Table 1. Properties of soil and biochar

Property	ASTM method	Soil	Biochar
Specific gravity	ASTM D854-14	2.68	0.65
Grain-size distribution:	ASTM D6913/D6913M-17	—	—
Gravel (%)	—	3.7	45
Sand (%)	—	14.4	54
Fines (%)	—	81.9	1
D_{50} (mm)	—	0.009	4.3
C_c	—	—	0.82
C_u	—	—	2.42
Atterberg limits:	ASTM D4318-17e1	—	—
Liquid limit (%)	—	39	Nonplastic
Plastic limit (%)	—	22	—
Plasticity index (%)	—	17	—
USCS classification	ASTM D2487-17e1	CL	SP
Water holding capacity (w/w)	ASTM D2980-17e1	43.4	51.6
Hydraulic conductivity (cm/s)	ASTM D5084-16a/ASTM D2434-19	5.4×10^{-8}	4×10^{-3}
Organic content (%)	ASTM D2974-20e1	5.80	96.71
pH	ASTM D4972-19	7.6	6.5

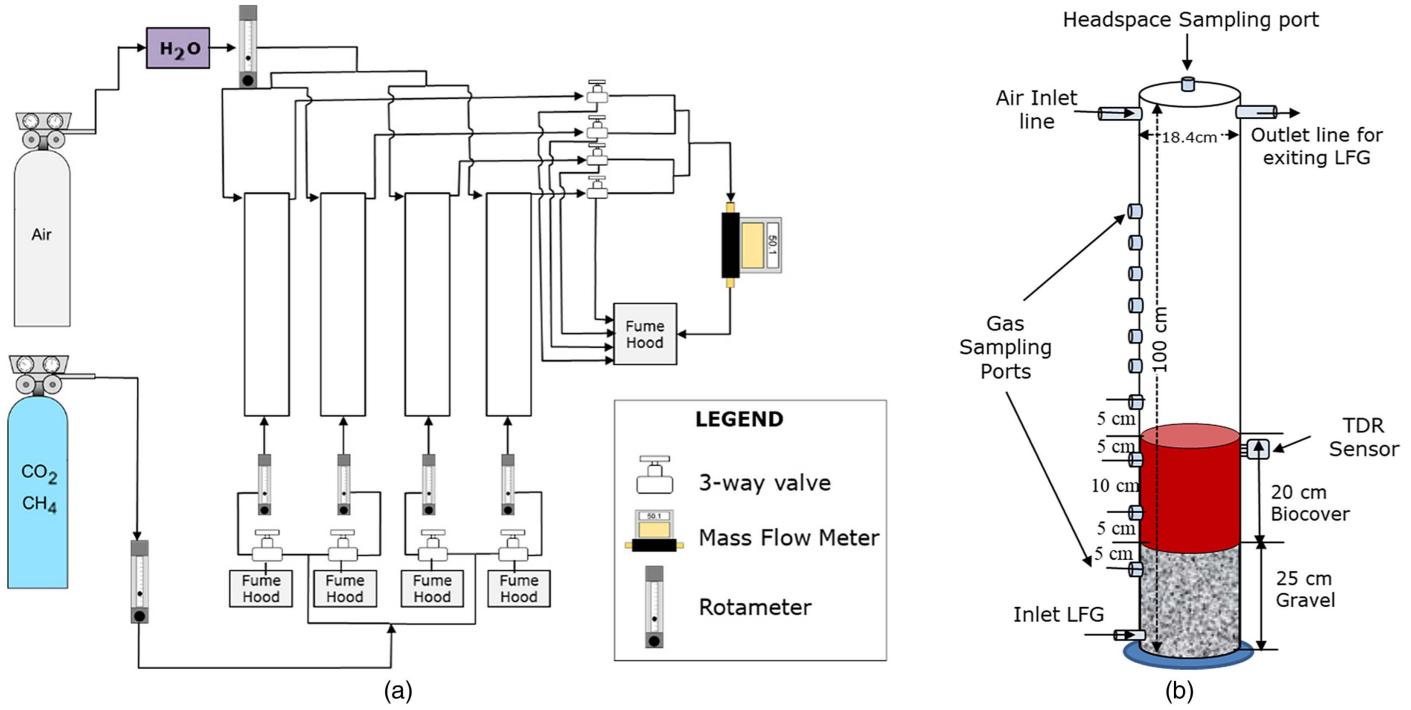

Fig. 1. Schematic of (a) column experimental setup; and (b) column configuration.

pH meter (Orion Research, Franklin, Massachusetts) at a liquid to solid ratio of 1:1.

Biochar Activation

A methanotrophic culture was prepared by enriching landfill cover soil in nitrate mineral salts (NMS) medium (Whittenbury et al. 1970) following procedures described by Rai et al. (2018) and Reddy et al. (2019). NMS solution was prepared by mixing 0.2 g $MgSO_4$, 0.02 $CaCl_2$, 1 g KNO_3, 0.7 g KH_2PO_4, 1.5 g $Na_2HPO_4 \cdot 5H_2O$, and 1 mL trace element solution in 1 L distilled water. For enrichment cultures and MOB consortium preparations, 5 g soil (sieved through a 2-mm sieve) was mixed with 100 mL NMS solution in a 500-mL glass bottle and sealed with a long sleeve rubber septum. Approximately 80 mL air from the headspace was replaced with an equal volume of 50% by volume (v/v) CH_4 and 50% (v/v) CO_2 to obtain a headspace concentration of ~8%CH_4 and 8% CO_2 balanced in air. Headspace concentrations of CH_4 and CO_2 were monitored regularly using a gas chromatograph (GC) (SRI 9300 GC, SRI Instruments, Torrance, California) to evaluate the activity of methanotrophs. Headspace gas was replenished twice during the incubation of 20 days. Thereafter, the supernatant was separated from each enrichment bottle and transferred into two acrylic columns each with a volume of 2,356 cm^3. Biochar (1,050 g) was added to the culture media such that the biochar was completely soaked, and the columns were sealed immediately. A port was provided at the top of the column for gas measurement. Approximately 500 mL of headspace was replaced with an equal volume of a 50:50 (v/v) mixture of CO_2 and CH_4. The headspace concentration of CO_2, CH_4, and O_2 was monitored regularly to evaluate the activity of the methanotrophs and ensure that the biochar was activated. The gases in the headspace were replenished regularly when the concentrations became significantly low (<1%v/v). The biochar was soaked in the MOB culture for nearly 56 days before mixing into landfill cover soil. The biochar was separated from the culture supernatant before adding to the soil. The CH_4 consumption rates of biochar during activation period are shown in Fig. S1 in the Supplemental Materials.

Column Setup

Four biocover profiles were tested in the column incubation experiments: soil only or soil control (CS), soil + 10% (w/w) biochar (B10), soil + 5% (w/w) activated biochar (AB5), and soil +10% (w/w) activated biochar (AB10). The columns were made of acrylic tubing, and were cylindrical, with a height of 100 cm and an internal diameter (ID) of 18.40 cm. The columns were provided with a flange top and bottom plates with rubber O rings screwed together to ensure airtight sealing. The experimental setup is shown in Fig. 1(a). A 25-cm-thick layer of pea gravel was placed at the bottom of each column to serve as a gas distribution layer (GDL). A 20-cm-thick biocover layer was placed on top of the GDL. A geotextile fabric was used as a separator between the GDL and the biocover layer. Water was added to achieve a moisture content of 15% (w/w) in each biocover material. Biocover materials were mixed thoroughly before placement into the columns, resulting in moisture contents ranging from 15.3% to 16.6% (Table 2). These measured values, exceeding 15%, were due to the hydroscopic moisture present in the soil samples. Biochar was soaked in the MOB consortia for activation purposes as described previously, leading to a high moisture content (~98%w/w). The moisture content in activated biochar-amended soils was adjusted accordingly to obtain an overall moisture content of 15% (w/w). The biocover materials were placed in 5-cm layers with light tamping by a 3.1-kg tamping rod to achieve the bulk densities ranging from ~1.3 to 1.5 g/cm^3 (Table 2). The four columns were connected in parallel [Fig. 1(a)]. The inlet synthetic LFG was fed to the bottom of the column, and air was fed from the top of the column at a flow rate of ~60 mL/min. The inlet LFG flow rates were controlled by rotameters (Cole-Parmer, Vernon Hills, Illinois). Similarly, the rate of outflow LFG was monitored by a rotameter connected at the outlet of each column. Two gas sampling ports were located within the biocover layer, one port was located above

Table 2. Properties of control soil and biochar-amended soils during placement in column reactors

Property	CS	B10	AB5	AB10
Substrate	Soil only	Soil + 10% biochar	Soil + 5% activated biochar	Soil + 10% activated biochar
Bulk density (g/cm^3)	1.53	1.37	1.51	1.25
Dry density (g/cm^3)	1.31	1.18	1.31	1.08
Total porosity	0.49	0.52	0.47	0.56
Air-filled porosity	0.27	0.32	0.27	0.39
Water-filled porosity	0.22	0.20	0.20	0.17
Initial moisture (% w/w)	16.6	16.0	15.3	15.7

the biocover layer, and one port was located below the biocover layer [Fig. 1(b)]. A time-domain reflectometry (TDR) sensor (CS655-L, Campbell Scientific, Logan, Utah), capable of measuring volumetric water content (VWC), temperature, and electrical conductivity (EC), was installed within the biologic layer to monitor the changes in physical parameters during column incubation. Parameters were recorded every 15 s and averaged over 4-h intervals by the data acquisition system (Campbell ScientificCR1000 Data Logger equipped with an AM16/32 B multiplexer).

Gas Concentration and Methane Oxidation Efficiency

Gas concentrations along the depth of the columns were determined by extracting gas samples from sampling ports 1–2 times/week. Gas samples were analyzed using an SRI 9300 GC equipped with a thermal conductivity detector (TCD) (detection limit = 500 ppm) and a flame ionization detector/flame photometric detector (FID/FPD) (detection limit = 1 ppm) capable of measuring CO_2, CH_4, and H_2S simultaneously (Chetri et al. 2020). An SRI Model 110 standalone detector chassis with electronic pressure control equipped with a FID/FPD was connected to the host GC with a heated transfer line for the detection of H_2S. The host GC was fitted with a HayeSep-D packed column (SRI Instruments, Torrance, California) 1829 mm × 3.2 mm (6 ft × 1/8 in.) for separation of CH_4 and CO_2, and a 60-m MXT-1 capillary column (SRI Instruments, Torrance, California) for separation of H_2S. The TCD and FID/FPD used helium and hydrogen as the carrier gases, respectively. For O_2 concentration measurements, the carrier gas was switched to nitrogen in the TCD. Gas samples were withdrawn from sampling ports using a 1-mL syringe equipped with a filter and stopper.

The four phases tested in the column experiments are summarized in Table 3. Phase 1 was the acclimation phase, in which the covers were exposed to a low flow of 50% (v/v) CH_4 and 50% (v/v) CO_2 (inlet flux 50 $g\,CH_4\,m^{-2}\,day^{-1}$) to allow the microbes to acclimatize to the LFG conditions. After the gas profiles stabilized in Phase 1, the inflow gas configuration was switched to a mixture of 48.25% (v/v) CH_4, 50% (v/v) CO_2, and 1.75% (v/v) H_2S in Phase 2 to evaluate the effect of the presence of H_2S in LFG on microbial CH_4 oxidation and community structure. LFG from MSW landfills can contain H_2S generated from the decomposition of sulfur-containing organic wastes such as paper, food, and sewage sludge from waste treatment plants (Ko et al. 2015). In addition, the inflow gas composition was switched to pure CH_4 (~99%v/v CH_4) in Phase 3, maintaining the same CH_4 flux as in Phase 2 to investigate the resilience of microbial communities to changing gas compositions. A 10-cm-thick layer of field sand was added on top of the biocover layer in each column during Phase 3 to stabilize gas profiles and reduce the effect of dilution. The incubation phase before adding sand was termed Phase 3a, and that after adding sand was termed Phase 3b. A schematic of the column set up in Phase 3b is shown in Fig. S2 in the Supplemental Materials. The main aim of conducting these three phases was to understand how well the microbial communities adapted to the changing gas conditions.

Batch Tests and Methane Oxidation Rates

At the end of Phase 2, batch incubation experiments were performed on soil-biochar samples extracted from the biocover layer of each reactor to quantify their CH_4 oxidation rates. Approximately 10–11 g of sample was extracted from each of the biocover layer from top 0 to 5 cm during Phase 2 by pushing a thin-walled stainless-steel sampler with an inner diameter of 2.5 cm (1 in.) fabricated specifically for the sample extraction. The cavity created by sampling was filled with an equivalent amount of fresh biocover material. Batch incubation tests were performed on 5 g each of the extracted sample in a 125-mL glass serum vial (Wheaton, Millville, New Jersey). The glass vials were hermetically sealed with rubber septa and aluminum crimps after samples were placed inside. A volume of 20 mL air was withdrawn from each vial and replaced with a mixture of 50% (v/v) CH_4 and 50% (v/v) CO_2 to obtain the headspace concentration of ~8% (v/v) CH_4 and 8% (v/v) CO_2 balanced in air. The headspace gas concentrations were monitored at regular intervals until the headspace CH_4 concentrations were completely depleted. Methane oxidation rates were estimated by linear regression of CH_4 concentrations versus time plots following zero-order kinetics (Reddy et al. 2019). Batch tests were performed in duplicate.

Analysis of Microbial Community Structure

Soil samples (~1 g) were obtained from the top layer (0–5 cm from the surface) of column reactors during each incubation phase (Phases 1, 2, and 3a) to characterize the structure of microbial communities in response to the experimental conditions. Genomic DNA was extracted from each sample using a DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) implemented on a QIAcube instrument (Qiagen, Hilden, Germany). Each sample was weighed

Table 3. Description of different incubation phases of column experiments

Phase	Phase description	Duration (days)	Inlet gas (% v/v)	Avg inlet Q (mL/min)	Avg CH_4 influx ($g\,CH_4\,m^{-2}\,day^{-1}$)
1	Acclimation stage	90	50% CH_4, 50% CO_2	2.8	50
2	Exposure to mixed LFG	81	48.25% CH_4, 50% CO_2, 1.75 H_2S	6–8	103–138
3a	Exposure to pure methane	22	99% CH_4	2.8	100
3b	Exposure to pure methane-after addition of sand	37	99% CH_4	2.9	102

before DNA extraction for absolute quantification. Bead-beating was performed off-instrument prior to automated extraction using an MP FatsPrep-24 5G homogenizer (MP Biomedicals, Irvine, California) 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. A standard TaqMan assay (Thermo Fisher Scientific, Waltham, Massachusetts) was used. The final concentration of each primer in the 10-μL qPCR reaction was 500 nM and the probe had a concentration of 250nM. Cycling conditions were standard TaqMan cycling conditions: 50°C for 2 min, 95°C for 20 s, and then 40 cycles of 95°C for 1 s and 60°C for 20 s. The calculated primer efficiency for this specific assay was between 84% and 94%, with an average of 90%. Analysis was performed using a ViiA7 real-time PCR instrument (Thermo Fisher Scientific), with an 8-order-of-magnitude dilution series for absolute quantification. Genomic DNA was used as a template for amplification of microbial 16S rRNA gene amplicons using a two-stage PCR protocol described by Naqib et al. (2018). The primer set 515F-806R was employed (Parada, et al. 2016; Apprill et al. 2015), and libraries were sequenced using an Illumina (San Diego) MiniSeq instrument, employing paired-end 2x153 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. Forward and reverse reads were merged using the software package PEAR (Zhang et al. 2014). Merged reads were trimmed to remove ambiguous nucleotides and primer sequences, and were trimmed based on a 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 the 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 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 UIC.

The estimated normalized abundance of methylotrophs was calculated by multiplying their relative abundance by the total number of 16S rRNA gene copies. Because gene copy numbers vary between different organisms, the estimated normalized methylotrophic abundance was calculated only for comparison among the groups. It did not represent the actual methylotrophic gene copies.

Data Archive

Raw sequence data (FASTQ files) were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA), under BioProject PRJNA750501.

Statistical Analysis

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

Results and Discussions

Phase 1 Incubation

Gas Concentration Profiles during Phase 1

During Phase 1, columns were exposed to low flow (~2.8 mL/min) of 50% (v/v) CH_4 and 50% (v/v) CO_2 to allow the biocovers to acclimatize to the synthetic LFG conditions. The columns were incubated for 90 days until the gas concentration profiles along the depth of the biocover stabilized and did not fluctuate significantly. During Phase 1, three distinct stages were observed; Stage 1, in which the CH_4 concentration within the biocover remained relatively high, suggesting a lag phase with minimal oxidation; Stage 2, in which the CH_4 concentration within the biocover decreased gradually, indicating adaptation and growth of methanotrophs and microbial oxidation; and Stage 3, in which CH_4 concentrations remained relatively stable, suggesting a steady state (Fig. S3). The average CH_4 oxidation efficiencies during each stage are shown in Fig. 2(a). During Stage 1, all biocovers had low CH_4

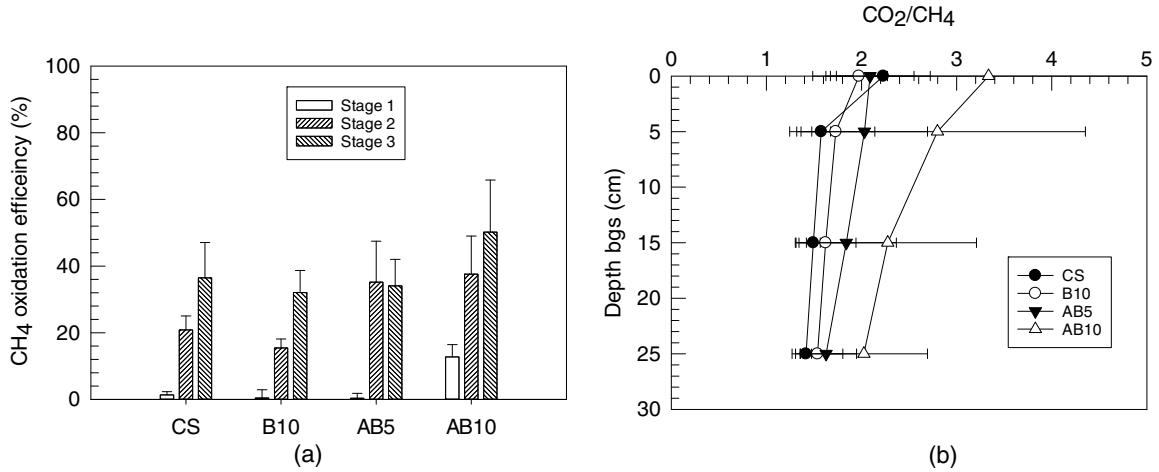

Fig. 2. (a) Average CH_4 oxidation efficiencies at various stages during Phase 1; and (b) average CO_2/CH_4 concentration ratios for all four columns during Phase 1. The higher CH_4 oxidation efficiency in all three stages and the CO_2/CH_4 ratio shows that 10% MOB-activated biochar was successful in reducing the lag phase as well as enhancing the microbial CH_4 oxidation.

J. Environ. Eng., 2022, 148(4): 04022009

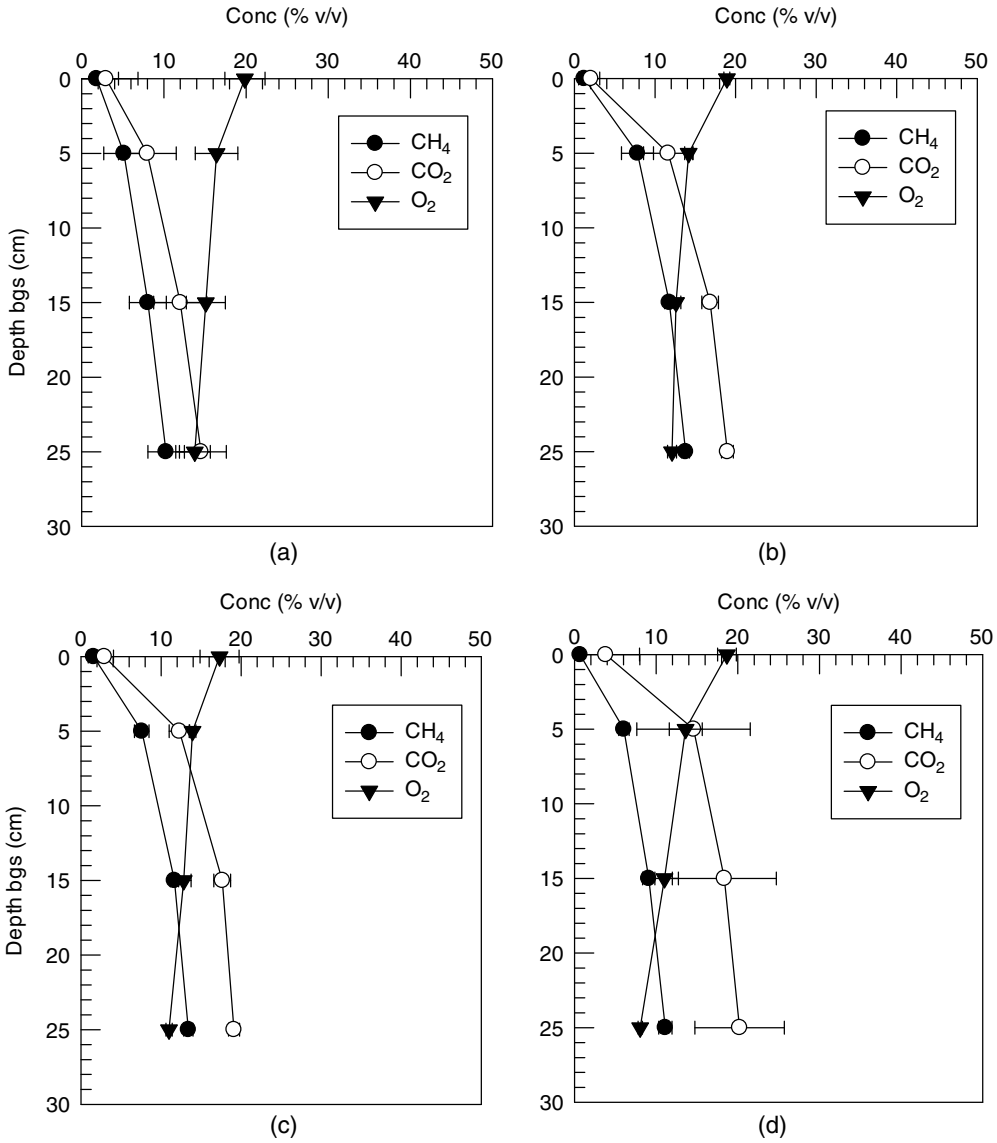

Fig. 3. Average gas concentration profiles along the depth of the biocovers during Stage 3 (steady state) of Phase 1 (exposure to 50% CH_4 and 50% CO_2): (a) soil control (CS); (b) soil + 10% biochar (B10); (c) soil + 5% activated biochar (AB5); and (d) soil + 10% activated biochar (AB10). The CO_2 concentrations were greater than CH_4 concentrations along the depth of the biocovers, confirming CH_4 oxidation activity. The differences in CH_4 and CO_2 concentrations were highest in AB10, confirming higher CH_4 oxidation activity.

oxidation efficiencies (1%–3%), with the exception of the AB10 (~15%). This observation is consistent with our hypothesis that activated biochar can minimize the methanotrophy lag phase. However, soil amendment with 5% activated biochar did not appear to be effective in reducing the lag phase. After an initial lag phase, CH_4 oxidation efficiency increased gradually in all the biocovers and reached a stable oxidation state in Stage 3, resulting the efficiencies of 32%– 50%, with AB10 having the highest CH_4 oxidation efficiency. The thickness of the biocover layer in each column was just 20 cm, which led to significant dilution from air injected from the top, resulting in reduced concentrations of CH_4 and CO_2 and making it hard to quantify CH_4 oxidation efficiencies from flux measurement. Hence, the ratio of CO_2/CH_4 was evaluated along the depth of the biocover layer, with the assumption that CO_2 was equally affected by dilution as CH_4. A CO_2/CH_4 ratio greater than 1 indicated generation of CO_2 due to CH_4 oxidation. Fig. 2(b) shows the average ratio of CO_2/CH_4 during Stage 3 of Phase 1. The CO_2/CH_4 ratios were greater than 1 in all four columns, confirming that CH_4 oxidation was occurring in the biocovers [Fig. 2(b)]. For reference, the inlet CO_2/CH_4 ratio was 1. The ratio was higher in AB5 and AB10, which further confirms greater CH_4 oxidation efficiency in activated biochar–amended soils. B10 (nonactivated biochar –amended soil) had marginally less CH_4 oxidation efficiency than CS (soil control). A plausible explanation for this observation is that soil by itself had a higher microbial load than the mixture of soil and nonactivated biochar. Therefore, biochar-amended soil had lower CH_4 oxidation efficiencies at the start of the experiment and took longer for acclimation and full development of the methanotrophic communities. This observation is consistent with that of Rai et al. (2019), who also observed lower CH_4 oxidation rates in 10% (w/w) nonactivated biochar–amended soils than in the soil control during the initial 50 days of batch incubation. Fig. 3 shows average CH_4, CO_2, and O_2 concentration profiles along the depth of the biocovers during Stage 3 (steady-state) of Phase 1. The biocover layers were fully aerated (Fig. 3) due to the low layer thickness and high porosity

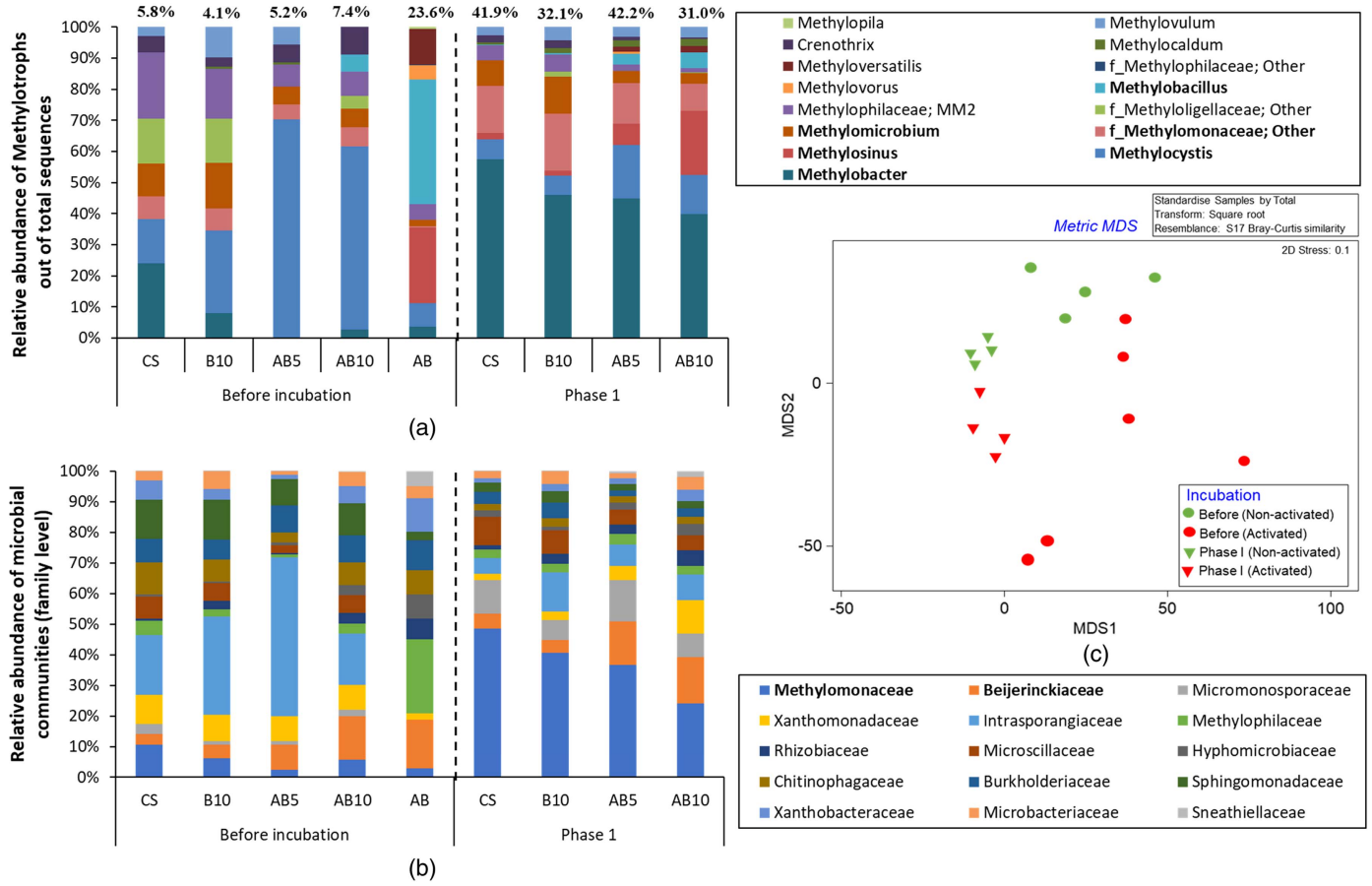

Fig. 4. Microbial community structure in biocover samples before and after incubation in Phase 1 (exposure to 50% CH_4 and 50% CO_2) based on 16S rRNA gene amplicon sequencing: (a) average relative abundance of methylotrophic communities (genus level) in biocover samples before incubation and after incubation in Phase 1, with percentages representing the relative abundance of methylotroph sequences out of all the 16S rRNA gene sequences for each sample; (b) average relative abundance of total microbial communities (family level) in the biocover samples before and after incubation in Phase 1; and (c) metric multidimensional scaling plot of total microbial community distribution (genus level) before and after incubation in Phase 1; communities before incubation were significantly different from those after incubation in Phase 1 (ANOSIM, $R = 0.358$, $p = 0.002$, 999 permutations). CS = soil control; B10 = soil + 10% biochar; AB5 = soil + 5% activated biochar; AB10 = soil + 10% activated biochar; and AB = activated biochar.

(Table 2). Therefore, O_2 limitation, one of the factors affecting CH_4 oxidation efficiency (Rachor et al. 2011; Rachor et al. 2011; Scheutz et al. 2009), was not a limiting factor in our study.

Microbial Community Distribution during Phase 1

Samples were extracted from each of the biocovers during the end of Phase 1 to assess the development of microbial community along the column incubation process. Fig. 4 shows the microbial community distribution in the biocover samples before adding them to the column and after incubation in Phase 1 [exposure to 50% (v/v) CH_4 and 50% (v/v) CO_2]. The activated biochar had significantly higher relative abundance of putative methylotrophs (~23.6%) than did the nonactivated landfill cover soil and biochar-amended soil (~4%–6%) [Fig. 4(a)]. The low methylotrophic abundance in the landfill cover soil before column incubation likely was the reason for the relatively longer lag phase for CH_4 oxidation in Phase 1 (Fig. S3). The landfill soil used in the study, despite being stored for 3 years without CH_4, contained a diverse methanotrophic community, although in low relative abundance [Fig. 4(a)], which shows that methanotrophs are resilient to CH_4 starvation conditions. In a study by Kightley et al. (1995) in which CH_4 supply was interrupted for 8 days, CH_4 oxidation rates recovered quickly upon reinstating CH_4 supply, demonstrating that methanotrophs can be sustained even under CH_4 starvation. Reactors amended with activated biochar had microbial communities with substantial contributions of methane-oxidizing methylotrophs to the total microbial community. In these systems, Type II methanotrophs such as *Methylosinus* (5.8%) and *Methylocystis* (1.8%) were dominant, whereas the relative abundance of Type I methanotrophs was relatively low [e.g., *Methylobacter* (0.9%)]. Our cultivation conditions, with some gas diffusion limitations, may have favored conditions suitable for Type II methanotrophs to thrive, because the biochar was completely soaked in the consortium during activation. Type II methanotrophs are known to grow in nitrogen-limited conditions at low O_2 and high CH_4 concentrations (Graham et al. 1993; Amaral and Knowles 1995; Pfluger et al. 2011). Reddy et al. (2019) also observed significantly higher abundances of Type II methanotrophs such as *Methylocystis* in enrichment cultures at room temperature (23°C) than in landfill cover soil incubated at the same temperature. In addition to methane-oxidizing methanotrophs, activated biochar samples also had higher relative abundances of non-methane-oxidizing methylotrophs such as *Methylobacillus* (9.5% of total sequences) that grow on methane-oxidation intermediates such as methanol (Hanson and Hanson 1996; Gilman et al. 2017).

After acclimation in Phase 1 for nearly 90 days, the relative abundance of methylotrophs increased significantly from 4%–7% before incubation to 31%– 42% [Fig. 4(a)]. The methylotrophic communities contained methane-oxidizing methanotrophs (Type I and Type II) and non-methane-oxidizing methylotrophs, consistent with previous studies of landfill cover soils (Yargicoglu and Reddy 2018; Reddy et al. 2019). The significant increase in methylotrophic bacteria after column incubation suggests that biocover conditions were conducive for microbial growth. pH and temperature are two of the important factors that affect methylotrophic diversity in landfill cover soil (Reddy et al. 2019, 2020a). The optimum pH for methanotrophic activity has been reported to be in the range 6–8 (Zhao et al. 2021; Reddy et al. 2020a). The pH of the soil and biochar used in this study were 7.6 and 6.5, respectively, which are within the optimum range, further suggesting favorable condition for methanotrophic activity. Similarly, optimum temperature for microbial oxidation has been reported to vary from 25°C to 30°C (Reddy et al. 2019; Majdinasab and Yuan 2017), which means the column incubation temperatures in this study (23°C ± 2°C) were in the conducive range.

Soil bacteria from the genus *Methylobacter*, Type I methanotrophs, dominated the samples and constituted nearly 12%–24% of the total 16S rRNA gene sequences (40%–55% of the total methylotrophic abundance). Type I methanotrophs have been reported to grow faster than Type II methanotrophs (Wilshusen et al. 2004; Henckel et al. 2000), and this may be the reason for the dominance of *Methylobacter* during column incubations. Bacteria from the genus *Methylobacter* were relatively abundant in landfill cover soil prior to column incubation (1.4% of total sequences), which is associated with the CH_4 exposure in the landfill prior to sampling (Yargicoglu and Reddy 2017b), and their relative abundance increased significantly after incubation in Phase 1 (24% of total sequences). The relative abundance of bacteria from the genus *Methylobacter* was significantly lower in 10% biochar–amended soil samples (14.8% and 12.3% in B10 and AB10, respectively) than in CS ($p = 0.009$, ANOVA), whereas AB5 had a comparable relative abundance (19.0%). AB5 had a higher proportion of landfill soil, and this likely led to higher relative abundances of *Methylobacter*. Other Type I methanotrophic taxa, such as *Methylomicrobium*, *Methylovolum*, *Methylocaldum*, *Crenothrix*, and *Methylomonaceae* (family) also were detected in the samples, although at relatively lower abundance (~6%–12% combined) compared with *Methylobacter*. In addition to Type I methanotrophs, Type II methanotrophs such as *Methylocystis* and *Methylosinus* were present (combined relative abundance of 3.64%, 2.48%, 10.21%, and 10.26% of total sequences in CS, B10, AB5, and AB10, respectively) in the biocover samples, but primarily in activated biochar–amended soils. Similarly, Huang et al. (2019) reported the dominance of Type II methanotrophic bacteria in biochar-amended soil after inoculation with CH_4-oxidizing bacterial consortia. Prior studies reported that Type II methanotrophs are not exceedingly sensitive to changing conditions (Henckel et al. 2000; Gebert et al. 2009), and this may explain why Type II

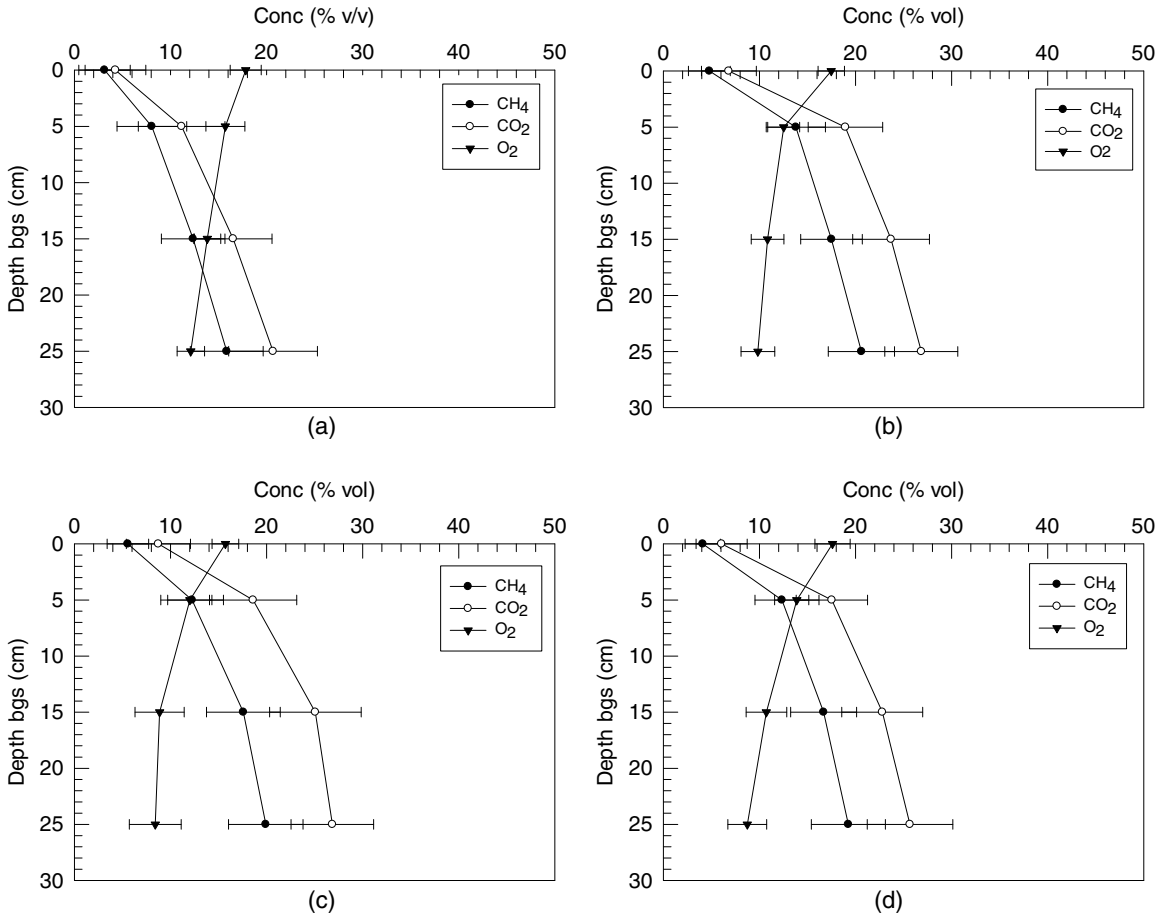

Fig. 5. Average gas concentration profiles along the depth of biocovers: (a) soil alone; (b) soil + 10% biochar-amended soil; (c) soil + 5% activated biochar–amended soil; and (d) soil + 10% activated biochar–amended soil in Phase 2. (Exposure to mixture of 48.25% CH_4, 50% CO_2, and 1.75% H_2S.)

J. Environ. Eng., 2022, 148(4): 04022009

methanotrophs were retained in soils from activation to column incubation in Phase 1.

Fig. 4(b) shows the relative abundance of bacterial families in biocover samples before and after incubation in Phase 1. Across all samples, the most abundant methylotrophic taxa were *Methylomonaceae* and *Beijerinckiaceae* after incubation in Phase 1. The relative abundance of family *Methylomonaceae* in CS was significantly different from that in the 10% biochar–amended groups ($p = 0.03$, ANOVA), with averages of 36.4%, 26.6%, and 17.3% in CS, B10, and AB10, respectively, and was not significantly different from that in AB5 (28.1%), which shows that the *Methylomonaceae* was enriched in the landfill cover soil from CH_4 exposure prior to sampling. The relative abundance of bacteria from the family *Beijerinckiaceae* (including *Methylocystis* and *Methylosinus*) was significantly ($p = 0.008$, ANOVA) more abundant in activated biochar (~11% in both AB5 and AB10) than in nonactivated samples [B10 (2.7%) and CS (3.6%)]. Column incubation with activated biochar appears to have created a favorable environment for growth of Type II methanotrophs. A clear shift in microbial community structure was observed as a result of incubation [Fig. 4(c)], with significance testing performed by ANOSIM ($R = 0.358$, $p = 0.002$). Similarly, a significant difference was observed between activated biochar–amended samples and nonactivated samples after incubation (ANOSIM, $R = 0.969$ and $p = 0.029$).

Phase 2 Incubation

Gas Concentration Profiles

In Phase 2, columns were exposed to a synthetic LFG mixture of 48.25% (v/v) CH_4, 50% (v/v) CO_2 and 1.75% (v/v) H_2S at an inlet flux of 103–138 g CH_4 m^{-2} day^{-1} for ~80 days. At the beginning of the Phase 2, transient white globules, which disappeared after 10 days, formed in each biocover surface [Fig. S4(a)] and may have been sulfur globules produced by sulfur oxidizing bacteria (Kleinjan et al. 2003; Grant and Bathmann 1987). Grant and Bathmann (1987) observed the formation of white sulfur mats on the surface of sediments in coastal environments from sulfur bacteria which oxidize sulfur compounds and produce deposits of elemental sulfur. Because no H_2S was detected in the gas sampling ports located within the biocover, and black precipitates appeared on the gravel surface in the GDL, abiotic interactions with the gravel surface also appear to have played a role in sulfur cycling in these reactors [Fig. S4(b)].

The average concentrations of CH_4, CO_2, and O_2 along the depth of the biocovers during Phase 2 are shown in Fig. 5. CO_2 concentrations were consistently higher than CH_4 concentrations in all biocovers as a result of CH_4 oxidation. Although some studies reported an inhibitory effect of H_2S on the CH_4 oxidation capacity of landfill cover soils (Xia et al. 2015; Lee et al. 2015; Long et al. 2013; Lee et al. 2011), such a phenomenon was not observed in the present study. CH_4 oxidation efficiencies in the presence of H_2S were higher than in Phase 1 ($p < 0.001$, ANOVA), ranging from 88% to 93%. Studies have reported that competitive inhibition of CH_4 oxidation by H_2S occurs at lower CH_4 concentrations (5% v/v) (Long et al. 2013). Because the injected CH_4 concentrations in this study were substantially higher (~50%v/v), competitive inhibition may not have been high enough to impede the CH_4 oxidation process. In addition, some H_2S appears to have been removed by abiotic processes in the pea gravel layer beneath the biocover, thereby reducing the H_2S flux into the biocover layer.

Batch Incubation and Methane Oxidation Rates

In column incubation, several processes interact concurrently, including dilution, diffusion, advection, and oxidation, and as a result, absolute CH_4 oxidation rates are difficult to calculate (De Visscher et al. 1999). Therefore, at the end of Phase 2, samples were extracted from biocover layer in each column and subjected to batch incubations for CH_4 oxidation rate measurement and microbial community structure analysis. Fig. 6(a) shows variation of headspace CH_4 and CO_2 concentrations over time during batch incubations. CH_4 concentrations decreased linearly with time in all the biocover samples with concomitant increases in CO_2 concentration [Fig. 6(a)]. The biochar-amended soils did not have any lag phase, confirming that the methanotrophic communities were well established during column incubation. However, CS samples had an initial lag phase of ~4 days, which could be due to the drying of the soil samples from continuous exposure to LFG and air in the column reactors causing water stress and reduced microbial activity. Prior studies also reported reduced microbial activity and CH_4 oxidation capacity due to a reduction in moisture content (Boeckx and Van Cleemput 1996; Spokas and Bogner 2011; Yargicoglu and Reddy 2017a; Chetri and Reddy 2021). The better moisture retention capacity of biochar-amended soils help to sustain microbial colonization under prolonged exposure to LFG and air

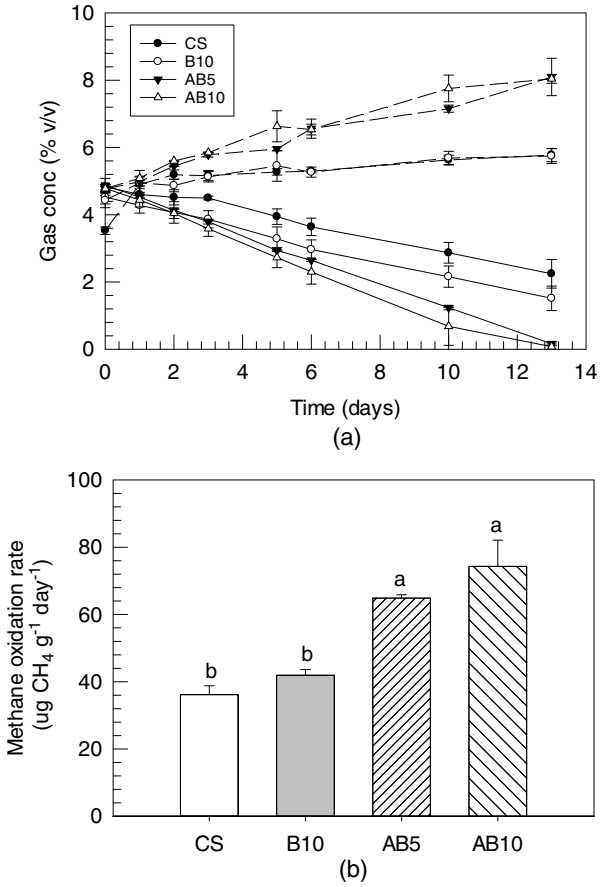

Fig. 6. (a) Variation of CH_4 and CO_2 with time (dotted lines represent CO_2 and solid lines represent CH_4); and (b) CH_4 oxidation rates obtained during batch incubation of biocover samples obtained during Phase 2 [lowercase letters on top of the bars show significant difference ($p < 0.05$) in CH_4 oxidation rates among samples]; the CH_4 oxidation rates support the hypothesis that the activated biochar addition helps to increase the microbial CH_4 oxidation in landfill cover soil.

(Yargicoglu and Reddy 2017a), and this was reflected in the absence of a lag phase in batch incubations seeded with biochar-amended soils [Fig. 6(a)].

The CH_4 oxidation rates in the four biocover samples were significantly different ($p < 0.01$, ANOVA), and AB10 had the highest CH_4 oxidation rate (74.3 $\mu g\, g^{-1}_{dry\ soil}\, day^{-1}$) followed by AB5 (64.8 $\mu g\, g^{-1}_{dry\ soil}\, day^{-1}$). Batch reactors seeded with nonactivated biochar–amended soil had a higher CH_4 oxidation rate (41.9 $\mu g\, g^{-1}_{dry\ soil}\, day^{-1}$) than did the soil control batch reactors (36.1 $\mu g\, g^{-1}_{dry\ soil}\, day^{-1}$) [Fig. 6(b)]. The CH_4 oxidation rates determined from the batch incubations can be extrapolated to field scale; for example, a rate of 36.1 $\mu g\, g^{-1}_{dry\ soil}\, day^{-1}$ (CS) corresponds to ~151 $g\, CH_4\, m^{-2}\, day^{-1}$. Thus, all the biocovers in the column tests, assuming that they had the same oxidation potential as that obtained through batch testing, had the potential to oxidize all the CH_4 injected into the column during Phase 2 [flux rates 103–138 $g\, CH_4\, m^{-2}\, day^{-1}$ (Table 3)]. The positive effect of biochar amendment is reflected in the higher CH_4 oxidation rates of biochar-amended soils than of the CS. In addition, the significantly higher CH_4 oxidation rates in activated biochar (AB5 and AB10) than in the nonactivated biochar (B10) emphasizes that the enhanced microbial oxidation was not merely because of biochar addition, but was due to the combined effect of biochar and its infusion with MOB.

Microbial Characterization during Phase 2

Fig. 7(a) shows total bacterial 16S rRNA gene copies per gram soil in each biocover sample during Phase 1 and Phase 2 of column incubation. The relative abundance of methylotrophs, as assessed by 16S rRNA gene sequencing, during each of the incubation phases are shown in Fig. S5. The estimated normalized abundance of methylotrophs is shown in Fig. 7(b). The total bacterial gene copies as well as the estimated normalized methylotrophic abundances were slightly lower in Phase 2 than in Phase 1. Except for AB10, the differences were not significant ($p = 0.17$, ANOVA). The methylotrophic diversity and their relative abundance were fairly similar in Phase 1 and Phase 2 (Fig. S7), which further suggests a minimal impact of the presence of H_2S in LFG on the methylotrophic community composition and abundance. In addition, no sulfur-oxidizing bacterial sequences were detected in the biocover samples. The methylotrophic communities were notably different between activated and nonactivated biocover groups in both Phase 1 ($R = 0.604$, $p = 0.0029$) and Phase 2 ($R = 0.748$, $p = 0.0001$)

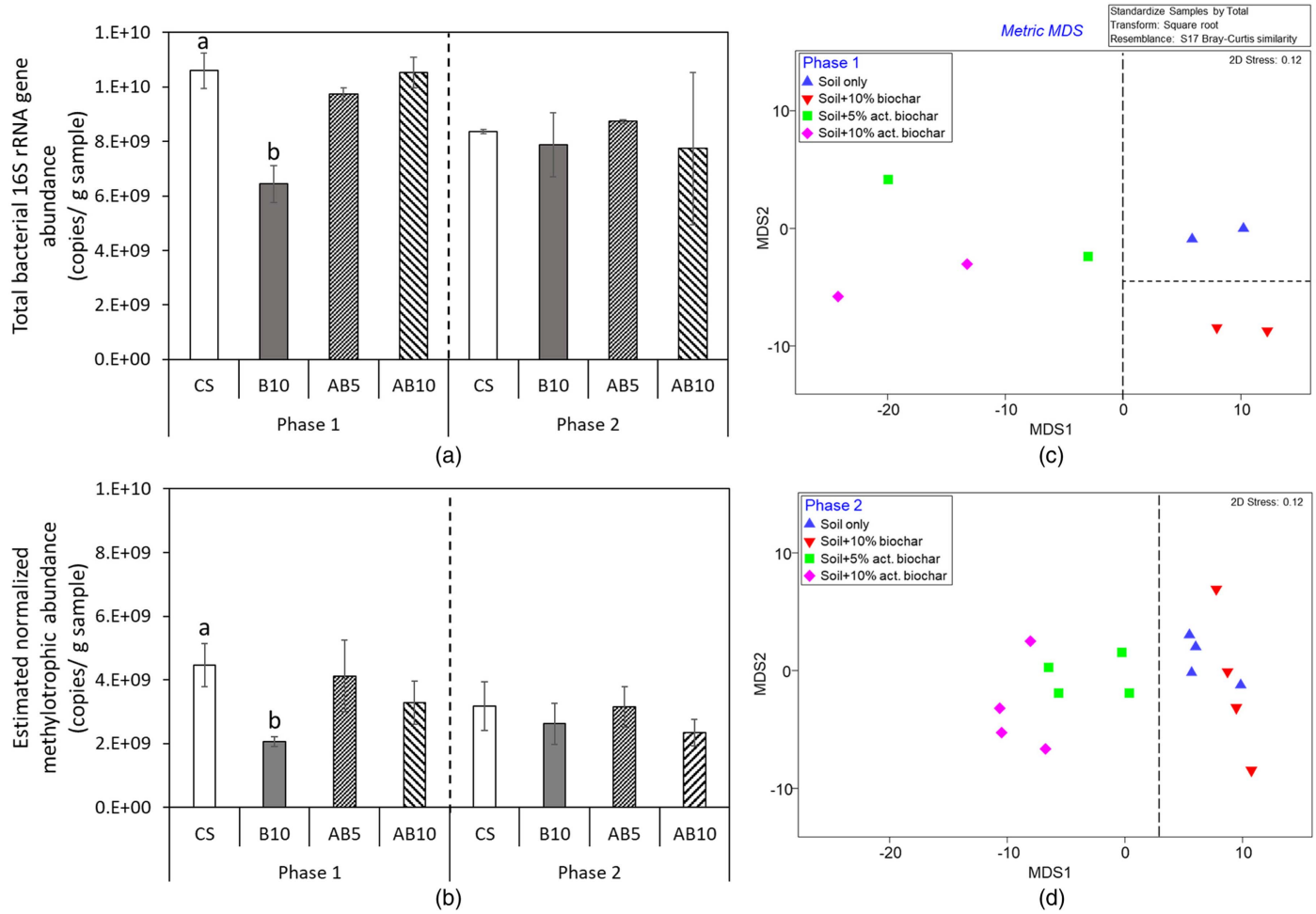

Fig. 7. Microbial communities in biocover samples extracted from columns during Phase 1 and Phase 2: (a) total bacterial abundance, as assessed by qPCR of 16S rRNA genes; (b) estimated total methylotrophic count (relative abundance of methylotrophs determined by 16S rRNA gene amplicon sequencing multiplied by total bacterial 16S rRNA gene copies); (c) metric multidimensional scaling plot of microbial communities in biocover sample during Phase 1 (exposure to 50%CH_4 and 50%CO_2); and (d) metric multidimensional scaling plot of microbial communities in biocover samples during Phase 2 (exposure to 48.25% CH_4, 50% CO_2, and 1.75% H_2S). During both phases, the microbial community structures in the four biocover samples were significantly different from each other (global ANOSIM, $R = 0.604$, $p = 0.0029$ and $R = 0.748$, $p = 0.0001$ in Phases 1 and 2, respectively). Lowercase letters on top of the bars represent significant difference in bacterial population in the samples.

J. Environ. Eng., 2022, 148(4): 04022009

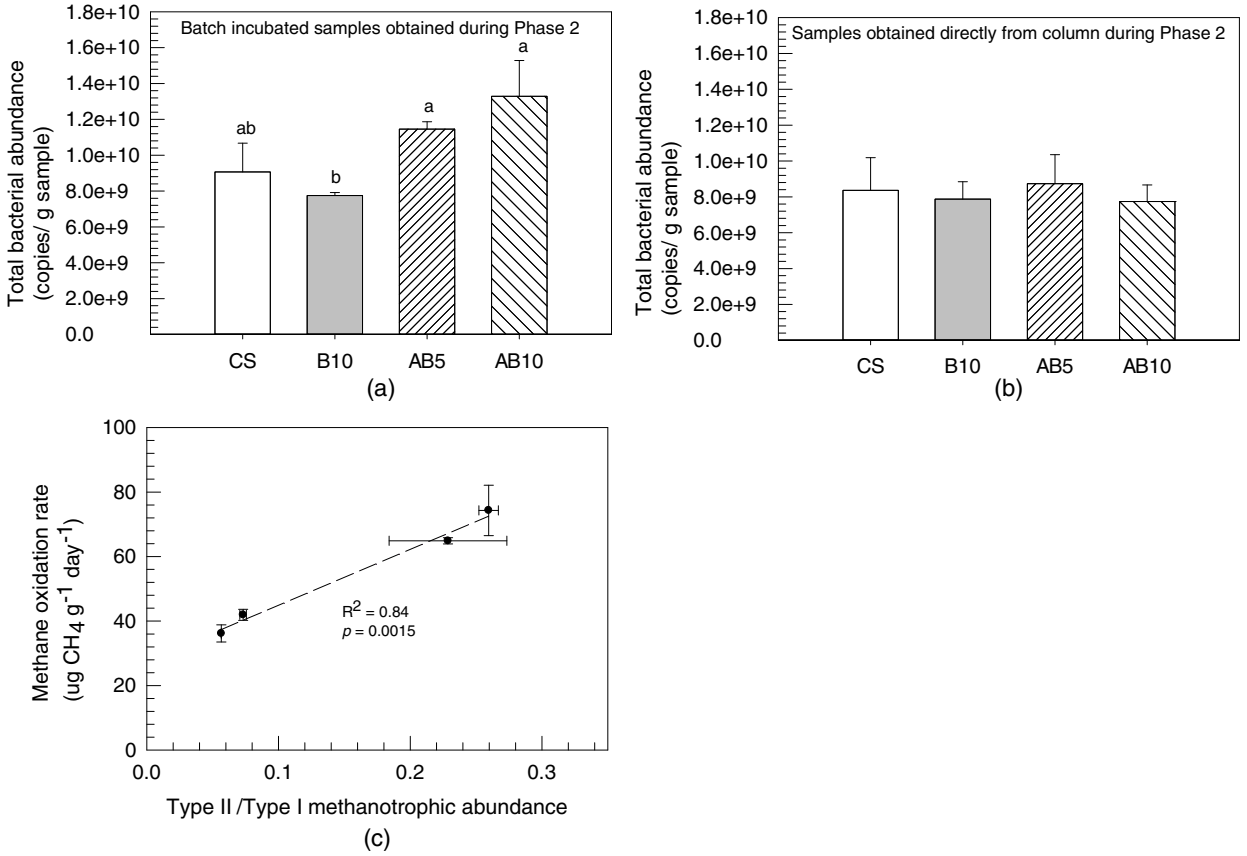

Fig. 8. Relationship between methanotrophic microbial community structure and methane oxidation in Phase 2 column soils: (a) total bacterial abundance, as assessed by qPCR, in batch-incubated samples obtained during Phase 2; (b) total bacterial abundance, as assessed by qPCR, in samples extracted from columns during Phase 2 based on the qPCR analysis (bacterial abundance is significantly different in batch and column samples, $p = 0.015$); and (c) relationship between methane oxidation rates and ratio of Type II/Type I methanotrophic relative abundance in batch incubated samples ($R^2 = 0.84$, $p = 0.0015$, from regression analysis in EXCEL 2019). Lowercase letters on top of the bars represent significant difference in bacterial population in the samples.

[Figs. 7(c and d)]. The batch-incubated samples obtained from column incubations during Phase 2 also were analyzed for bacterial and methylotrophic abundance. Fig. 8(a) shows total bacterial copies in the batch incubated samples. The estimated normalized methylotrophic abundance was 2.92×10^9, 2.14×10^9, 2.82×10^9, and 3.62×10^9 in CS, B10, AB5, and AB10, respectively.

A significant positive correlation was observed between CH_4 oxidation rates and ratio of Type II/Type I methanotrophic relative abundance in batch-incubated samples [Fig. 8(b)] ($R^2 = 0.84$, $p = 0.0015$, from regression analysis in EXCEL 2019), suggesting an important role for Type II methanotrophs in CH_4 oxidation in these columns. Type II methanotrophs have a clear dominant role in landfill CH_4 oxidation, likely due to resilience to changing and adverse environmental conditions. For example, Kumaresan et al. (2009) also found Type II methanotrophs (mainly *Methylocystis*) to be more resilient to changing environmental conditions in a landfill cover soil. Fig. 8(c) shows total bacterial abundance (16S rRNA gene copies per gram soil) in the biocover samples obtained directly from column during Phase 2. A significant difference was noticed between samples obtained directly from column incubation and the samples incubated in batch microcosms ($p = 0.015$, ANOVA). Similar observations were reported by Yargicoglu and Reddy (2017b), in which batch incubated samples had higher methylotrophic relative abundance than samples obtained directly from the column. These differences may be attributed to the differences between batch and column incubation conditions such as a closed system (batch) versus a continuous flow system (column) affecting the gas retention time and interaction of microbes with the gases.

Phase 3 Incubation

Gas Profiles and Methane Oxidation during Phase 3

In Phase 3, the soil columns were exposed to 99% (v/v) CH_4 concentrations at similar inflow rates (~100 g CH_4 m^{-2} day^{-1}) as in Phase 2. Fig. 9 shows the average gas concentration profiles along the depth of the biocovers in Phase 3a, and demonstrates that all biocovers were fully aerated. The appearance of CO_2 is an indication of CH_4 oxidation activity in Phase 3a. However, relatively lower CO_2 concentrations may be an effect of dilution, because CH_4 concentrations also were reduced significantly. Kightley et al. (1995) also observed that the maximum CO_2 concentration generated during column experiments exposed to 99% (v/v) CH_4 influx were less than 5% (v/v) and were reduced further in the upper 20 cm of the biocover layer due to the dilution from air ingress. Another tenable reason for lower CO_2 production could be the presence of non-methane-oxidizing methylotrophs feeding on the intermediate products of CH_4 oxidation, such as methanol, formaldehyde, and so forth, thereby limiting the production of CO_2 (Huang et al. 2019). The amount of CO_2 produced in the CS was less ($p = 0.017$, ANOVA) than that in the biochar-amended

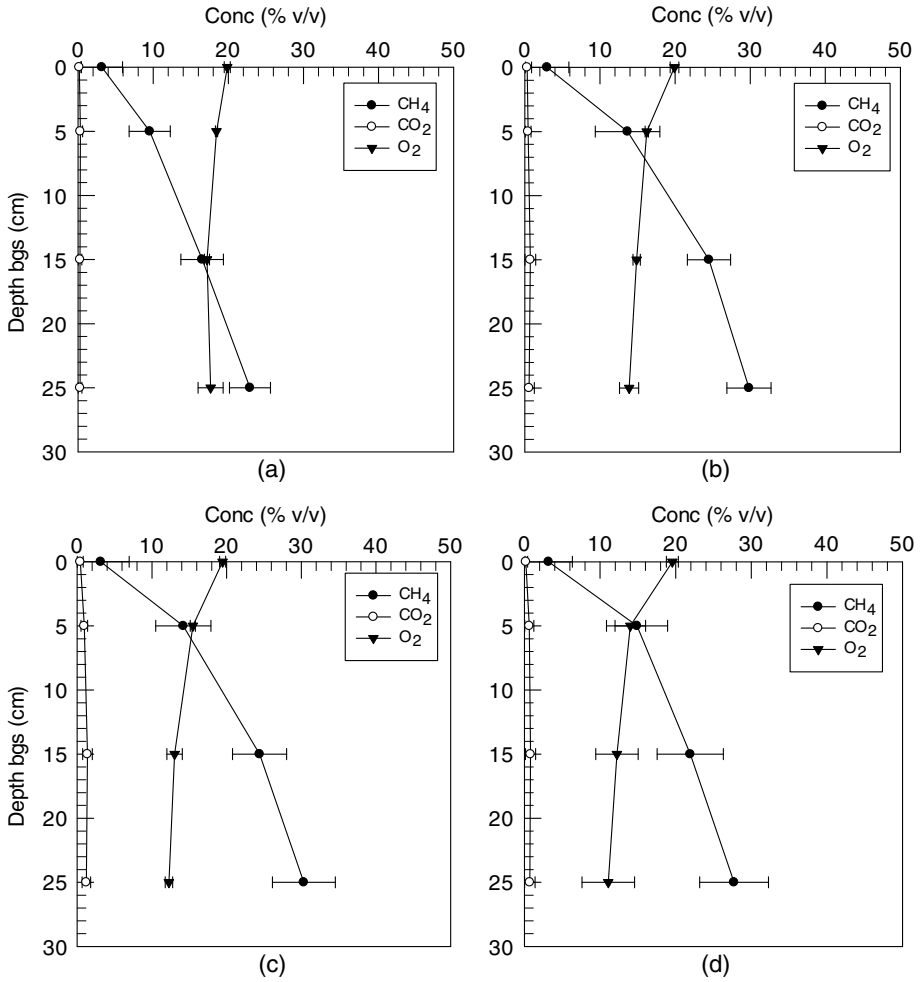

Fig. 9. Average gas profiles along the depth of the biocovers in Phase 3a: (a) soil control (CS); (b) soil + 10% biochar (B10); (c) soil + 5% activated biochar (AB5); and (d) soil + 10% activated biochar (AB10). The presence of CO_2 gas along the depth of the biocovers confirms CH_4 oxidation activity in the covers. It also shows resilience of the microbial communities to changing gas compositions, which generally is encountered in MSW landfills due to heterogeneity in the waste composition.

soils, demonstrating higher CH_4 oxidation rates in the biochar-amended soils.

In Phase 3b, after the addition of a 10-cm-thick sand layer, a sharp decrease in CH_4 concentration was observed in the top 10 cm of the biocover layer, indicating a likely zone of maximum oxidation (Fig. S6). However, in the case of AB5, the zone extended to the entire 20 cm of the biocover layer, suggesting that CH_4 oxidation was occurring deeper into the biocover layer. The depth of maximum CH_4 oxidation normally is governed by the degree of soil aeration, and most soils are well aerated toward the surface (Cabral et al. 2010; Rachor et al. 2011; Roncato and Cabral 2012). However, the biocovers in this study were well aerated throughout their depth, allowing oxidation across the entire soil column. A possible cause of the oxidation occurring in the upper layers despite full aeration could be the high CH_4 load ($127\ g\,CH_4\,m^{-2}\,day^{-1}$). Prior studies have shown shifting of CH_4 oxidation zones toward the surface under such CH_4 loads (Rachor et al. 2011).

Effect of Phase 3 Incubation on Microbial Community

Methylotrophic community structures in biocover samples from Phase 3a were similar to those observed in the previous incubation phases [Fig. 10(a) and Fig. S6]. Bacteria from the genus *Methylobacter* (Type I methanotroph) continued to dominate the biocover samples in Phase 3. Bacteria from the genera *Methylocystis and Methylosinus* (Type II methanotrophs) were found in greater abundance in activated biochar-amended soil samples than in the CS and B10 ($p < 0.001$, ANOVA), consistent with the previous column incubation phases (Table 4). The changes in gas composition did not have a significant effect on the microbial community composition, as they remained similar across all the incubation phases. The stability of the microbial community could be a result of maintaining equivalent inflow CH_4 fluxes (Table 3), because CH_4 flux is a key driver of microbial community composition in landfill cover soil (Gebert et al. 2009; Yargicoglu and Reddy 2017a, b). In addition, the bacterial communities were well established in Phases 1 and 2, so they were resilient to changing gas compositions. Unlike other incubation phases, the total bacterial gene abundance in the biocover samples in Phase 3a was significantly different among the biocovers ($p = 0.014$, ANOVA), and was remarkably higher in biochar-amended soil samples [Fig. 10(b)]. Fig. 10(c) shows the estimated normalized abundance of methylotrophs in the biocover samples, which was significantly different among the biocover samples ($p < 0.001$, ANOVA).

Batch Tests to Verify H_2S Removal by Gravel

The darkening of the pea gravel prompted further study to assess the reactivity of the gravel with H_2S. Batch tests were performed

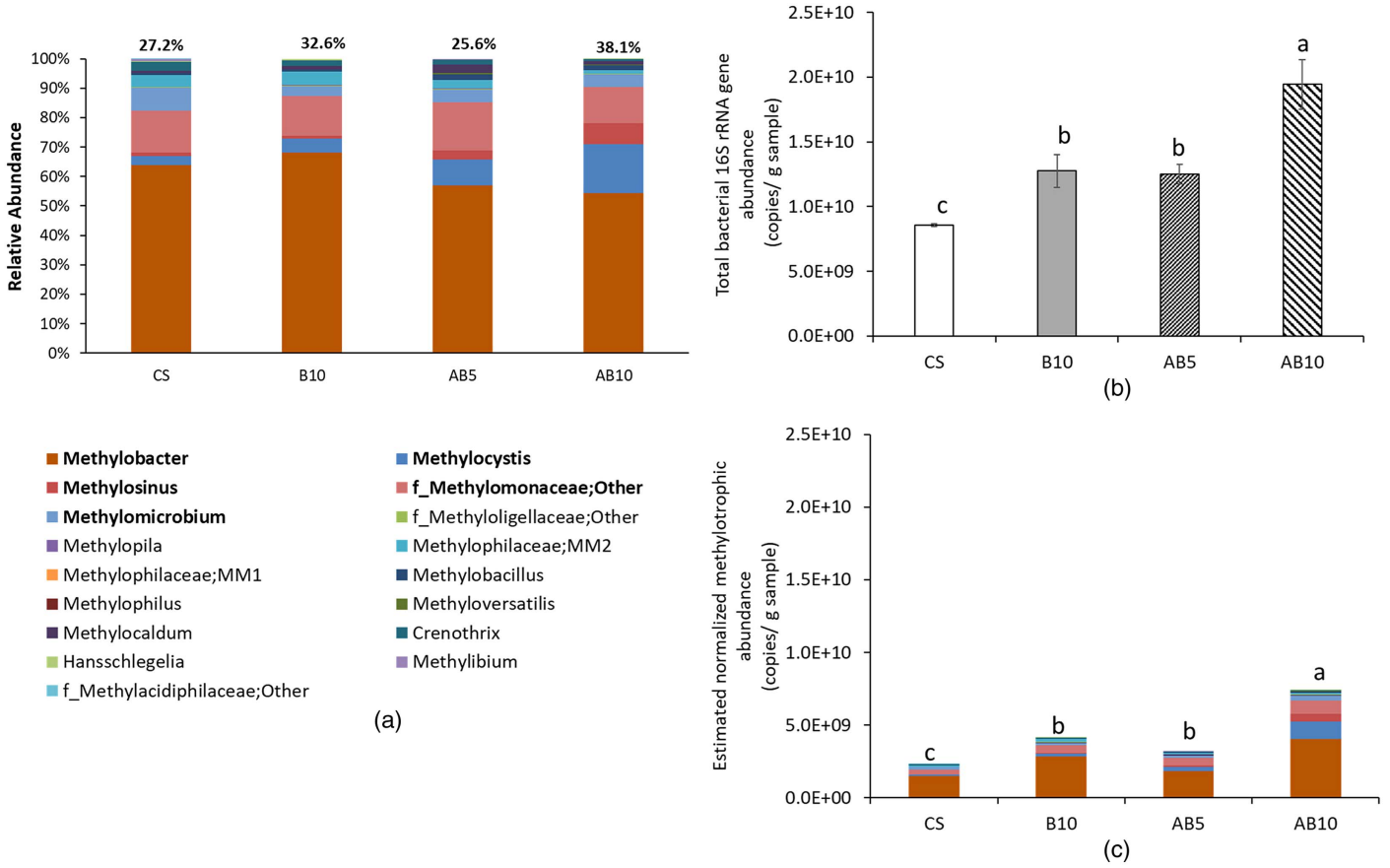

Fig. 10. Microbial community structure in biocover samples during Phase 3a: (a) relative abundance of methylotrophs as assessed by 16S rRNA gene amplicon sequencing (numbers above each column indicate the relative abundance of methylotroph sequences out of all recovered microbial sequences); (b) total bacterial abundance in each biocover samples extracted during Phase 3a of column incubation based on qPCR analysis (bacterial abundance significantly different among the samples, $p = 0.014$, ANOVA); and (c) estimated absolute abundance of methylotrophs calculated by multiplying the relative abundance of each genus by the total bacterial 16S rRNA gene abundance measured by qPCR for the corresponding sample (methylotrophic abundance significantly different among the samples, $p < 0.001$, ANOVA). Lowercase letters on top of the bars represent significant difference in bacterial population among the samples.

with the same pea gravel used in the column studies, and were performed following similar procedure as described previously, with the exception that in these batch tests, the vials containing 5 g of the substrate were purged with a mixture of 48.25% (v/v) CH_4, 50% (v/v) CO_2, and 1.75% (v/v) H_2S. The tested substrates included pea gravel, clean Ottawa sand, field sand, and controls without substrate. The headspace gas concentration was monitored over time to assess absorption of H_2S by each substrate (Fig. S7). Pea gravel and field sand showed significant reactivity with H_2S, whereas clean Ottawa sand and controls showed negligible reactivity. These batch tests affirmed that the H_2S was reacting with the gravel layer in the columns, thereby reducing the H_2S flux into the overlying biocover layer.

The darkening of the gravel could be attributed to the reaction of metals with H_2S leading to formation of metal sulfides. Wohlers and Feldstein (1966) studied the darkening of paints due to H_2S exposure and attributed it to the reaction of heavy metals, including iron salts. Similarly, Chetri et al. (2020) showed a reaction of H_2S with basic oxygen furnace (BOF) steel slag, which is high in iron, leading to blackening of the BOF slag. Hence, it is likely that the pea gravel contained iron salts which reacted with H_2S, leading to the formation of black iron sulfide precipitates on the surface. These observations from batch tests further support our hypothesis that a significant amount of H_2S did not reach the overlying biocover layer to affect the CH_4 oxidation efficiency negatively in Phase 2. However, further investigation is warranted to confirm the reaction mechanisms of H_2S in the column reactors.

Practical Implications and Future Perspectives

Biocovers have emerged as an environmentally friendly alternative to landfill CH_4 mitigation. Biochar based biocover offers advantages over many organics-based biocovers such as compost in terms of long-term endurance due to low susceptibility of biochar to degradation under dynamic environmental conditions (Sadasivam and Reddy 2014). Biochar amendment also helps to prevent desiccation cracking and formation of preferential flow paths due to high moisture retention abilities of biochar (Yargicoglu and Reddy 2017a), which is a common issue in conventional soil covers (Barlaz et al. 2004). Hence, biochar amendment to landfill cover soil helps to prevent the formation of CH_4 hotspots. Similarly, long-term stability of biochar-amended soil reduces the requirement for regular maintenance, and in turn reduces maintenance costs required for repairing cracks and regular monitoring of surface emissions to identify hotspots. Activation of biochar with MOB offers advantage in accelerating the CH_4 oxidation activity in the biocover in addition to the benefits of biochar. Biochar-based landfill cover soil has applicability not only in the US setting, but also in global settings where fugitive landfill gas emissions is a

Table 4. Relative abundance of methylotrophs in tested biocover samples during different phases of column incubation based on 16S rRNA gene amplicon sequencing

Column incubation phase	Percentage of 16S rRNA gene amplicon sequences	Activated biochar		Soil control (CS)		Soil + 10% biochar (B10)		Soil + 5% activated biochar (AB5)		Soil + 10% activated biochar (AB10)	
		Average	Standard deviation	Average	Standar ddeviation	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
Before incubation	Total methylotrophs	23.6	3.7 ($n = 2$)	5.8	1.7 ($n = 2$)	4.1	0.0 ($n = 2$)	5.2	0.0 ($n = 2$)	7.4	0.0 ($n = 2$)
	Methanotrophs	8.9	1.1 ($n = 2$)	3.7	1.1 ($n = 2$)	2.9	0.1 ($n = 2$)	4.8	0.4 ($n = 2$)	6.2	3.3 ($n = 2$)
	Type I	1.4	0.7 ($n = 2$)	2.9	1.0 ($n = 2$)	1.8	0.1 ($n = 2$)	1.1	1.1 ($n = 2$)	1.8	0.1 ($n = 2$)
	Type II	7.5	0.4 ($n = 2$)	0.8	0.03 ($n = 2$)	1.1	0.02 ($n = 2$)	3.6	1.5 ($n = 2$)	4.4	3.2 ($n = 2$)
Phase 1	Total methylotrophs	—	—	41.9	1.7 ($n = 2$)	32.1	2.0 ($n = 2$)	42.2	0.6 ($n = 2$)	30.9	2.2 ($n = 2$)
	Methanotrophs	—	—	39.8	1.8 ($n = 2$)	29.7	2.1 ($n = 2$)	39.1	1.5 ($n = 2$)	28.3	2.5 ($n = 2$)
	Type I	—	—	36.1	1.4 ($n = 2$)	27.2	1.8 ($n = 2$)	28.9	5.6 ($n = 2$)	18.0	3.7 ($n = 2$)
	Type II	—	—	3.7	0.4 ($n = 2$)	2.5	0.3 ($n = 2$)	10.2	4.1 ($n = 2$)	10.3	1.3 ($n = 2$)
Phase 2	Total methylotrophs	—	—	37.8	3.7 ($n = 4$)	32.8	3.8 ($n = 4$)	36.0	2.5 ($n = 4$)	30.4	4.5 ($n = 4$)
	Methanotrophs	—	—	35.9	3.4 ($n = 4$)	31.3	4.1 ($n = 4$)	34.0	2.1 ($n = 4$)	28.2	4.7 ($n = 4$)
	Type I	—	—	32.6	3.3 ($n = 4$)	29.0	3.9 ($n = 4$)	28.5	2.9 ($n = 4$)	21.2	4.1 ($n = 4$)
	Type II	—	—	3.2	0.2 (n = 4)	2.3	0.1 (n = 4)	5.5	0.9 (n = 4)	7.0	0.8 (n = 4)
Phase 3a	Total methylotrophs	—	—	27.1	0.6 ($n = 2$)	32.6	0.1 ($n = 2$)	25.6	0.5 ($n = 2$)	38.1	4.8 ($n = 2$)
	Methanotrophs	—	—	25.2	0.4 ($n = 2$)	30.6	0.3 ($n = 2$)	24.1	0.8 ($n = 2$)	36.8	4.8 ($n = 2$)
	Type I	—	—	24.4	0.3 ($n = 2$)	28.7	0.3 ($n = 2$)	21.1	0.1 ($n = 2$)	27.8	4.4 ($n = 2$)
	Type II	—	—	1.1	0.1 ($n = 2$)	1.9	0.1 ($n = 2$)	3.1	0.1 ($n = 2$)	9.0	0.5 ($n = 2$)

problem and constructing advanced gas control systems is not economically and practically feasible.

The study evaluated the CH_4 mitigation efficiency of activated and nonactivated biochar–amended landfill cover soil based on column studies. The future goal is to evaluate the ability of the activated and nonactivated biochar–amended soil to mitigate CH_4 emissions under biogeochemical cover conditions. In the newly proposed biogeochemical cover system, it is proposed that basic oxygen furnace steel slag should be placed over biochar-amended soil to mitigate CH_4, CO_2, and H_2S simultaneously (Reddy et al. 2018). Because BOF slag is highly alkaline in nature and rich in calcium-containing minerals, it is crucial to understand the effect of BOF slag on the microbial activity in the underlying biochar-amended soil. In addition, the performance of biochar-amended soil will be evaluated in field-scale experiments to better understand the effect of environmental conditions. Moreover, biochar's properties vary based on the type of feedstock and pyrolysis conditions; hence, further studies should be conducted with different types of biochar to verify the outcomes of this study to validate the widespread applicability of biochar for landfill CH_4 mitigation.

Conclusions

The study evaluated the performance of four different biocovers in column incubation studies in terms of CH_4 oxidation efficiency and CH_4 oxidation rates. The findings of the study supported our hypothesis that biochar activation with MOB helps reduce the initial lag phase in CH_4 oxidation observed in control and nonactivated biochar soil columns, and significantly enhances the CH_4 oxidation potential of the biochar-amended landfill cover soil. The use of activated biochar also resulted in higher CH_4 oxidation rates and methylotrophic abundance in soil columns. A shift in methylotrophic communities was not observed during various incubation phases under diverse gas compositions and CH_4 flux rates, highlighting the resilience of methylotrophic communities to changing LFG conditions. H_2S presence in LFG did not hamper the methylotrophic community composition and CH_4 oxidation; however, the exact reaction mechanism could not be elucidated due to likely absorption of H_2S in the gravel layer. The activated biochar and activated biochar-amended soils had higher relative abundances of Type II methanotrophs (*Methylocystis* and *Methylosinus*) across all the incubation phases, and a positive correlation between CH_4 oxidation rates and Type II/Type I methanotrophic abundance was observed, highlighting the importance of Type II methanotrophs in higher CH_4 turnover rates. However, the findings of this study need to be verified with field-scale demonstration as the conditions in the real field vary significantly. In addition, the physicochemical properties of biochar vary depending on the feedstock type and pyrolysis temperature, due to which the observations obtained in this study may differ upon using different biochar. Hence, further studies need to be performed to confirm methanotrophic activities in different types of biochar.

Data Availability Statement

All data generated during the study appear in this article.

Acknowledgments

This research is a part of comprehensive project Innovative Biochar-Slag-Soil Cover System for Zero Emissions at Landfills

funded by the National Science Foundation (CMMI# 1724773), which is gratefully acknowledged. The authors are grateful to the Genomic Research Core in the Research Resources Center at the University of Illinois at Chicago for microbial analysis.

Supplemental Materials

Figs. S1–S7 are available online in the ASCE Library (www.ascelibrary.org).

References

Amaral, J. A., and R. Knowles. 1995. "Growth of methanotrophs in methane and oxygen counter gradients." *FEMS Microbiol. Lett.* 126 (3): 215–220. https://doi.org/10.1111/j.1574-6968.1995.tb07421.x.

Apprill, A., S. McNally, R. Parsons, and L. Weber. 2015. "Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton." *Aquat. Microb. Ecol.* 75 (2): 129–137. https://doi.org/10.3354/ame01753.

ASTM. 2014. *Standard test methods for specific gravity of soil solids by water pycnometer.* ASTM D854-14. West Conshohocken, PA: ASTM.

ASTM. 2016. *Standard test methods for measurement of hydraulic conductivity of saturated porous materials using a flexible wall permeameter.* ASTM D5084-16a. West Conshohocken, PA: ASTM.

ASTM. 2017a. *Standard practice for classification of soils for engineering purposes (Unified soil classification system)*. ASTM D2487-17e1. West Conshohocken, PA: ASTM.

ASTM. 2017b. *Standard test method for saturated density, moisture-holding capacity, and porosity of saturated peat materials*. ASTM D2980-17e1. West Conshohocken, PA: ASTM.

ASTM. 2017c. *Standard test methods for liquid limit, plastic limit, and plasticity index of soils*. ASTM D4318-17e1. West Conshohocken, PA: ASTM.

ASTM. 2017d. *Standard test methods for particle-size distribution (gradation) of soils using sieve analysis*. ASTM D6913/D6913M-17. West Conshohocken, PA: ASTM.

ASTM. 2019a. *Standard test methods for permeability of granular soils (constant head)*. ASTM D2434-19. West Conshohocken, PA: ASTM.

ASTM. 2019b. *Standard test methods for pH of soils*. ASTM D4972-19. West Conshohocken, PA: ASTM.

ASTM. 2020. *Standard test methods for determining the water (moisture) content, ash content, and organic material of peat and other organic soils*. ASTM D2974-20e1. West Conshohocken, PA: ASTM.

Barlaz, M. A., R. B. Green, J. P. Chanton, C. D. Goldsmith, and G. R. Hater. 2004. "Evaluation of a biologically active cover for mitigation of landfill gas emissions." *Environ. Sci. Technol.* 38 (18): 4891–4899. https://doi.org/10.1021/es049605b.

Boeckx, P., and O. Van Cleemput. 1996. "Methane oxidation in a neutral landfill cover soil: Influence of moisture content, temperature, and nitrogen-turnover." *J. Environ. Qual.* 25 (1): 178–183. https://doi.org/10.2134/jeq1996.00472425002500010023x.

Bogner, J., K. Spokas, E. Burton, R. Sweeney, and V. Corona. 1995. "Landfills as atmospheric methane sources and sinks." *Chemosphere* 31 (9): 4119–4130. https://doi.org/10.1016/0045-6535(95)80012-A.

Bogner, J. E., K. A. Spokas, and J. P. Chanton. 2011. "Seasonal greenhouse gas emissions (methane, carbon dioxide, nitrous oxide) from engineered landfills: Daily, intermediate, and final California cover soils." *J. Environ. Qual.* 40 (3): 1010–1020. https://doi.org/10.2134/jeq2010.0407.

Cabral, A. R., M. A. Capanema, J. Gebert, J. F. Moreira, and L. B. Jugnia. 2010. "Quantifying microbial methane oxidation efficiencies in two experimental landfill biocovers using stable isotopes." *Water Air Soil Pollut.* 209 (1): 157–172. https://doi.org/10.1007/s11270-009-0188-4.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. "DADA2: High-resolution sample inference from Illumina amplicon data." *Nat. Methods* 13 (7): 581–583. https://doi.org/10.1038/nmeth.3869.

Chetri, J. K., and K. R. Reddy. 2021. "Climate-resilient biogeochemical cover for waste Containment Systems." In *Geo-extreme*. Reston, VA: ASCE.

Chetri, J. K., K. R. Reddy, and D. G. Grubb. 2020. "Carbon-dioxide and hydrogen-sulfide removal from simulated landfill gas using steel slag." *J. Environ. Eng.* 146 (12): 04020139. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001826.

Clarke, K. R., and R. N. Gorley. 2015. "Getting started with PRIMER v7." *PRIMER-e: plymouth, plymouth marine laboratory*, 20. Auckland, New Zealand: PRIMER-e.

De Visscher, A., D. Thomas, P. Boeckx, and O. Van Cleemput. 1999. "Methane oxidation in simulated landfill cover soil environments." *Environ. Sci. Technol.* 33 (11): 1854–1859. https://doi.org/10.1021/es9900961.

Edgar, R. C. 2010. "Search and clustering orders of magnitude faster than BLAST." *Bioinformatics* 26 (19): 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

Gebert, J., B. K. Singh, Y. Pan, and L. Bodrossy. 2009. "Activity and structure of methanotrophic communities in landfill cover soils." *Environ. Microbiol. Rep.* 1 (5): 414–423. https://doi.org/10.1111/j.1758-2229.2009.00061.x.

Gilman, A., Y. Fu, M. Hendershott, F. Chu, A. W. Puri, A. L. Smith, M. Pesesky, R. Lieberman, A. C. D. Beck, and M. E. Lidstrom. 2017. "Oxygen-limited metabolism in the methanotroph *Methylomicrobium buryatense* 5GB1C." *PeerJ* 5 (Oct): e3945. https://doi.org/10.7717/peerj.3945.

Glöckner, F. O., et al. 2017. "25 years of serving the community with ribosomal RNA gene reference databases and tools." *J. Biotechnol.* 261 (Nov): 169–176. https://doi.org/10.1016/j.jbiotec.2017.06.1198.

Graham, D. W., J. A. Chaudhary, R. S. Hanson, and R. G. Arnold. 1993. "Factors affecting competition between type I and type II methanotrophs in two-organism, continuous-flow reactors." *Microb. Ecol.* 25 (1): 1–17. https://doi.org/10.1007/BF00182126.

Grant, J., and U. V. Bathmann. 1987. "Swept away: Resuspension of bacterial mats regulates benthic-pelagic exchange of sulfur." *Science* 236 (4807): 1472–1474. https://doi.org/10.1126/science.236.4807.1472.

Hanson, R. S., and T. E. Hanson. 1996. "Methanotrophic bacteria." *Microbiol. Rev.* 60 (2): 439–471. https://doi.org/10.1128/mr.60.2.439-471.1996.

Henckel, T., P. Roslev, and R. Conrad. 2000. "Effects of O_2 and CH_4 on presence and activity of the indigenous methanotrophic community in rice field soil." *Environ. Microbiol.* 2 (6): 666–679. https://doi.org/10.1046/j.1462-2920.2000.00149.x.

Huang, D., L. Yang, J. H. Ko, and Q. Xu. 2019. "Comparison of the methane-oxidizing capacity of landfill cover soil amended with biochar produced using different pyrolysis temperatures." *Sci. Total Environ.* 693 (Nov): 133594. https://doi.org/10.1016/j.scitotenv.2019.133594.

Huber-Humer, M., J. Gebert, and H. Hilger. 2008. "Biotic systems to mitigate landfill methane emissions." *Waste Manage. Res.* 26 (1): 33–46. https://doi.org/10.1177/0734242X07087977.

Kaza, S., L. Yao, P. Bhada-Tata, and F. Van Woerden. 2018. *What a waste 2.0: A global snapshot of solid waste management to 2050*. Washington, DC: World Bank Publications.

Kightley, D., D. B. Nedwell, and M. Cooper. 1995. "Capacity for methane oxidation in landfill cover soils measured in laboratory-scale soil microcosms." *Appl. Environ. Microbiol.* 61 (2): 592–601. https://doi.org/10.1128/aem.61.2.592-601.1995.

Kjeldsen, P., and C. Scheutz. 2018. "Landfill gas management by methane oxidation." In *Solid waste landfilling*, 477–497. Amsterdam, Netherlands: Elsevier.

Kleinjan, W. E., A. de Keizer, and A. J. Janssen. 2003. "Biologically produced sulfur." In *Elemental sulfur and sulfur-rich compounds I*, edited by R. Steudel, 167–188. Berlin: Springer.

Ko, J. H., Q. Xu, and Y. C. Jang. 2015. "Emissions and control of hydrogen sulfide at landfills: A review." *Crit. Rev. Environ. Sci. Technol.* 45 (19): 2043–2083. https://doi.org/10.1080/10643389.2015.1010427.

Kumaresan, D., G. C. Abell, L. Bodrossy, N. Stralis-Pavese, and J. C. Murrell. 2009. "Spatial and temporal diversity of methanotrophs in a landfill cover soil are differentially related to soil abiotic factors."

Environ. Microbiol. Rep. 1 (5): 398–407. https://doi.org/10.1111/j.1758-2229.2009.00059.x.

La, H., J. P. A. Hettiaratchi, G. Achari, J.-J. Kim, and P. F. Dunfield. 2018. "Investigation of biologically stable biofilter medium for methane mitigation by methanotrophic bacteria." *J. Hazard. Toxic Radioact. Waste* 22 (3): 04018013. https://doi.org/10.1061/(ASCE)HZ.2153-5515.0000406.

Lee, E.-H., K.-E. Moon, T. G. Kim, S.-D. Lee, and K.-S. Cho. 2015. "Inhibitory effects of sulfur compounds on methane oxidation by a methane-oxidizing consortium." *J. Biosci. Bioeng.* 120 (6): 670–676. https://doi.org/10.1016/j.jbiosc.2015.04.006.

Lee, E.-H., T. W. Yi, K.-E. Moon, H. J. Park, H. W. Ryu, and K.-S. Cho. 2011. "Characterization of methane oxidation by a methanotroph isolated from a landfill cover soil, South Korea." *J. Microbiol. Biotechnol.* 21 (7): 753–756. https://doi.org/10.4014/jmb.1102.01055.

Lehmann, J., J. Gaunt, and M. Rondon. 2006. "Bio-char sequestration in terrestrial ecosystems–A review." *Mitig. Adapt. Strateg. Glob. Change* 11 (2): 403–427. https://doi.org/10.1007/s11027-005-9006-5.

Long, Y.-Y., Y. Liao, K. Zhang, L.-F. Hu, C.-R. Fang, and D.-S. Shen. 2013. "Can H_2S affect the methane oxidation in a landfill?" *Ecol. Eng.* 60 (Part 1): 438–444. https://doi.org/10.1016/j.ecoleng.2013.09.006.

Majdinasab, A., and Q. Yuan. 2017. "Performance of the biotic systems for reducing methane emissions from landfill sites: A review." *Ecol. Eng.* 104 (Part A): 116–130. https://doi.org/10.1016/j.ecoleng.2017.04.015.

Nadkarni, M. A., F. E. Martin, N. A. Jacques, and N. Hunter. 2002. "Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set." *Microbiology (Reading)* 148 (1): 257–266. https://doi.org/10.1099/00221287-148-1-257.

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

Parada, A. E., D. M. Needham, and J. A. Fuhrman. 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. https://doi.org/10.1111/1462-2920.13023.

Pfluger, A. R., W.-M. Wu, A. J. Pieja, J. Wan, K. H. Rostkowski, and C. S. Criddle. 2011. "Selection of Type I and Type II methanotrophic proteobacteria in a fluidized bed reactor under non-sterile conditions." *Bioresour. Technol.* 102 (21): 9919–9926. https://doi.org/10.1016/j.biortech.2011.08.054.

Powelson, D. K., J. Chanton, T. Abichou, and J. Morales. 2006. "Methane oxidation in water-spreading and compost biofilters." *Waste Manage. Res.* 24 (6): 528–536. https://doi.org/10.1177/0734242X06065704.

Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2012. "The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools." *Nucleic Acids Res.* 41 (1): D590–D596. https://doi.org/10.1093/nar/gks1219.

Rachor, I., J. Gebert, A. Gröngröft, and E.-M. Pfeiffer. 2011. "Assessment of the methane oxidation capacity of compacted soils intended for use as landfill cover materials." *Waste Manage.* 31 (5): 833–842. https://doi.org/10.1016/j.wasman.2010.10.006.

Rai, R. K., J. K. Chetri, S. J. Green, and K. R. Reddy. 2018. "Identifying active methanotrophs and mitigation of CH_4 emissions in landfill cover soil." In *Proc., Int. Congress on Environmental Geotechnics*, 308–316. Singapore: Springer.

Rai, R. K., J. K. Chetri, and K. R. Reddy. 2019. "Effect of methanotrophic-activated biochar-amended soil in mitigating CH_4 emissions from landfills." In *Proc. 4th Int. Conf. on Civil and Environmental Geology and Mining Engineering*. Trabzon, Turkey: Nevsehir Bektas Veli Univ. and Giresun Univ.

Reddy, K. R., D. G. Grubb, and G. Kumar. 2018. "Innovative biogeochemical soil cover to mitigate landfill gas emissions." In *Proc. Int. Conf. on Protection and Restoration of the Environment XIV.* Hoboken, NJ: Stevens Institute of Technology.

Reddy, K. R., R. K. Rai, S. J. Green, and J. K. Chetri. 2019. "Effect of temperature on methane oxidation and community composition in landfill cover soil." *J. Ind. Microbiol. Biotechnol.* 46 (9–10): 1283–1295. https://doi.org/10.1007/s10295-019-02217-y.

Reddy, K. R., R. K. Rai, S. J. Green, and J. K. Chetri. 2020a. "Effect of pH on methane oxidation and community composition in landfill cover soil." *J. Environ. Eng.* 146 (6): 04020037. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001712.

Reddy, K. R., E. N. Yargicoglu, and J. K. Chetri. 2020b. "Effects of biochar on methane oxidation and properties of landfill cover soil: Long-term column incubation tests." *J. Environ. Eng.* 147 (1): 04020144. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001829.

Roncato, C. D., and A. R. Cabral. 2012. "Evaluation of methane oxidation efficiency of two biocovers: field and laboratory results." *J. Environ. Eng.* 138 (2): 164–173. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000475.

Sadasivam, B. Y., and K. R. Reddy. 2014. "Landfill methane oxidation in soil and bio-based cover systems: A review." *Rev. Environ. Sci. Bio/Technol.* 13 (1): 79–107. https://doi.org/10.1007/s11157-013-9325-z.

Sadasivam, B. Y., and K. R. Reddy. 2015a. "Adsorption and transport of methane in biochars derived from waste wood." *Waste Manage.* 43 (9): 218–229. https://doi.org/10.1016/j.wasman.2015.04.025.

Sadasivam, B. Y., and K. R. Reddy. 2015b. "Adsorption and transport of methane in landfill cover soil amended with waste-wood biochars." *J. Environ. Manage.* 158 (8): 11–23. https://doi.org/10.1016/j.jenvman.2015.04.032.

Scheutz, C., P. Kjeldsen, J. E. Bogner, A. De Visscher, J. Gebert, H. A. Hilger, M. Huber-Humer, and K. Spokas. 2009. "Microbial methane oxidation processes and technologies for mitigation of landfill gas emissions." *Waste Manage. Res.* 27 (5): 409–455. https://doi.org/10.1177/0734242X09339325.

Shackley, S., S. Sohi, R. Ibarrola, J. Hammond, O. Mašek, P. Brownsort, A. Cross, M. Prendergast-Miller, and S. Haszeldine. 2013. "Biochar, tool for climate change mitigation and soil management." In *Geoengineering responses to climate change*, 73–140. New York: Springer.

Sharma, H. D., and K. R. Reddy. 2004. *Geoenvironmental engineering: Site remediation, waste containment, and emerging waste management technologies*. Hobeken, NJ: Wiley.

Spokas, K. A., and J. E. Bogner. 2011. "Limits and dynamics of methane oxidation in landfill cover soils." *Waste Manage.* 31 (5): 823–832. https://doi.org/10.1016/j.wasman.2009.12.018.

USEPA. 2020. "Basic information about landfill gas." Accessed October 27, 2020. https://www.epa.gov/lmop/basic-information-about-landfill-gas.

USEPA. 2021. "Sustainable materials management (SMM) web academy webinar: Material characterization and economic impacts of recycling—2020 reports." Accessed March 3, 2021. https://www.epa.gov/smm/sustainable-materials-management-smm-web-academy-webinar-material-characterization-and-economic.

Whittenbury, R., K. C. Phillips, and J. F. Wilkinson. 1970. "Enrichment, isolation and some properties of methane-utilizing bacteria." *Microbiology (Reading)* 61 (2): 205–218. https://doi.org/10.1099/00221287-61-2-205.

Wilshusen, J. H., J. P. A. Hettiaratchi, A. De Visscher, and R. Saint-Fort. 2004. "Methane oxidation and formation of EPS in compost: Effect of oxygen concentration." *Environ. Pollut.* 129 (2): 305–314. https://doi.org/10.1016/j.envpol.2003.10.015.

Wohlers, H. C., and M. Feldstein. 1966. "Hydrogen sulfide darkening of exterior paint." *J. Air Pollut. Control Assoc.* 16 (1): 19–21. https://doi.org/10.1080/00022470.1966.10468434.

Xia, F.-F., H.-T. Zhang, X.-M. Wei, Y. Su, and R. He. 2015. "Characterization of H_2S removal and microbial community in landfill cover soils." *Environ. Sci. Pollut. Res.* 22 (23): 18906–18917. https://doi.org/10.1007/s11356-015-5070-x.

Xie, T., B. Y. Sadasivam, K. R. Reddy, C. Wang, and K. Spokas. 2016. "Review of the effects of biochar amendment on soil properties and carbon sequestration." *J. Hazard. Toxic Radioact. Waste* 20 (1): 04015013. https://doi.org/10.1061/(ASCE)HZ.2153-5515.0000293.

Yargicoglu, E. N., and K. R. Reddy. 2017a. "Effects of biochar and wood pellets amendments added to landfill cover soil on microbial methane

oxidation: A laboratory column study." *J. Environ. Manage.* 193 (May): 19–31. https://doi.org/10.1016/j.jenvman.2017.01.068.

Yargicoglu, E. N., and K. R. Reddy. 2017b. "Microbial abundance and activity in biochar-amended landfill cover soils: Evidence from large-scale column and field experiments." *J. Environ. Eng.* 143 (9): 04017058. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001254.

Yargicoglu, E. N., and K. R. Reddy. 2018. "Biochar-amended soil cover for microbial methane oxidation: Effect of biochar amendment ratio and cover profile." *J. Geotech. Geoenviron. Eng.* 144 (3): 04017123. https://doi.org/10.1061/(ASCE)GT.1943-5606.0001845.

Yargicoglu, E. N., B. Y. Sadasivam, K. R. Reddy, and K. Spokas. 2015. "Physical and chemical characterization of waste wood derived biochars." *Waste Manage.* 36 (Feb): 256–268. https://doi.org/10.1016/j.wasman.2014.10.029.

Zhang, J., K. Kobert, T. Flouri, and A. Stamatakis. 2014. "PEAR: A fast and accurate Illumina Paired-End reAd merger." *Bioinformatics* 30 (5): 614–620. https://doi.org/10.1093/bioinformatics/btt593.

Zhao, Q., Y. Wang, Z. Xu, and Z. Yu. 2021. "How does biochar amendment affect soil methane oxidation? A review." *J. Soil. Sediment* 21 (Apr): 1575–1586. https://doi.org/10.1007/s11368-021-02889-z.