Effect of Basic Oxygen Furnace (BOF) Slag-infiltrated Water on Methane Oxidation and Community Composition in Biogeochemical Landfill Cover System

Krishna R. Reddy, F.ASCE

Professor, University of Illinois at Chicago, Department of Civil & Materials Engineering, 842 West Taylor Street, Chicago, IL 60607, USA, e-mail: kreddy@uic.edu (Corresponding author)

Raksha K. Rai, SM.ASCE

Graduate Research Assistant, University of Illinois at Chicago, Department of Civil & Materials Engineering, 842 West Taylor Street, Chicago, IL 60607, USA, e-mail: rrai5@uic.edu

Stefan J. Green

Director, Sequencing Core, Resources Center & Department of Biological Sciences, University of Illinois at Chicago, 835 S. Wolcott, Chicago, IL 60612, USA, e-mail: greendna@uic.edu

Jyoti K. Chetri, SM.ASCE

Graduate Research Assistant, University of Illinois at Chicago, Department of Civil & Materials Engineering, 842 West Taylor Street, Chicago, IL 60607, USA, e-mail: jkc4@uic.edu

Manuscript Submitted to:

Journal of Hazardous, Toxic, and Radioactive Waste Management

July 20, 2020

Abstract: A sustainable biogeochemical cover system, consisting of a biochar-amended soil layer overlain by Basic Oxygen Furnace (BOF) slag layer, is being developed to mitigate fugitive emissions of methane (CH_4) and carbon dioxide (CO_2) at municipal solid waste (MSW) landfills. The effectiveness of such a cover system is highly dependent on the survival and activity of methanotrophs in the soil under highly alkaline conditions induced by the presence of the slag. In this paper, laboratory microcosm tests were conducted to investigate the effect of BOF slag-infiltrated water on CH_4 oxidation in soil and in enrichment culture. Effects of BOF slag-infiltrated water at different proportions in soil (0, 5, 20, 60 and 100%) and in enrichment culture (0, 11, 25 and 100%) were studied. CH_4 oxidation rates in the soil were 113, 116, 115, 108 and 97 μg CH_4 $g^{-1}d^{-1}$ at 0, 5, 20, 60 and 100% slag-infiltrated water content, respectively, and in enrichment culture, the CH_4 oxidation rates were 36, 27, 20 and 17 μg CH_4 $mL^{-1}d^{-1}$ at 0, 11, 25 and 100% slag-infiltrated water content, respectively. The results showed significant decrease in CH_4 oxidation rates with increase in slag-infiltrated water content (> 11%) in enrichment culture and a marginal decrease in soil microcosm. Furthermore, no substantial change in microbial community composition was noted in soil microcosms across all the slag-infiltrated water contents and were generally dominated by the species *Methylobacter luteus*. However, the enrichment culture, which was dominated by *Methylobacter* at 0% slag-infiltrated water content showed decrease in the abundance of *Methylobactor* and increase in the abundance of *Methylosinus* with increase in the slag-infiltrated water content.

Keywords: Basic Oxygen Furnace (BOF) Slag; Biogeochemical cover; Landfill gas; Methane oxidation; Methanotrophs; Slag-infiltrated water

Introduction

Industrial waste disposal is currently a major environmental and societal issue. These wastes are either reused within industry or stock-pilled for disposal in landfills. Slag, a byproduct from steel mills, is generated in enormous quantities during iron making and steel making process. Iron and steel slags composition varies depending upon the type of production process or type of furnace used in the production process. Two main types of slag are generated by the steel industry: blast furnace (BF) slag, an iron making slag, and steel slag, a steel making slag. Steel slag is further classified by technology used, including basic oxygen furnace (BOF) slag, ladle furnace (LF) slag, and electric arc furnace (EAF) slag. Among the types of iron and steel slags, BF slag has found its application in civil and construction industry, while steel slags have limited applications due to the finer particle size and volumetric instability resulting in the stockpiling as well as landfilling of the steel slag (Reddy et al. 2019a).

In the recent years, steel slag has found its application in CO_2 sequestration and reduction of CO_2 emissions into the atmosphere (Huijgen et al 2005; Bonenfant et al. 2008; Chang et al. 2013). Steel slags are rich in alkaline metal oxides such as calcium oxide (CaO) which react with CO_2 to form stable carbonates. Similarly, steel slag has many calcium containing minerals such as as free lime (CaO), portlandite ($Ca(OH)_2$) and larnite (Ca_2SiO_4) which bind CO_2 to form carbonates. Accelerated carbonation of BOF slags have been used to treat volumetric instability of the BOF slag (Ko et al 2015). The alkaline minerals present in the steel slag makes them favorable for the CO_2 sequestration applications, however, the high alkalinity and high pH of steel slag could be a problem for the terrestrial and aquatic environments. The most widespread impact is that of slag leachate on receiving water bodies by drastically increasing the pH of the water, depleting O_2

69 in the water bodies, and increasing salinity and metal concentrations. An alkaline pH of 8.5-10 has
70 been reported to cause severe disturbance to fish life leading to death (Saha et al. 2002). Excess
71 carbonate precipitation in Onondaga Lake, New York, USA has shown reduced diversity of
72 zooplankton and growth of macrophytes (Effler and Matthews 2003). In contrast, the dumping of
73 industrial slag since 1880s in the low-lying Lake Calumet area of southeast Chicago and north-
74 west Indiana, has shown diverse microbial communities that inhabits alkaliphiles, iron-reducing
75 and sulfur reducing bacteria in extremely alkaline groundwater (Roadcap et al. 2006).

76 Recently, Reddy et al. (2018) proposed the concept of an innovative biogeochemical cover
77 consisting of biochar-amended soil and BOF slag to mitigate fugitive CH_4 and CO_2 emissions from
78 the municipal solid waste (MSW) landfills. The use of biochar amended soil has been studied
79 previously in our laboratory showing promising results in mitigating CH_4 emissions from landfills
80 due to its physico-chemical properties such as water holding capacity, high porosity and surface
81 area for proliferation of microbes (Yargicoglu and Reddy, 2017a, b). The CH_4 oxidizing bacteria
82 present in the landfill soil utilize CH_4 as the sole source of carbon and energy and oxidize it to CO_2
83 in the presence of O_2 as shown in Eq. 1 (Reddy et al. 2014). The methanotrophic bacteria produce
84 enzyme CH_4 monooxygene (MMO) which catalyze the CH_4 oxidation reaction (Reddy et al. 2014).

85 $$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \quad (1)$$

86 Another important aspect of biogeochemical cover is the steel slag which has the potential
87 to sequester CO_2. Although mineral carbonation and CO_2 sequestration by steel slag had been
88 explored extensively in the recent years, none of the studies explored the use of BOF slag in
89 sequestering CO_2 under landfill gas conditions. Reddy et al. (2019b, c, d) investigated the CO_2
90 sequestration capacity of BOF slag under simulated landfill conditions and showed steel slag has
91 significant CO_2 sequestration potential under landfill conditions. The governing reactions for CO_2

92 sequestration by steel slag are shown in Eq. 2 to 5 (Reddy et al. 2019b). Further information on
93 carbonation of BOF slag under landfill conditions can be found in Reddy et al. (2019b, c, d).

94 $$CaO + H_2O = Ca^{2+} + 2OH^- \quad (2)$$

95 $$Ca(OH)_2 = Ca^{2+} + 2OH^- \quad (3)$$

96 $$CO_2 + H_2O = CO_3^{2-} + 2H^+ \quad (4)$$

97 $$Ca^{2+} + CO_3^{2-} = CaCO_3 \quad (5)$$

98 In the biogeochemical cover, steel slag is used in conjunction with the biochar amended
99 soil to mitigate CO_2 and CH_4 simultaneously which are the major components of the landfill gas.
100 Since, BOF slag is highly alkaline in nature with pH ~ 12, it is crucial to understand the effect of
101 BOF slag on the general microbial community, and specifically the methylotrophs which play a
102 pivotal role in the oxidation of CH_4 in biochar-amended soil. Preliminary investigations from our
103 laboratory showed inhibited CH_4 oxidation when BOF slag was mixed with soil or biochar-
104 amended soil in contrast to an isolated or layered system (soil/biochar amended soil isolated from
105 slag) that showed substantial CH_4 oxidation, affirming feasibility of the layered system in the
106 biogeochemical landfill cover (Rai and Reddy 2019). However, during precipitation events, water
107 may percolate through the steel slag layer and seep into the underlying biochar amended soil layer.
108 The water infiltrating through steel slag may have high alkalinity and affect the microbial activity
109 in the soil, which needs to be investigated. Hence, the specific goals of this study are: (i) to
110 investigate the effect of slag-infiltrated water on CH_4 oxidation in a landfill cover system
111 consisting of BOF slag underlain by biochar amended soil; (ii) to evaluate the effect of slag-
112 infiltrated water on the microbial activity and community composition of microbes in soil; and (iii)
113 to compare the effect of slag-infiltrated water on the community composition of microbes in soil

and methanotrophic enrichment culture to delineate the important factors affecting the microbial communities.

Materials and Methods

Soil

Soil was collected from the Zion landfill, located in Zion, Illinois, USA. Soil samples were collected from an interim cover at a depth of ~1 to 2 feet and were shipped to the Geotechnical and Geoenvironmental Engineering Laboratory at the University of Illinois at Chicago (UIC), where they were stored at room temperature (23 ± 2°C). Soil samples were air dried (moisture content <0.5%), pulverized and screened through a 2 mm sieve prior to conducting the experiments. It is important to choose the right location for collection of soil in order to have indigenous methanotrophic bacteria acclimated to CH_4 influx. In this study, soil was collected at the points at interim cover which were likely be exposed to CH_4 such as locations away from the gas collection wells.

BOF Slag

The BOF slag used in this experiment was obtained from Indiana Harbor East (IHE) of Arcelor Mittal steel plant, located in East Chicago, Indiana, USA. The BOF slag, was sampled from the steel plant in September 2017, and is designated as IHE 9/17 (samples collected from Indiana Harbor East on Sep 17, 2017). All the tests were performed using the bulk slag sample as obtained from the plant. The steel slag was also oven-dried at 105°C prior to conducting the experiments.

Characteristics of Landfill Soil and BOF Slag

The landfill soil and BOF slag were characterized for their physical, chemical and geotechnical properties as listed in Table 1. Particle size distribution was determined as per American Society for Testing and Materials (ASTM) D422. The specific gravity, soil classification, and water holding capacity (WHC) were determined according to ASTM D584, D2487 and D2980, respectively. Loss on ignition (LOI) was determined as per ASTM D2974. Hydraulic conductivity was measured according to ASTM D2434 standard procedure using a rigid wall permeameter for slag and according to ASTM 5084 using a flexible wall triaxial setup for soil. The pH and oxidation-reduction potential (ORP) were measured using an ORION Model 720A pH meter at a liquid to solid ratio of 1:1. The pH meter was pre-calibrated using standard buffer solutions of pH 4, 7 and 10 before use. Electrical conductivity (EC) was measured using a Corning 311 Conductivity Meter and pre-calibrated using standard solution of 12.9 mS/cm.

The elemental content analysis of BOF slag-infiltrated water was conducted by inductively coupled plasma optical emission spectrometry (ICP-OES) following EPA method SW6020A (SW3005A). The mercury content analysis was done as per EPA method SW7470A.

BOF Slag-infiltrated Water Collection

An acrylic glass column of 30 cm height and 2.5 cm inner diameter was filled with BOF slag in three layers each of 5 cm with slight tamping to a total height of 15 cm. **Fig. 1.** shows a picture of BOF slag-infiltrated water collection procedure. The glass column was secured with bed support mesh screen and screw cap at the bottom to prevent spillage of the fines. To simulate infiltration, the headspace of the column was filled with regular tap water, and was allowed to percolate through the slag, and infiltrate was collected at the bottom after passing through Ahlstrom grade

55 filter paper (**Fig. 1**). Around 20 pore volumes (PVs) of infiltrate (1 PV = 29. 23 mL) was collected for the experiments. The collected infiltrate samples were tested for pH and electrical conductivity. It was observed that for all the PVs tested, the pH and electrical conductivity remained nearly constant (12.32-12.38 and 8.30-8.60 mS/cm, respectively).

Soil Microcosm Batch Tests

For batch testing, 10 g of soil was placed in 125 mL-serum vials and moisture content was adjusted to 20% (w/w) using BOF slag-infiltrated water and deionized water (DI) mixture. The slag infiltrate was mixed with DI water at different proportions: 0% (control - 2 mL DI water), 5% (0.1 mL slag-infiltrated water + 1.9 mL DI water), 20% (0.4 mL slag-infiltrated water + 1.6 mL DI water), 60% (1.2 mL slag-infiltrated water + 0.8 mL DI water), and 100% (2 mL slag-infiltrated water). The vials were sealed airtight using butyl rubber septa and secured using crimp cap. 20 mL of air from the headspace was replaced with equal volume of synthetic landfill gas (LFG) comprising of 50% (v/v) CH_4 and 50% (v/v) CO_2 to achieve a headspace concentration of 5-6% CH_4 (v/v), 5-6% CO_2 (v/v) and a balance (~88–90%) of air. The change in the headspace concentrations was determined by collecting and analyzing the gas samples on a regular basis using gas chromatography (GC) until the headspace concentration of CH_4 dropped below 1%. All the experiments were conducted in triplicate along with the controls (with synthetic LFG without any material) to account for the errors and inconsistencies in sampling. The controls were monitored along with the samples to ensure that the change in headspace concentration was only due to CH_4 oxidation and not due to leakage. The CH_4 oxidation rates were calculated from linear regression analysis of CH_4 concentration versus elapsed time, based on the zero-order kinetics.

Enrichment Culture Batch Tests

Serum vials, rubber septa and pipettes were sterilized using a Napco Model 8000-DSE autoclave operated at >120°C for a minimum of 60 minutes to ensure sterilization. A mixed methanotrophic culture was cultivated in the laboratory by enriching soil in Nitrate Mineral Salts (NMS) media along with CH_4 and O_2 as previously described in Rai et al. (2018). The enrichment cultures from the above was used in the experiments and inoculated with BOF slag-infiltrated water at different proportions : 0%, 11% (1 mL slag-infiltrated water and 9 mL enrichment culture), 25% (2 mL slag-infiltrated water and 8 mL enrichment culture) and 100% (5 mL slag-infiltrated water and 5 mL enrichment culture). Similar procedure as microcosm batch tests were followed in the culture batch tests to achieve a headspace concentration of ~5-6% (v/v) CH_4, ~5-6% (v/v) CO_2 and a balance (~88-90%) of air. All the experiments were conducted in triplicate along with the controls (media-NMS). The pH of each sample was measured at the beginning and end of the experiment.

Gas Analysis

Gas samples were analyzed at regular time intervals and analyzed for CH_4 and CO_2 concentrations using an SRI 9300 Gas Chromatograph (GC) equipped with a thermal conductivity detector (TCD). Gas samples were withdrawn using 1 mL syringe where 0.5 mL of the sample was discarded and remaining 0.5 mL was injected into the GC to reduce any pressure effects due to sampling. A calibration curve for a minimum of three points was established using high purity standard gas mixtures ranging from 1% to 50% CH_4 and 5% to 50% CO_2.

Microbial Community Structure Analysis

Genomic DNA (gDNA) was extracted from soil samples and from cell pellets using a DNeasy PowerSoil Kit (Qiagen) based on manufacturer's instructions with a slight modification. Samples were heated at 65°C for 10 min before homogenizing with FastPrep-24 5G bead-beating device (MP Biomedicals) at 6 m/s for 40 sec. After homogenization, extraction protocols were automated on a QIAcube instrument (Qiagen), according to the manufacturer's instructions. Genomic DNA was processed for microbial community analysis using shotgun metagenome sequencing (SMS), using a Nextera XT library preparation kit (Illumina, San Diego, CA) according to the manufacturer's instructions. Libraries were pooled and sequenced on a NextSeq500 instrument, employing a 300 cycle (2x150 paired end reads) mid-output kit. Sequence data were analyzed using the online annotation server from OneCodex, as described previously (Minot et al. 2015). DNA extraction, library preparation and sequencing were performed at the University of Illinois at Chicago Sequencing Core (UICSQC).

Data Archive

Raw sequence data files were submitted in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI). The BioProject identifiers of the SMS samples is PRJNA540300.

Statistical Analysis

Statistical analysis of batch test results was tested using one-way (ANOVA) and t-tests (equivalency of sample means) using Microsoft Excel-2018. A significance level of 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 multidimensional scaling (MDS) plots. Significant differences in community structure between experimental conditions were assessed using analysis of similarity (ANOSIM).

Results and Discussion

Landfill Soil and BOF Slag Characterization

The physical and chemical properties of the landfill soil and BOF slag are summarized in **Table 1**. The specific gravity of the BOF slag (3.5) was relatively high and is attributed to high iron content (30.2%). The WHC of the BOF slag was 20% and that of soil was 45.9%. The higher WHC of soil is beneficial for moisture retention and prevents early drying out of the cover soil. The pH of the slag was 12.4, indicating slag to be highly alkaline in nature. The hydraulic conductivity of BOF slag was 1.1 x 10^{-3} cm/s which is similar to sand. It means the slag layer will have relatively higher permeability and the slag-infiltrated water may seep into the underlying biochar amended soil layer in a biogeochemical cover system.

The constituents of concern (COC) metals concentrations in the BOF slag-infiltrated water are presented in **Table 2.** The metal concentrations are observed to be below the Resource Conservation and Recovery Act (RCRA) limits, demonstrating BOF slag to be non-hazardous. BOF slag is a complex mixture with many insoluble silicates and strongly pH buffered minerals. Studies have shown low leaching of heavy metals from the BOF slag (Proctor et al. 2000; Motz and Geiseler; 2001; Chand et al. 2017; Reddy et al. 2019 b) indicating safe disposal of slag into the environment. In contrast, few studies have shown oxidation of Vanadium (oxidation state +3) to Vanadium (oxidation state +5) during leaching that is highly mobile and toxic (Chaurand et al. 2007; De Windt et al. 2011). The BOF slag-infiltrated water shows negligible leaching of heavy

metals (**Table 2**) which is consistent with most of the studies mentioned above.

Methane Oxidation Potential in Soil and Enrichment Culture

The CH_4 oxidation rates in soil microcosms at different slag-infiltrated water content (0, 5, 20, 60, 100%) ranged between 97 to 116 μg CH_4 $g^{-1}d^{-1}$ as shown in **Fig. 2a**. A marginal decrease in the CH_4 oxidation rates with increased slag-infiltrated water content was observed, which could be due to the slight increase in the pH of the soil-slag-infiltrated water mixture with increase in the slag-infiltrated water content. **Table 3** shows the pH of the soil with slag-infiltrated water content ranging from 0% to 40% (w/w). The slag-infiltrated water mixing limit of 40% was chosen based on the water holding capacity (WHC) of the soil. The pH of the soil and slag-infiltrated water mixture was much lower than the original pH of the slag which was ascribed to the high buffering capacity of the soil, thereby resisting change in pH. At different proportions of slag-infiltrated water, the pH fluctuated within a narrow range of 7.87-8.04 which is close to the optimum pH range for CH_4 oxidation as observed by Scheutz and Kjeldsen (2004). Statistical analysis (Single factor- one-way ANOVA) showed that the CH_4 oxidation rates in soil at different slag-infiltrated water content were not significantly different from each other ($p = 0.16$). This can be associated with the lower leachability of heavy metals from the BOF slag (**Table 2),** suggesting heavy metal leaching is not a concern for CH_4 oxidation rates in biogeochemical cover in landfill. Prior studies have shown CH_4 oxidation in agricultural soils contaminated with lead, zinc and nickel at a concentration of 60, 120 and 35 μg g^{-1}, respectively (Walkiewicz et al. 2016). In contrary, Contin et al. (2012) showed higher inhibition rate in sewage sludge treated soil with zinc at a dose of 1500 μg g^{-1} when compared to sewage sludge treated soil with lead at a dose of 1000 mg g^{-1} in arable and grassland soils. Wnuk et al. (2016) showed considerable CH_4 oxidation in lead-contaminated

mineral soils optimal at 13% (v/v) moisture content. Chromium significantly inhibited CH_4 oxidation in the alluvial rice soil at concentration > 20 µg g^{-1} and 60% WHC, while zinc inhibited CH_4 oxidation under flooded conditions in both alluvial and laterite rice soils (Mohanty et al. 1990). Bowman and Hayward (1990) reported few of the culturable methanotrophic species sensitive to mercury and cadmium at concentrations > 0.5 and 10 µM, respectively but relatively resistant to copper, chromium and zinc. However, in this study, even though the elemental composition of the slag is high, the metal constituents in the slag-infiltrated water were very low indicating heavy metals leaching is not a concern for the methanotrophic activity when using BOF slag in the landfill cover.

Fig. 2b shows the CH_4 oxidation rates in enrichment culture inoculated with slag-infiltrated water at proportions of 0%, 11%, 25% and 100%. **Fig. 2b** shows a significant decrease in the CH_4 oxidation rates with increase in slag-infiltrated water content, with rates ranging from 11 to 36 µg CH_4 $mL^{-1}d^{-1}$. The lower oxidation rates in enrichment culture as opposed to soil microcosms is a result of diffusion limitation in aqueous phase when compared to gaseous phase (Whalen et al. 1990; Park et al. 2005). CH_4 oxidation rates with different slag-infiltrated water content were significantly different from each other (ANOVA, $p = 0.003$). **Table 4** shows pH of the cultures at different slag-infiltrated water content. The pH of the cultures varied from an initial pH of 8.2 at 11% to 11.3 at 100% slag-infiltrated water content. The higher pH at higher slag-infiltrated water content may be a result of calcium leaching from the slag matrix. Likely due to the metabolic activity (*i.e.*, aerobic respiration leading to CO_2 production) of heterotrophic microorganisms, a significant drop in the pH over the course of incubation was observed, with near neutral pH values measured at the end of the experiment (6.7-7.3). Despite an eventual decrease in pH, this study showed significantly lower rates of CH_4 oxidation rates in incubations with elevated slag-

297 infiltrated water content, and CH_4 oxidation rates were inversely correlated with initial pH levels.
298 The microbial community composition was analyzed only at the end of the tests, and likely a shift
299 in the methanotrophic microbial community accompanied the decreasing pH over the course of
300 the experiment, as most methanotrophs favor circumneutral pH (Hanson and Hanson, 1996).
301 However, some methanotrophs have been identified in highly acidic (Semrau et al. 2008; Baesman
302 et al. 2015) or alkaline environments (Khmelenina et al. 1997; Kalyuzhnaya et al. 2008). pH is
303 thus a likely driver of observed community structure and CH_4 oxidation rate; as the BOF slag
304 showed low metal leaching properties (**Table 2**), the effect of heavy metals in the slag on the CH_4
305 oxidation is assumed to be minimal.
306
307 ***Methylotroph Microbial Community in Soil and Enrichment Cultures***
308 Soil microcosms and enrichment cultures were inoculated with slag-infiltrated water in batch
309 serum vials at different proportions from 0 to 100%, incubated for 5-6 days (enrichments) and 13-
310 28 days (soil microcosms) at 23 ± 2°C. Microbial communities in enrichments were profiled using
311 shotgun metagenome sequencing **(Fig. 3 and 4).** Majority of the microbial community that were
312 found dominant in the soil microcosms belonged to the genus *Methylobacter* (~18%), *Micromonospora* (~8.4%), *Bradyrhizobium* (~3.3%), *Streptomyces* (~2.7%), others (< 2%).
314 However, the enrichment cultures were dominated by the bacteria from the genus *Methylosinus*
315 (~10.1%), *Brevundimonas* (~5.3%), *Variovorax* (~5.4%), *Methylobacter* (~5.3%) and others (<
316 5%). The methylotrophic community in soil microcosms was dominated by the bacteria from the
317 genus *Methylobacter* and species *M. luteus* (**Fig. 3**). In addition to *Methylobacter* (82-84% of all
318 methylotroph-annotated sequences), other genera of methylotrophs were detected, including
319 *Methylocystis* (2.1-2.7%), *Methylosarcina* (3-3.7%), *Methylomicrobium* (2.7-3.3%) and others

(<1-2%). Overall, sequences from MOB represented from 20 to 23% of all annotated sequences. Bacteria from the genus *Methylobacter* are type I methanotrophs, found most commonly in landfills (Chen et al. 2007; Chi et al. 2015; Yargicoglu and Reddy, 2017) and other ecosystems (Amaral and Knowles, 1995; Henckel et al. 2000), and are known to be mesophilic with an optimal pH of 6.5 -7 (Bowman et al. 1993). Prior studies have shown that type I methanotrophs can be favored under low CH_4 and high oxygen conditions (Chi et al. 2015; Chen et al. 2007; Li et al. 2013), and these conditions are consistent with our microcosms.

Analysis of similarity (ANOSIM) was used to determine if microbial community structure was significantly different between groups in soil microcosms inoculated with slag-infiltrated water. The global ANOSIM R statistic was 0.119, with a p-value of 0.079 (999 permutations), indicating that microbial communities in soil microcosms were not significantly different between groups at different slag-infiltrated water contents (**Fig. 5**). This is likely a result of the high buffering capacity of the soil, which shields the methanotrophic community from the alkalinity of slag-infiltrated water. This finding suggests that in the field, any water infiltrating through the slag layer will not pose significant impact on the microbial activities in the biochar-amended soil layer. Pairwise comparisons also had R values ranging from -0.037 to 0.333, but due to the limited number of replicates, significance could not be properly assessed. Two-dimensional ordination resulted in substantial loss of information, as assessed by 2D stress value of 0.34.

Fig. 4 shows the structure of methylotrophic communities in enrichment cultures inoculated with slag-infiltrated water at proportions varying from 0-100%. In contrast to soil microcosms, enrichment culture microbial communities were significantly affected by the slag-infiltrated water **(Fig. 4 and 6).** Methanotrophs of the genus *Methylobacter* and species *Methylobacter luteus* were most abundant in enrichments with no slag-infiltrated water, but the

relative abundance of these organisms was significantly lower in enrichments with slag-infiltrated water (ANOVA, p=0.0075 and p=0.0017). The relative abundance of the genus *Methylobacter* decreased from 65% of all methylotrophic sequences to 33%, 22% and 14% in enrichments with 11%, 25% and 100% slag-infiltrated water content, respectively. However, the relative abundance of methylotroph sequences was consistent, ranging from 17-27% of all annotated sequences across all enrichment conditions (**Fig. 4**). The decrease in relative abundance of *Methylobacter* sequences was largely mirrored with an increase in sequences from bacteria of the genus *Methylosinus*. The relative abundance of the genus *Methylosinus* represented 9%, 37%, 35% and 57% of all methylotroph sequences at 0%, 11%, 25% and 100%, respectively. The shift in microbial community was associated with a decrease in CH_4 oxidation rates (**Fig. 2b)**, and this may be a result of different maximum oxidation rates of bacteria from the genera *Methylobacter* and *Methylosinus*, or the result of inhibition due to high initial pH levels in enrichments (**Table 4**), or both. Although methylotroph communities were dominated by bacteria from the genera *Methylobacter* and *Methylosinus*, other methanotrophic taxa were identified in the enrichment cultures, including *Methylosarcina* (Type I methanotrophs), *Methylomicrobium* (Type I methanotrophs) and *Methyloversatilis* (methylotrophs). Analysis of similarity (ANOSIM) was used to determine if microbial community structure in enrichment cultures was significantly different between groups. The global ANOSIM R statistic was 1, with a p-value of 0.001 (999 permutations), indicating significant differences in microbial communities. Pairwise comparisons also had R values of 1, but due to the limited number of replicates, significance could not be properly assessed.

The results of both soil microcosm and enrichment culture tests demonstrate that the CH_4 oxidation rates were more affected in the enrichment culture relative to soil, likely due to the

366 elevated initial pH conditions induced by the slag-infiltrated water. In soil microcosm tests, a
367 minimal pH change was observed due to the high buffering capacity of the soil, and CH_4 oxidation
368 rates were largely stable across all slag-infiltrated water contents. Furthermore, the slag-infiltrated
369 water had no significant effect on the methylotroph community composition in soil microcosms,
370 whereas a strong effect was observed in enrichments. Thus, due to the high buffering capacity of
371 the soil it is anticipated that the slag-infiltrated water will have a minimal effect on the CH_4
372 oxidation potential of biochar-amended soil layers underlying the slag layer in the biogeochemical
373 cover.
374

375 ## Conclusions

376

377 An investigation of slag-infiltrated water on CH_4 oxidation and community composition was
378 performed in soil and enrichment cultures. The soil microcosms and enrichment cultures
379 responded differently to slag-infiltrated water, with soil microcosms remaining largely unaffected.
380 No significant change in oxidation rates and methylotrophic microbial community structure were
381 observed in soil microcosms, and bacteria from the genus *Methylobacter* dominated the soil
382 microcosm. Conversely, enrichment culture showed a steep decline in oxidation rates with
383 increased slag-infiltrate water content, and a concomitant shift in dominant methanotrophic taxon
384 from the genus *Methylosinus* to the genus *Methylobacter*. The landfill soil is shown to possess
385 significant buffering capacity which neutralizes the effect of elevated pH of the slag-infiltrated
386 water, thereby facilitating effective CH_4 oxidation rates at varying slag-infiltrated water contents.
387 These results suggest that the use of BOF slag (isolated system) in the biogeochemical cover will
388 be viable for maintaining CH_4 oxidation *in situ*. Long term column studies and field scale studies

are ongoing to evaluate the overall performance of CH_4 oxidation and CO_2 sequestration in the slag isolated biochar-amended soil cover systems.

Data Availability

All data generated during the study appear in this article.

Acknowledgement

This research is a part of comprehensive project titled “Innovative Biochar-Slag-Soil Cover System for Zero Emissions at Landfills” funded by the National Science Foundation (CMMI# 1724773) which is gratefully acknowledged.

References

Amaral, J. A., and Knowles, R. (1995). “Growth of methanotrophs in methane and oxygen counter gradients”. *FEMS Microbiology Letters*, 126(3), 215-220.

Baesman, S., Miller, L., Wei, J., Cho, Y., Matys, E., Summons, R., and Oremland, R. (2015). “Methane oxidation and molecular characterization of methanotrophs from a former mercury mine impoundment”. *Microorganisms*, 3(2), 290-309.

Bonenfant, D., Kharoune, L., Sauve, S., Hausler, R., Niquette, P., Mimeault, M., and Kharoune, M. (2008). "CO_2 sequestration potential of steel slags at ambient pressure and temperature."

411 *Industrial & Engineering Chemistry Research*, 47(20), 7610-7616.
412 Bowden, L. I., Jarvis, A. P., Younger, P. L., and Johnson, K. L. (2009). "Phosphorus removal from
413 waste waters using basic oxygen steel slag". *Environmental Science & Technology*, 43(7),
414 2476-2481.
415 Bowman, J. P., Sly, L. I., and Hayward, A. C. (1990). "Patterns of tolerance to heavy metals among
416 methane-utilizing bacteria". *Letters in Applied Microbiology*, 10(2), 85-87.
417 Bowman, J. P., SLY, L. I., NICHOLS, P. D., and Hayward, A. C. (1993). "Revised taxonomy of
418 the methanotrophs: description of Methylobacter gen. nov., emendation of Methylococcus,
419 validation of Methylosinus and Methylocystis species, and a proposal that the family
420 Methylococcaceae includes only the group I methanotrophs". *International Journal of*
421 *Systematic and Evolutionary Microbiology*, 43(4), 735-753.
422 Chand, S., Paul, B., and Kumar, M. (2017). "Short-term leaching study of heavy metals from LD
423 slag of important steel industries in Eastern India". *Journal of Material Cycles and Waste*
424 *Management*, 19(2), 851-862.
425 Chang, E. E., Chiu, A. C., Pan, S. Y., Chen, Y. H., Tan, C. S., and Chiang, P. C. (2013).
426 "Carbonation of basic oxygen furnace slag with metalworking wastewater in a slurry reactor."
427 *International Journal of Greenhouse Gas Control*, 12, 382-389.
428 Chaurand, P., Rose, J., Briois, V., Olivi, L., Hazemann, J. L., Proux, O., and Bottero, J. Y. (2007).
429 "Environmental impacts of steel slag reused in road construction: A crystallographic and
430 molecular (XANES) approach". *Journal of Hazardous Materials*, 139(3), 537-542
431 Chen, Y., Dumont, M. G., Cébron, A., and Murrell, J. C. (2007). "Identification of active
432 methanotrophs in a landfill cover soil through detection of expression of 16S rRNA and
433 functional genes". *Environmental Microbiology*, 9(11), 2855-2869.

Chi, Z. F., Lu, W. J., and Wang, H. T. (2015). “Spatial patterns of methane oxidation and methanotrophic diversity in landfill cover soils of Southern China”. *Journal of Microbiology & Biotechnology*, 25(4), 423-430.

Clarke, K. R., and R. N. Gorley. "Getting started with PRIMER v7". PRIMER-E: Plymouth, Plymouth Marine Laboratory (2015)

Contin, M., Goi, D., and De Nobili, M. (2012). “Land application of aerobic sewage sludge does not impair methane oxidation rates of soils”. *Science of the Total Environment*, 441, 10-18.

De Windt, L., Chaurand, P., and Rose, J. (2011). “Kinetics of steel slag leaching: batch tests and modeling”. *Waste Management*, 31(2), 225-235.

Effler, S.W., Matthews, D.A. (2003). “Impacts of a soda ash facility on Onondaga Lake and the Seneca River, NY”. *The Lake Reservoir & Management*, 19, 285e306.

Hanson, R. S., and Hanson, T. E. (1996). “Methanotrophic bacteria”. *Microbiol. Molecular. Biology. Review*, 60(2), 439-471

Henckel, T., Roslev, P., and Conrad, R. (2000). “Effects of O2 and CH4 on presence and activity of the indigenous methanotrophic community in rice field soil”. *Environmental Microbiology*, 2(6), 666-679.

Huijgen, W. J., Witkamp, G. J., and Comans, R. N. (2005). “Mineral CO_2 sequestration by steel slag carbonation”. *Environmental Science & Technology*, 39(24), 9676-9682.

Ko, M. S., Chen, Y. L., and Jiang, J. H. (2015). "Accelerated carbonation of basic oxygen furnace slag and the effects on its mechanical properties." *Construction and Building Materials*, 98, 286-293.

Kalyuzhnaya, M. G., Khmelenina, V., Eshinimaev, B., Sorokin, D., Fuse, H., Lidstrom, M., and Trotsenko, Y. (2008). “Classification of halo (alkali) philic and halo (alkali) tolerant

457 methanotrophs provisionally assigned to the genera Methylomicrobium and Methylobacter
458 and emended description of the genus Methylomicrobium". *International Journal of*
459 *Systematic and Evolutionary Microbiology*, 58(3), 591-596.
460 Khmelenina, V. N., Kalyuzhnaya, M. G., Starostina, N. G., Suzina, N. E., and Trotsenko, Y. A.
461 (1997). "Isolation and characterization of halotolerant alkaliphilic methanotrophic bacteria
462 from Tuva soda lakes". *Current Microbiology*, 35(5), 257-261.
463 Minot, S. S., Krumm, N., & Greenfield, N. B. (2015). "One codex: A sensitive and accurate data
464 platform for genomic microbial identification". *BioRxiv*, 027607.
465 Mohanty, S. R., Bharati, K., Deepa, N., Rao, V. R., and Adhya, T. K. (2000). "Influence of heavy
466 metals on methane oxidation in tropical rice soils". *Ecotoxicology and Environmental*
467 *Safety*, 47(3), 277-284.
468 Motz, H., and Geiseler, J. (2001). "Products of steel slags an opportunity to save natural
469 resources". *Waste Management*, 21(3), 285-293.
470 Park, J. R., Moon, S., Ahn, Y. M., Kim, J. Y., and Nam, K. (2005). "Determination of
471 environmental factors influencing methane oxidation in a sandy landfill cover
472 soil". *Environmental Technology*, 26(1), 93-102.
473 Proctor, D. M., Fehling, K. A., Shay, E. C., Wittenborn, J. L., Green, J. J., Avent, C., and Zak, M.
474 A. (2000). "Physical and chemical characteristics of blast furnace, basic oxygen furnace, and
475 electric arc furnace steel industry slags". *Environmental Science & Technology*, 34(8), 1576-
476 1582.
477 Rai, R. K., Chetri, J. K., Green, S. J., and Reddy, K. R. (2018). "Identifying active methanotrophs
478 and mitigation of CH_4 emissions in landfill cover soil". *Proceedings International Congress*
479 *on Environmental Geotechnics* (pp. 308-316). Springer, Singapore.

480 Rai, R. K., and Reddy, K. R. (2019). "Methanotrophic methane oxidation in new biogeochemical
481 landfill cover system." *In Proc. 34th International Conference on Solid Waste Technology*
482 *and Managemen*t, Annapolis, MD, USA.
483 Reddy, K. R., Grubb, D. G., and Kumar, G. (2018). "Innovative biogeochemical soil cover to
484 mitigate landfill gas emissions". *Proceedings International Conference on Protection and*
485 *Restoration of the Environment XIV*, Thessaloniki, Greece.
486 Reddy, K. R., Gopakumar, A., and Chetri, J. K., (2019, a). "Critical review of applications of iron
487 and steel slags for carbon sequestration and environmental remediation". *Reviews in*
488 *Environmental Science and Bio/Technology*, DOI: 10.1007/s11157-018-09490-w.
489 Reddy, K. R., Chetri, J. K., Kumar, G., and Grubb, D. G. (2019, b). "Effect of basic oxygen furnace
490 slag type on carbon dioxide sequestration from landfill gas emissions". *Waste*
491 *Management*, 85, 425-436.
492 Reddy, K. R., Gopakumar, A., Rai, R. K., Kumar, G., Chetri, J. K., and Grubb, D. G. (2019, c).
493 "Effect of basic oxygen furnace slag particle size on sequestration of carbon dioxide from
494 landfill gas". *Waste Management & Research*, 0734242X18823948
495 Reddy, K. R., Gopakumar, A., Chetri, J. K., Kumar, G., and Grubb, D. G. (2019, d). "Sequestration
496 of landfill gas emissions using basic oxygen furnace slag: Effects of moisture content and
497 humid gas flow conditions." *Journal of Environmental Engineering*, 145(7), 04019033.
498 Roadcap, G. S., Sanford, R. A., Jin, Q., Pardinas, J. R., and Bethke, C. M. (2006). "Extremely
499 alkaline (pH> 12) ground water hosts diverse microbial community". *Groundwater*, 44(4),
500 511-517.
501 Saha, N., Kharbuli, Z.Y., Bhattacharjee, A., Goswami, C., Heaussinger, D. (2002). "Effect of
502 alkalinity (pH 10) on ureogenesis in the air-breathing walking catfish, Clarias batrachus".

503 *Comparative Biochemistry & Physiology Part A*, 132, 353e364.
504 Semrau, J. D., DiSpirito, A. A., and Yoon, S. (2010). "Methanotrophs and copper". *FEMS*
505 *Microbiology Reviews*, 34(4), 496-531.
506 Walkiewicz, A., Bulak, P., Brzezińska, M., Wnuk, E., and Bieganowski, A. (2016). "Methane
507 oxidation in heavy metal contaminated mollic gleysol under oxic and hypoxic
508 conditions". *Environmental Pollution*, 213, 403-411.
509 Whalen, S. C., Reeburgh, W. S., and Sandbeck, K. A. (1990). "Rapid methane oxidation in a
510 landfill cover soil". *Applied Environmental Microbiology*, 56(11), 3405-3411.
511 Wnuk, E., Walkiewicz, A., and Bieganowski, A. (2017). "Methane oxidation in lead-contaminated
512 mineral soils under different moisture levels". *Environmental Science and Pollution*
513 *Research*, 24(32), 25346-25354.
514 Yargicoglu, E. N., and Reddy, K. R. (2017 a). "Microbial abundance and activity in biochar-
515 amended landfill cover soils: evidence from large-scale column and field
516 experiments". *Journal of Environmental Engineering*, 143(9), 04017058
517 Yargicoglu, E. N., and Reddy, K. R. (2017 b). "Biochar-amended soil cover for microbial methane
518 oxidation: effect of biochar amendment ratio and cover profile". *Journal of Geotechnical and*
519 *Geoenvironmental Engineering*, 144(3), 04017123.
520

Table 1. Physical and chemical characteristics of BOF slag (IHE 9/17)

Properties	ASTM Method	Landfill Soil	BOF Slag (IHE 9/17)
Grain Size Distribution:	D422		
Gravel (%)		0	20.8
Sand (%)		7.2	74.3
Fines (%)		92.8	4.9
D_{50} (mm)			
C_c		-	0.7
C_u		-	18
Atterberg Limits:	D4318		
Liquid Limit (%)		30	Non-Plastic
Plastic Limit (%)		20	
Plasticity Index (%)		15	
USCS Classification	D2487	CL	SP-SM equivalent
Specific Gravity	D854	2.65	3.5
Dry Density (g/cm^3)		2.11	1.72
Water Holding Capacity (*w/w*)		45.9	20
Hydraulic Conductivity (cm/s)	D2434	2.75×10^{-8}	1.1×10^{-3}
Loss-on-Ignition (%)	D2974	4.47	1.6
pH (1:1)	D4972	7.04	12.4
Electrical Conductivity (mS/cm)	D4972	0.4	13.3
Redox Potential (mV)	D4972	-37.7	-313.3
Elemental Analysis	XRF		
Ca (%)			40.35
Fe (%)			30.25
Si (%)			9.55
Mg (%)			10.9
Mn (%)			2.2
Al (%)			3.95

C_c=Coefficient of curvature; C_u=Coefficient of uniformity

Table 2. Leaching properties of BOF slag (IHE 9/17)

Metals	Slag Infiltrated Water (mg/L)			RCRA Limit (mg/L)
	Sample 1	Sample 2	Average	
Aluminum	0.068	0.15	0.109	
Antimony	< 0.0060	< 0.0060	< 0.0060	
Arsenic	< 0.0040	< 0.0040	< 0.0040	5
Barium	0.16	0.16	0.16	100
Beryllium	< 0.0020	< 0.0020	< 0.0020	
Boron	0.13	0.26	0.195	
Cadmium	< 0.0020	< 0.0020	< 0.0020	1
Calcium	550	520	535	
Chromium	0.023	0.023	0.023	5
Cobalt	< 0.0040	< 0.0040	< 0.0040	
Copper	< 0.010	< 0.010	< 0.010	
Iron	< 0.10	< 0.10	< 0.10	
Lead	< 0.0020	< 0.0020	< 0.0020	5
Magnesium	< 0.10	< 0.10	< 0.10	
Manganese	< 0.0040	< 0.0040	< 0.0040	
Mercury	< 0.0012	< 0.0012	< 0.0012	0.2
Nickel	< 0.0040	< 0.0040	< 0.0040	
Potassium	2.0	2.0	2	
Selenium	< 0.0040	< 0.0040	< 0.0040	1
Silver	< 0.0040	< 0.0040	< 0.0040	5
Sodium	3.1	3.2	3.15	
Thallium	< 0.0020	< 0.0020	< 0.0020	
Tin	< 0.020	< 0.020	< 0.020	
Vanadium	< 0.0040	< 0.0040	< 0.0040	
Zinc	< 0.020	< 0.020	< 0.020	

Table 3. pH of the soil at different slag-infiltrated water content

Slag-Infiltrate Water Content (%)	pH
Soil + 10% slag-infiltrated water	7.87 ± 0.03
Soil + 20% slag-infiltrated water	7.92 ± 0.01
Soil + 30% slag-infiltrated water	8.01 ± 0.01
Soil + 40% slag-infiltrated water	8.04 ± 0.02

Table 4. pH of the enrichment culture at different slag-infiltrated water content

Slag-Infiltrated Water Content (%)	Initial pH	Final pH
Culture + 0% slag-infiltrated water	7.6	7.6
Culture + 11% slag-infiltrated water	8.2	6.83
Culture + 25% slag-infiltrated water	10.52	6.88
Culture + 100% slag-infiltrated water	11.35	7.36

Figure Captions

Fig. 1. BOF slag-infiltrated water collection

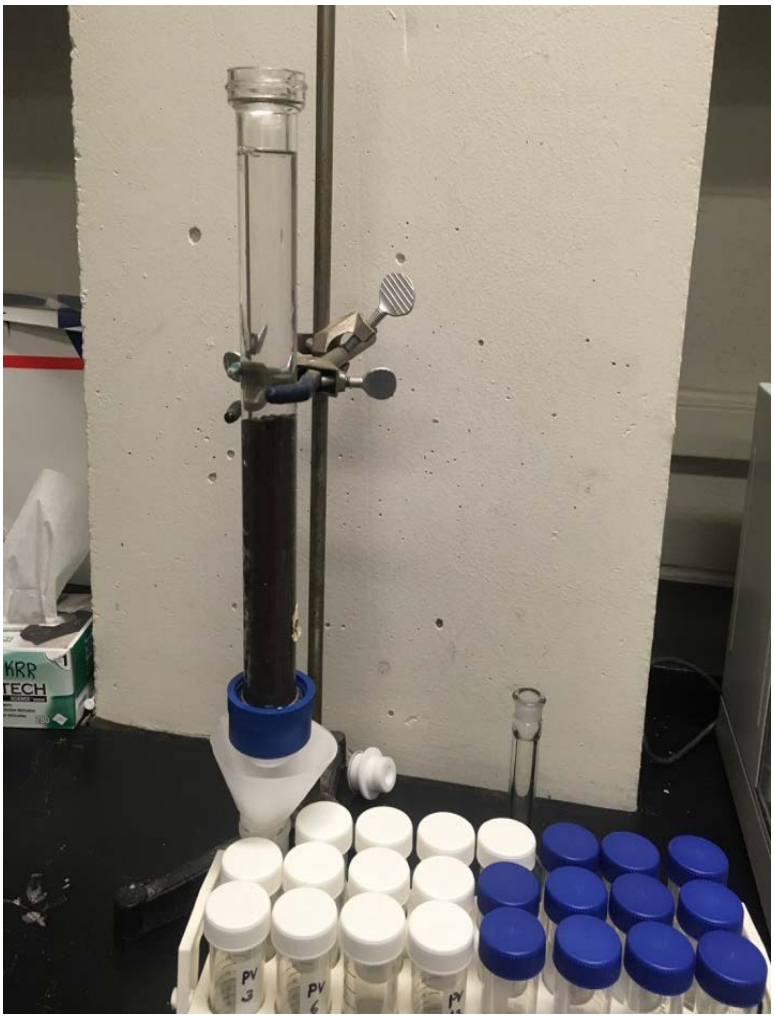
Fig. 2. Methane oxidation rates with different slag-infiltrated water contents in: (a) Soil, and (b) Enrichment Culture (* indicates values that are statistically different between two slag-infiltrated water contents at $p < 0.05$)

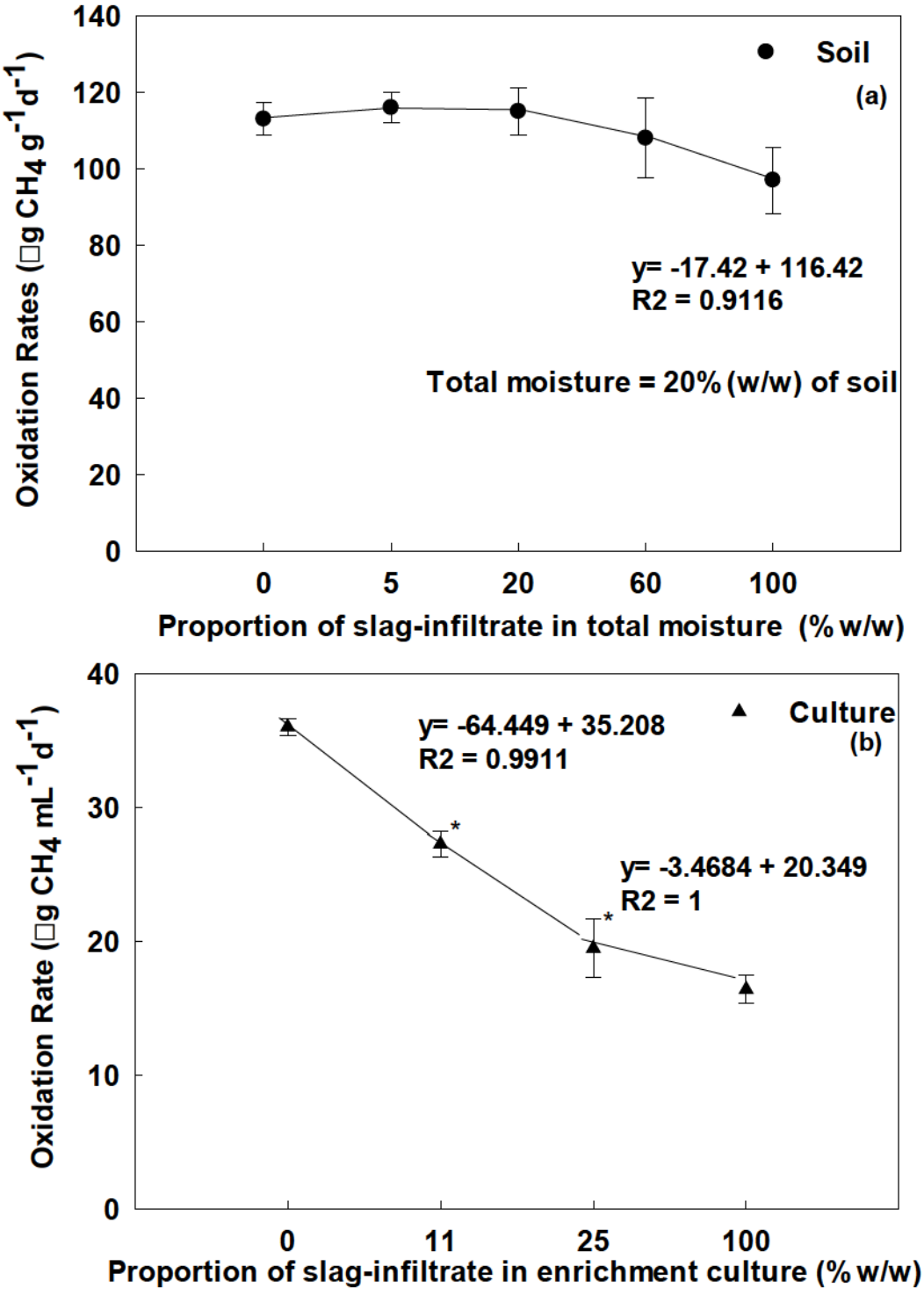
Fig. 3. Relative abundance (%) of sequences related to Methanotrophic and Methylotrophic community as determined by shotgun sequencing in soil microcosms, total of 20% moisture content (a) genera (b) species (percentage at top of each bars represent % of methanotrophic/methylotrophic community in total microbial community

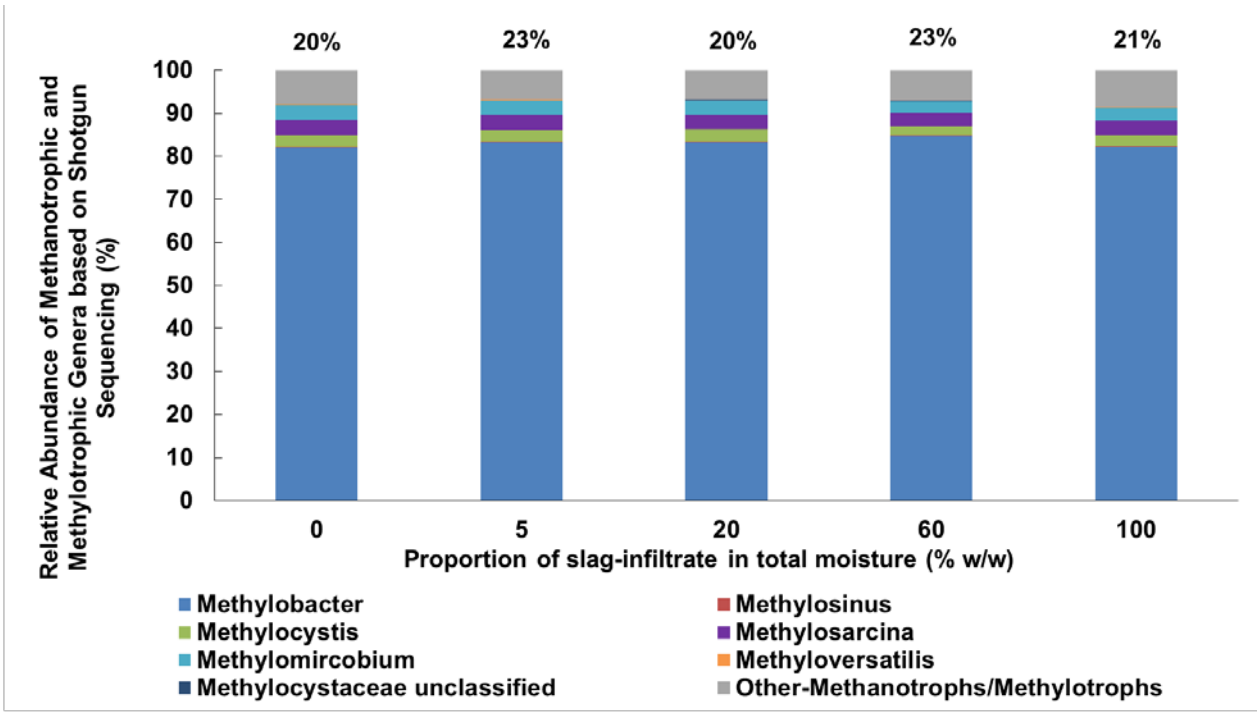
Fig. 4. Relative abundance (%) of sequences related to Methanotrophic and Methylotrophic community as determined by shotgun sequencing in enrichment culture (a) genera (b) species (percentage at top of each bars represent % of methanotrophic/methylotrophic community in total microbial community)

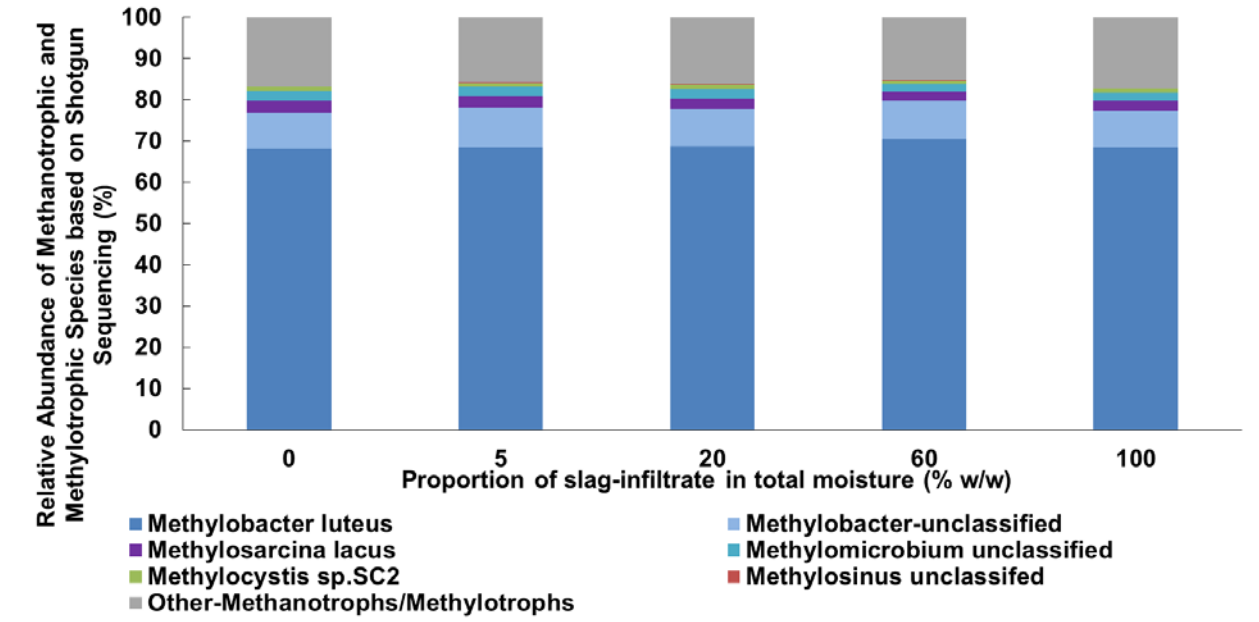
Fig. 5 Metric multi-dimensional scaling (mMDS) plot of microbial community structure (taxonomic level of species) in soil microcosms inoculated with slag-infiltrated water contents from 0 - 100% (n=3) using Bray-Curtis similarity metrics.

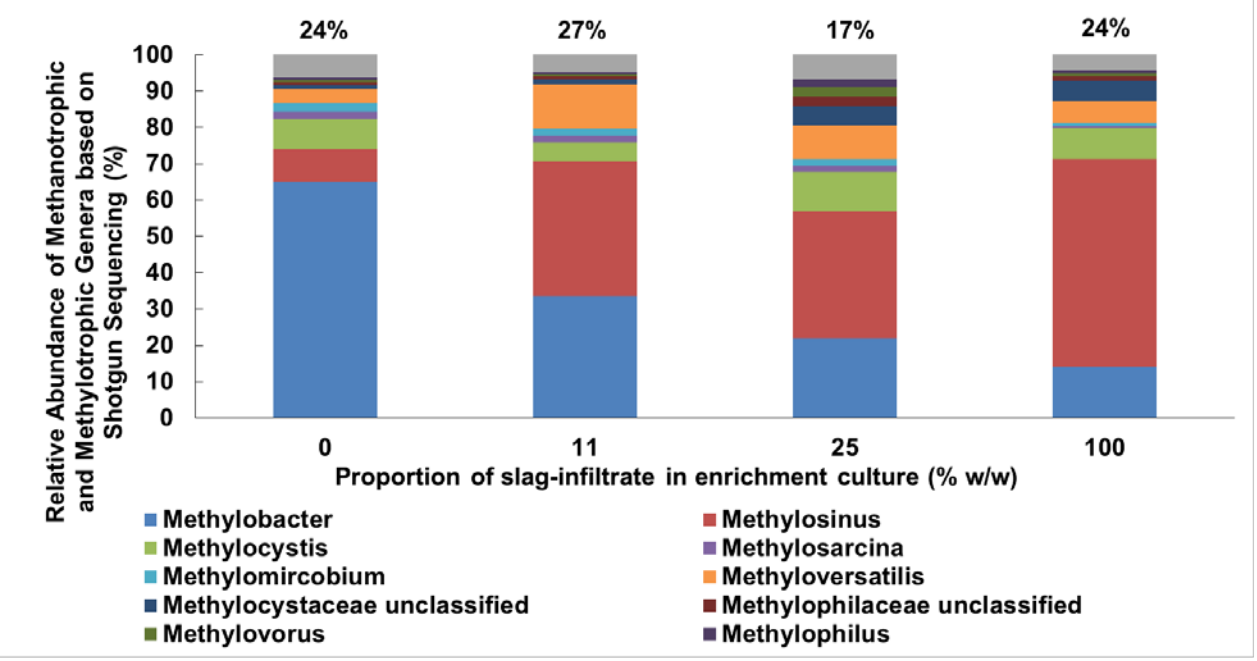
Fig. 6 Metric multi-dimensional scaling (mMDS) plot of microbial community structure (taxonomic level of species) in enrichment cultures inoculated with slag-infiltrated water contents from 0 - 100% (n=1 or 3) using Bray-Curtis similarity metrics.

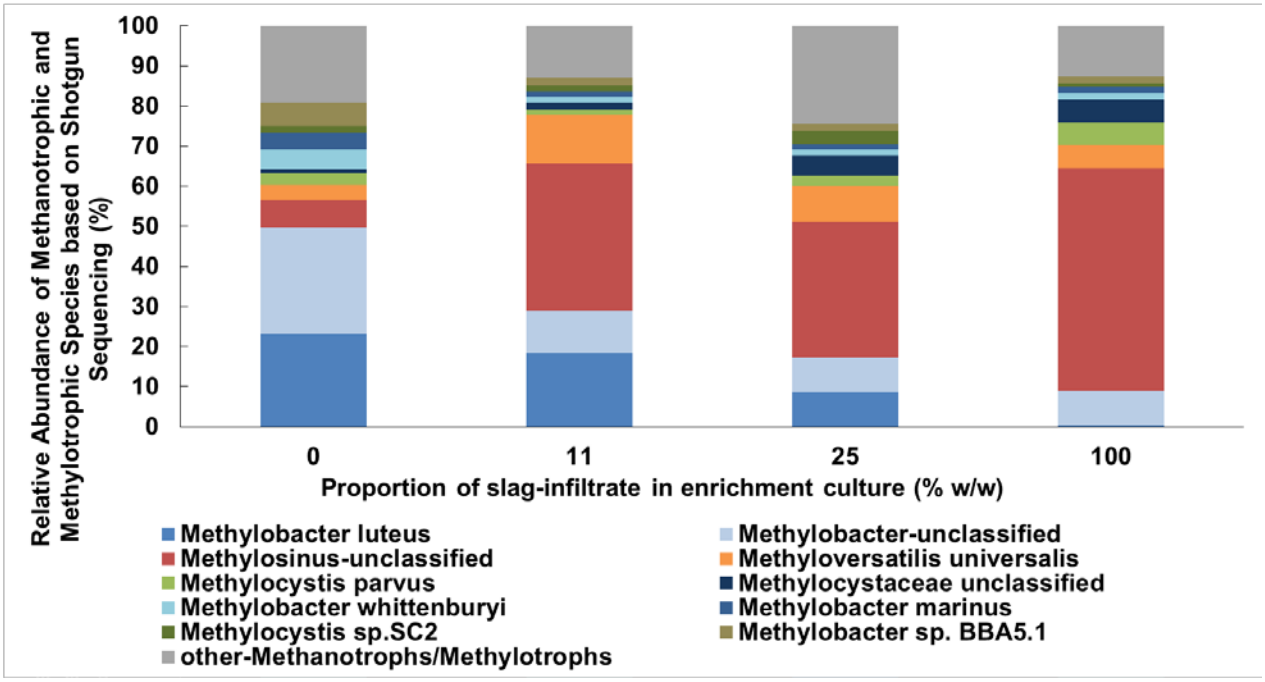


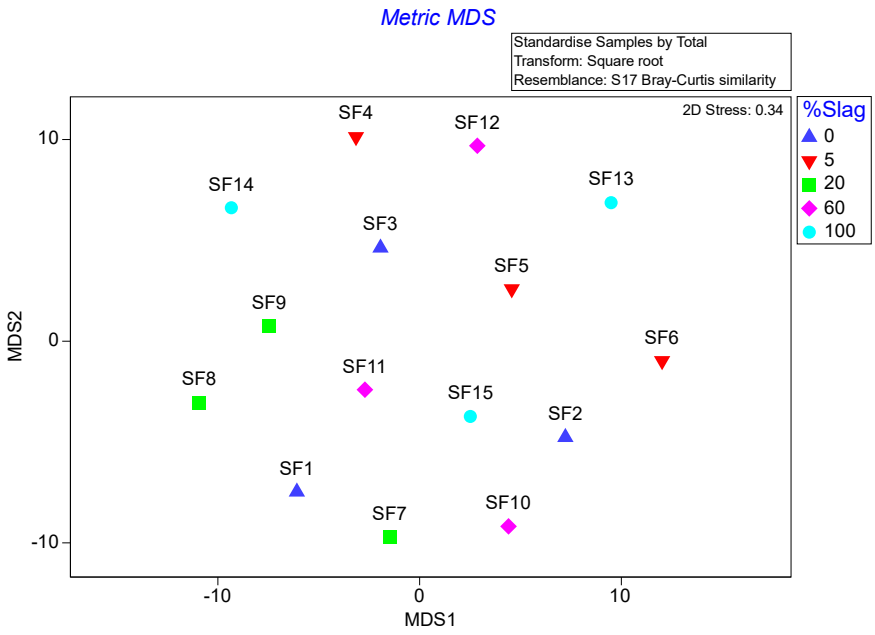

Soil
(a)
y= -17.42 + 116.42
R2 = 0.9116
Total moisture = 20% (w/w) of soil
Oxidation Rates (□g CH4 g-1d-1)
Proportion of slag-infiltrate in total moisture (% w/w)
Culture
(b)
y= -64.449 + 35.208
R2 = 0.9911
y= -3.4684 + 20.349
R2 = 1
Oxidation Rate (□g CH4 mL-1d-1)
Proportion of slag-infiltrate in enrichment culture (% w/w)

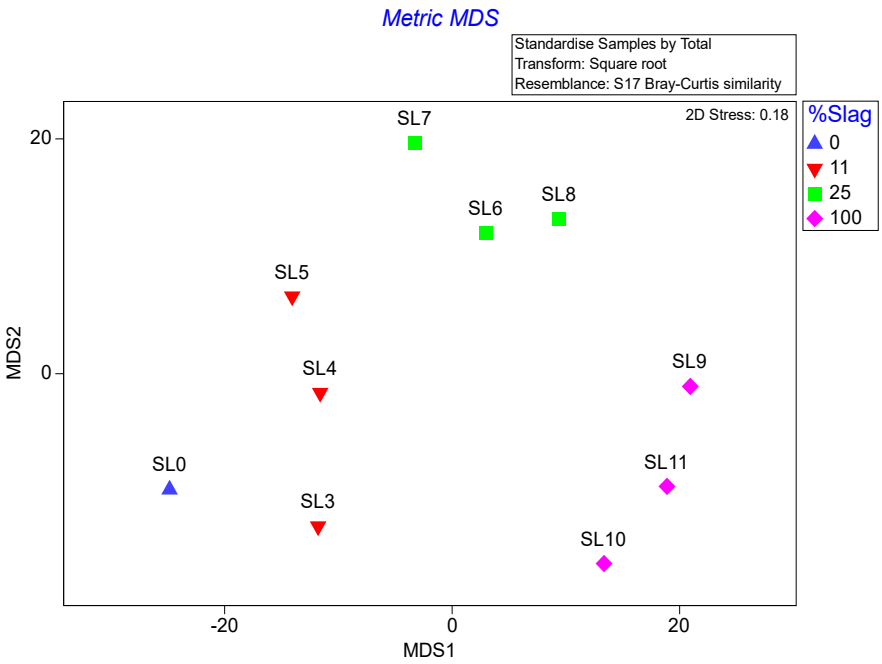

20%
23%
20%
23%
21%
Relative Abundance of Methanotrophic and Methylotrophic Genera based on Shotgun Sequencing (%)
100
90
80
70
60
50
40
30
20
10
0
0
5
20
60
100
Proportion of slag-infiltrate in total moisture (% w/w)
Methylobacter
Methylosinus
Methylocystis
Methylosarcina
Methylomircobium
Methyloversatilis
Methylocystaceae unclassified
Other-Methanotrophs/Methylotrophs


Relative Abundance of Methanotrophic and Methylotrophic Species based on Shotgun Sequencing (%)
100
90
80
70
60
50
40
30
20
10
0
0
5
20
60
100
Proportion of slag-infiltrate in total moisture (% w/w)
Methylobacter luteus
Methylobacter-unclassified
Methylosarcina lacus
Methylomicrobium unclassified
Methylocystis sp.SC2
Methylosinus unclassifed
Other-Methanotrophs/Methylotrophs


24%
27%
17%
24%
Relative Abundance of Methanotrophic and Methylotrophic Genera based on Shotgun Sequencing (%)
100
90
80
70
60
50
40
30
20
10
0
0
11
25
100
Proportion of slag-infiltrate in enrichment culture (% w/w)
Methylobacter
Methylosinus
Methylocystis
Methylosarcina
Methylomircobium
Methyloversatilis
Methylocystaceae unclassified
Methylophilaceae unclassified
Methylovorus
Methylophilus


Relative Abundance of Methanotrophic and Methylotrophic Species based on Shotgun Sequencing (%)
100
90
80
70
60
50
40
30
20
10
0
0
11
25
100
Proportion of slag-infiltrate in enrichment culture (% w/w)
Methylobacter luteus
Methylobacter-unclassified
Methylosinus-unclassified
Methyloversatilis universalis
Methylocystis parvus
Methylocystaceae unclassified
Methylobacter whittenburyi
Methylobacter marinus
Methylocystis sp.SC2
Methylobacter sp. BBA5.1
other-Methanotrophs/Methylotrophs


Metric MDS
Standardise Samples by Total
Transform: Square root
Resemblance: S17 Bray-Curtis similarity
2D Stress: 0.34
%Slag
0
5
20
60
100
SF4
SF12
SF14
SF13
SF3
SF5
SF9
SF6
SF8
SF11
SF15
SF2
SF1
SF10
SF7
10
0
-10
MDS2
-10
0
10
MDS1


Metric MDS
Standardise Samples by Total
Transform: Square root
Resemblance: S17 Bray-Curtis similarity
2D Stress: 0.18
%Slag
0
11
25
100
SL7
SL8
SL6
SL5
SL4
SL9
SL0
SL11
SL3
SL10
20
0
MDS2
-20
0
20
MDS1