Microbial community structure and diversity in a municipal solid waste landfill

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1. Introduction

Municipal solid waste (MSW) landfills are the most prevalent waste disposal method and constitute one of the largest sources of anthropogenic methane emissions in the world (IPCC, 2014). About 60% of MSW generated is disposed of in landfills in China and the United States (USEPA, 2009; Zhang et al., 2010), generating 112 and 178 million tons CO2eq, respectively (NCCC, 2013; USEPA, 2016). Microbial activities in disposed waste play a crucial role in greenhouse gas emissions; however, only a few studies have examined metagenomic microbial profiles in landfills. Here, the MiSeq high-throughput sequencing method was applied for the first time to examine microbial diversity of the cover soil and stored waste located at different depths (0–150 cm) in a typical MSW landfill in Yangzhou City, East China. The abundance of microorganisms in the cover soil (0–30 cm) was the lowest among all samples, whereas that in stored waste decreased from the top to the middle layer (30–90 cm) and then increased from the middle to the bottom layer (90–150 cm). In total, 14 phyla and 18 genera were found in the landfill. A microbial diversity analysis showed that Firmicutes, Proteobacteria, and Bacteroidetes were the dominant phyla, whereas Halanaerobium, Methylohalobius, Syntrophomonas, Fastidiosipila, and Spirochaeta were the dominant genera. Methylohalobius (methanotrophs) was more abundant in the cover layers of soil than in stored waste, whereas Syntrophomonas and Fastidiosipila, which affect methane production, were more abundant in the middle to bottom layers (90–150 cm) in stored waste. A canonical correlation analysis showed that microbial diversity in the landfill was most strongly correlated with the conductivity, organic matter, and moisture content of the stored waste.
Several methods have been applied to identify the microbial profiles in landfills, such as terminal restriction fragment length polymorphism (T-RFLP) (Sawamura et al., 2010; Gomez et al., 2011) and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) (Uchida et al., 2009; Song et al., 2015a). However, a previous study that used the T-RFLP and PCR-DGGE techniques was unable to produce a high-resolution microbial profile in landfills (Zhang et al., 2016). Novel high-throughput sequencing techniques (e.g., MiSeq) are an emerging tool to analyze microbial diversity and relative abundance and offer the advantages of high sensitivity and integral coverage on sequencing microorganisms. Thus, these techniques enable characterization of trace bacteria and uncultured microorganisms in a complex environment (Glenn, 2011). However, no study has used MiSeq to analyze the microbial communities in landfills.

In the present study, a typical MSW landfill located in Yangzhou, Jiangsu Province, East China was selected as the case study. Microbial diversity in the cover soil and stored waste was analyzed using the MiSeq platform. The study generated metagenomic datasets for both microbial and environmental studies (Oberholzer et al., 2011). However, no study has used MiSeq to analyze microbial diversity and relative abundance and offer the advantages of high sensitivity and integral coverage on sequencing microorganisms. Thus, these techniques enable characterization of trace bacteria and uncultured microorganisms in a complex environment (Glenn, 2011). However, no study has used MiSeq to analyze the microbial communities in landfills.

2. Materials and methods

2.1. Sampling method

Samples were collected from the Zhaozhuang landfill (latitude: 32.4713°N, longitude: 119.3189°E), a typical MSW landfill located in Yangzhou, Jiangsu Province, East China, with a humid and subtropical climate. The landfill began operations in August 2002 and now occupies an area of 150,000 m². It receives about 0.22 million tons of mixed MSW per year. The depth of the cover soil of each site was about 20 cm, and the stored waste was sampled at depths of 0–150 cm, in which the waste was stored for approximately 7 years after closure.

The cover soil and stored waste (both were solid samples) at two sampling sites (Site-1 and Site-2), which were located 10 m apart in the same landfill cell, were collected for parallel analysis. To examine the vertical distribution of the microbial community in stored waste, we sampled at 30-cm intervals to a depth of 150 cm. In total, we collected 12 samples, labeled _1_0, _1_3, _1_6, _1_9, _1_12, _1_15, _2_0, _2_3, _2_6, _2_9, _2_12, and _2_15. Labels _1_ and _2_ indicate Site 1 and Site 2, respectively; whereas label “_0” refers to the cover soil (0–20 cm) sample; labels “_3”, “_6”, “_9”, “_12”, and “_15” refer to the waste sampled from depths of 0–30, 30–60, 60–90, 90–120, and 120–150 cm, respectively. A shovel was used to excavate a 1.0 m x 1.5-m area in each sampling site. The cover soil and stored waste in each layer were removed and transferred to a sterilized plastic sheet, and the shovel was cleaned before excavating a new layer to avoid contamination between layers. Approximately 500 g of sample was collected at each site by the coning and quartering method, and each sample was placed in an icebox for a physicochemical analysis.

2.2. Physicochemical analysis

To test the physicochemical properties, each sample was mixed evenly and sieved through a 100-mesh screen to remove large particles (plastic bags, stone, glass, etc.). A soil–water (1:5, w/v) suspension was prepared 30 min prior to EC and pH measurements using the Professional Meter (PP-20, Sartorius, Göttingen, Germany). The concentration of organic matter was determined by Tyurin’s method (Nikolskii, 1963). Moisture content was measured gravimetrically. Cation exchange capacity (CEC) was measured using the sodium acetate exchange method (Rhoades, 1982). Total carbon (TC) and total nitrogen (TN) were tested by an elemental analyzer (vario PYRO cube; Elementar, Langenselbold, Germany). Ammonium in the samples was displaced by 100 mL of 0.01 M CaCl₂ for 60 min. The extract was filtered, and the NH₄-N concentration was measured using a continuous flow analytical system (Autoanalyzer 3, Bran + Luebbe: SPX Flow Technology, Norderstedt, Germany).

2.3. DNA extraction and polymerase chain reaction (PCR) amplification

To prepare samples for DNA extraction, a 10-mesh screen was used to remove large particles from the stored waste. A five-point sampling mode was applied for sub-sampling, and a 1.5–2.0-g sub-sample was collected for extracting DNA. The samples were stored at −80 °C before extraction. DNA in each sample was extracted using a MoBio Power Soil DNA extraction kit (MoBio, Carlsbad, CA, USA). The DNA concentration was quantified on a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The V4-V5 region of the microbial 16S ribosomal RNA gene was amplified by PCR (95 °C for 3 min, followed by 27 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 10 min) using primers 515F (5’–GTGCCAGCMGCCGCGG–3’) and 907R (5’–CCGTCAATTCMTTTRAGTTT–3’) (Xiong et al., 2012). A barcode and adapter were incorporated between the adapter and the forward primers. PCR reactions were performed in triplicate in 20-μL reaction mixtures containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, 10 ng of template DNA, and sterile double-distilled H₂O.

2.4. Illumina MiSeq sequencing

A mixture of equal quantities of the three PCR products from each sample was extracted from 2% agarose gels, purified using an Axyprep DNA Gel Extraction Kit (Axxygen Biosciences, Union City, CA, USA), and quantified using a Quantifluor-ST fluorometer (Promega, Madison, WI, USA), following the standard PCR procedure. The purified amplicons were pooled in equimolar amounts, detected with a NanoDrop 2000 spectrophotometer and then paired-end sequenced on an Illumina MiSeq platform.

2.5. Processing the sequencing data

Raw fastq files were demultiplexed and quality-filtered using QIIME (V 1.17) software (Caporaso et al., 2010) with the following criteria: (I) 250 bp reads were truncated at sites that received an average quality score <20 over a 10-bp sliding window, and truncated reads that were <50 bp were discarded; (II) exact barcode matching, two nucleotide mismatches in primer matching, reads containing ambiguous characters were removed; and (III) only sequences that had greater than a 10 bp overlap were assembled according to their overlap sequence. Reads that could not be assembled were discarded.

Chimeras were detected using the UCHIME algorithm, and the high-quality sequences were grouped into operational taxonomic units (OTUs), at a 97% sequence similarity threshold, using UPARSE (V 7.1) software (Edgar, 2013). RDP Classifier software (Wang et al., 2007) was used to analyze the phylogenetic affiliations of the 16S rRNA gene sequences against the SILVA (SSU119) 16S rRNA database (Quast et al., 2013) at a confidence threshold of more than 70%.
2.6. Multivariate analysis

Microbial community richness indices (Ace and Chao) and diversity indices (Shannon and Simpson estimators) were calculated using Mothur (V 1.30.1) software (Schloss et al., 2009). The R language platform was used to visualize microbial diversity and abundance datasets from the various samples. Canonical correlation analysis (CCA) was used to test the relationship between environmental variables and microbial diversity using Canoco (V 5.0) software. The sampling and analyzing methods are shown in Fig. 1.

3. Results and discussion

3.1. Characterization of soil and waste samples

The physicochemical properties of the studied samples are shown in Table 1. The moisture contents of the cover soil at Site-1 and Site-2 were 16.3% and 18.7%, respectively, whereas the moisture content of the stored waste increased with storage depth. The moisture content of the stored waste at Site-1 (18.3–28.8%) was lower than that at Site-2 (31.7–35.6%). The pH of the cover soil and the stored waste were alkaline, and the pH values of the cover soil (7.8 and 7.9) were lower than those of the stored waste (7.8–8.6). The EC of the stored waste was 1.6–20.0 times higher than that of the cover soil (5.4 and 6.7 ms/cm). The EC of the waste at Site-1 was also significantly higher at all depths (1.6–9.9 times) compared to the waste at Site-2, indicating more soluble salt content in the Site-1 samples. The highest EC was found at depths of 0–60 cm in the stored waste. CEC was not different in the cover soil and stored waste, ranging from 14.6 to 25.2 cmol/kg. The organic matter content in the stored waste (101.0–186.4 g/kg) was 18–38 times that in the cover soil (5 g/kg). Similarly, the total carbon (TC), total nitrogen (TN), and NH4-N contents of the stored waste were 31–118 times, 8–30 times, and 15–39 times higher than those of the cover soil, respectively. The highest levels of organic matter and TC and TN contents in stored waste were observed at a depth of 120–150 cm at Site-1 and at a depth of 30–60 cm at Site-2.

More than 70% of the stored waste was a soil-like material, which was formed by complex biochemical reactions in the organic waste over an extended period. Therefore, the organic matter, TC, and TN contents of the stored waste were comparatively higher than those of the cover soil. Similar to previous characterization studies on stored waste in landfills, the physicochemical properties of the stored waste varied with storage depth; however, these depth differences were rarely statistically significant (Zhou et al., 2015a,b). EC may strongly affect microbial activities in landfills. In this study, the EC of stored waste at Site-1 was similar to that reported previously (Zhou et al., 2015a,b); however, the EC was lower at Site-2.

3.2. Richness and diversity of the microbial community

The richness and diversity of the microbial community in the landfill are shown in Fig. 2. In total, 648,809 effective sequences with an average length of 395.46 bp were obtained from high-throughput sequencing, and the reads for each sample ranged from 31,831 to 54,233. A total of 2139 OTUs were assigned, and the sequencing coverage rate exceeded 99% (microbial population) for each sample, indicating that exhaustive information on the microbial community was obtained by MiSeq. An average of 1036 ± 219 OTUs was identified in stored waste, and the minimum values were 891 at Site-1 and 818 at Site-2, both at a depth of 60–90 cm. The Ace and Chao indices were used to show the richness of the microbial community. The average Ace and Chao index values for the microbial community in stored waste were 500 and 496, respectively, which were 2.5–2.6 times those in cover soil, indicating higher microbial richness in stored waste. A similar trend was evident in the Shannon and Simpson diversity indices (higher Shannon and lower Simpson values reflect higher microbial diversity). The average Shannon and Simpson values for the microbial community in stored waste were 4.6 and 0.05, which were 154% and 36% higher than those in cover soil, respectively, indicating higher microbial diversity in the stored waste. Interestingly, the microbial richness and diversity indicators showed a cross-depth trend: the Shannon and Simpson indices increased with storage depth, whereas the Ace and Chao indices decreased from the top (0–60 cm) to the middle layer (60–90 cm) and then increased in the bottom layer (90–150 cm).

The sequencing coverage rates in this study were higher than those obtained in a previous landfill microbial study (Song et al., 2015a), in which 454 pyrosequencing was used, indicating that a high-resolution microbial profile can be obtained by applying the MiSeq method. The richness and diversity of the microbial community in the stored waste were higher than those in the cover soil owing to the higher organic and nutrients contents in the stored waste. Changes in oxygen content may have resulted in the increased microbial diversity with storage depth. As oxygen content decreased with storage depth, the types of facultative anaerobic and anaerobic organisms likely increased. Microbe richness was lowest in the middle layer of stored waste, although the carbon and nutrient contents were moderate in all layers, indicating that the richness of the microbial community across depths in the stored waste may be affected by other environmental factors.

3.3. Microbial taxonomic analysis at the phylum level

High-throughput sequencing revealed the diversity of the microbial community in different samples at the phylum level (Fig. 3). These data have been uploaded to the National Center for Biotechnology Information Database (No. SRR5131582). We
found 14 phyla (each relative abundance > 1%) using MiSeq sequencing, whereas only five phyla were detected using the T-RFLP method in a previous study (Sawamura et al., 2010). The diversity of the microbial community in stored waste was significantly higher than that in the cover soil. The phyla Proteobacteria, Bacteroidetes, Firmicutes, Spirochaetes, Deinococcus-Thermus, Actinobacteria, Synergistetes, Thermotogae, and Tenericutes were detected in all 12 samples collected from the two sites in the landfill. Firmicutes (46.61%), Proteobacteria (23.84%), and Bacteroidetes (14.29%) were the most dominant phyla in the landfill. These phyla have also been observed in leachate, sludge (Qiu et al., 2013), and alkaline lake sediment (Xiong et al., 2012). In particular, the abundance of Firmicutes in cover soil was similar to those in samples 1_0 (33.54%) and 2_0 (41.81%), whereas the number of Firmicutes decreased initially in the stored waste, then increased along with the depth, and was most abundant at a depth of 30 cm.

Firmicutes is the most dominant phylum in landfills (Krishnamurthi and Chakrabarti, 2013; Van Dyke and McCarthy, 2002). Firmicutes are cellulose-degrading bacteria and are believed

<table>
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<th>Sampling sites</th>
<th>Sample ID</th>
<th>Moisture (%)</th>
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<th>EC (ms/cm)</th>
<th>CEC (cmol/kg)</th>
<th>Organic matter (g/kg)</th>
<th>TC (%)</th>
<th>TN (%)</th>
<th>NH4-N (mg/kg)</th>
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Table 1
The physical and chemical properties of cover soil and stored waste samples.
to play an important role in the anaerobic and methanogenic phases of refuse decomposition in landfills (Song et al., 2015b). The phyla Proteobacteria and Bacteroidetes are thought to play important roles in organic matter degradation and the carbon cycle (Newton et al., 2011). Gammaproteobacteria, Alphaproteobacteria, and Betaproteobacteria were detected in all samples.
The phyla Deinococcus-Thermus and Actinobacteria were subdominant at Site-1. Deinococcus-Thermus was previously found in both compost (Partanen et al., 2010) and a landfill (Song et al., 2015a). Actinobacteria is a phylum capable of accelerating biodegradation and was also a subdominant group in the microbial community at Site-1. The lower organic matter content at Site-1 compared with that at Site-2 may have been due to the biodegrading capacity of Actinobacteria (Table 1). The phyla Spirochaetae, Thermotogae, and Synergistetes were subdominant in the stored waste at a depth of 90–120 cm in Site-2. The phylum Spirochaetae, composed primarily of anaerobic heterotrophic microorganisms, was previously detected in landfills and in an anaerobic dynamic membrane bioreactor (Köchling et al., 2015; Xie et al., 2014). Synergistetes has been observed previously in anaerobic digestion reactors and may affect the fermentation of proteins and sugars during anaerobic processes (Morita et al., 2011; Tang et al., 2011).

3.4. Microbial taxonomic analysis at the genus level

The dominant microbial genera in the landfill included Halanaerobium, Lactococcus, Halocella, Methylohalobius, Truepera, Ignatzschineria, Pseudomonas, Bacillus, Syntrophomonas, Fastidiosipila, and Spirochaeta (Fig. 4). We are the first to report the predominance of the genera Halanaerobium and Ignatzschine-
ria in a landfill. Halanaerobium are anaerobic bacteria that produce hydrogen and were abundant in stored waste. As storage depth increased, the number of Halanaerobium first decreased and then increased. The abundance of Halanaerobium was lowest (6.28–6.74%) in samples taken from a depth of 60–120 cm, which also had a lower EC, indicating that Halanaerobium may grow better with higher salt content. Ignatzschineria was not found in cover soil, but was frequently found in stored waste; in particular, sample 1_9 had the highest abundance of Ignatzschineria (7.87%).

Two newly found genera, Halanaerobium and Ignatzschineria, could be helpful for examining the biological processes in landfills. Halanaerobium are hydrolytic bacteria that ferment complex organic matter and produce intermediary metabolites for other microbial groups, such as sulfate-reducing and methanogenic bacteria (Ivanova et al., 2011). Halanaerobium are highly enriched in lactate-and glucose-fed bioreactors (Zhou et al., 2015b). Ignatzschineria, formerly called Schineria, is a Gram-negative bacterial genus in class Gammaproteobacteria, (Gupta et al., 2011). Juteau et al. (2004) reported that Ignatzschineria was the second most abundant species in an aerobic thermophilic sequencing batch reactor; however, its function in the microbial community remains unclear.

Lactococcus and Methylohalobius were dominant in cover soil samples. The abundance of Methylohalobius was 19.76% in sample 1_0 and 23.95% in sample 2_0. Lactococcus is not only capable of degrading lignocelluloses but can also reduce humic acids (Benz et al., 1998). Methylohalobius are aerobic methane-oxidizing bacteria, with a crucial role in mitigating methane emissions from landfills (Nikiema et al., 2007).

Furthermore, the genus Pseudomonas has been widely detected in landfills and other contaminated environments (Gomez et al., 2011; Li et al., 2011). Pseudomonas are well-known pollutant-degrading bacteria that utilize a wide range of polycyclic aromatic hydrocarbons as their sole carbon source (Loick et al., 2009) and have roles in organic matter degradation (Horel et al., 2015) and denitrification (Lalucat et al., 2006). Bacillus are frequently found in landfills where they degrade cellulose (Westlake et al., 1995) and oxidize polycyclic aromatic hydrocarbons (Zeng et al., 2016) and chromium (Desai et al., 2008). Truera are mostly found in alkaline, moderately saline, and high temperature habitats (Ivanova et al., 2011) and are relatively abundant in agricultural waste (Covino et al., 2016). Halocella, which have previously been detected in landfills (Simankova et al., 1993), may be important in cellulose degradation, acid production, and methane production (Tang et al., 2011).

Syntrophomonas, Fastidiosiopia, Spirochaeta, and Thermovirga were abundant in the middle and bottom layers at Site-2. The abundance of these genera ranged from 4.56 to 8.30% at a depth of 60–150 cm. As most of these genera are anaerobes, they can survive in the deep layer in the absence of oxygen. The genera Syntrophomonas and Fastidiosiopia belong to the Clostridia class, which are efficient hydrogen producers (Kim et al., 2014). Syntrophomonas produce hydrogen and form a syntrophic relationship with hydrogen-using bacteria, such as Methanospirillum (Toumi et al., 2015). Fastidiosiopia, a Gram-positive genus, was one of the most abundant genera found in a membrane bioreactor for treating landfill leachate (Xie et al., 2014). Spirochaeta may help to enhance cellulose biodegradation of landfill leachate in symbiosis with Clostridium species (Pohlbroedrger et al., 1994). Most bacteria from the genus Ther-movirga are thermophilic and capable of utilizing carbohydrates, proteins, amino acids, and organic acids (Li et al., 2015).

**3.5. Correlations between microbial diversity and environmental factors**

Principal coordinates analysis (PCoA) is a non-constrained data dimensionality reduction method that was used to further analyze the environmental factors affecting the microbial community (Fig. 5). The amount of variance accounted by the two principle components was 40.7% and 31.6%. According to the PCoA analysis, the 12 samples were divided into four groups: (1) samples 1_0 and 2_0; (2) samples 2_9, 2_12, and 2_15; (3) samples 2_3 and 2_6; and (4) samples 1_3, 1_6, 1_9, 1_12, and 1_15.

The clustering of the 18 genera with an average relative abundance > 1% in all samples is shown in Fig. 6 (supporting data are shown in Table S1). Samples 1_3 and 1_6 had a significantly lower abundance of Halanaerobium and Ignatzschineria than samples 1_9, 1_12, and 1_15, which separated samples 1_3 and 1_6 from the other three samples at Site-1. Furthermore, samples from Site-2 were clustered into two groups due to a high abundance of Syn-trophomonas, Fastidiosiopia, Spirochaeta, and Thermovirga in samples 2_9, 2_12, and 2_15, whereas samples 2_3 and 2_6 exhibited low abundance. The microbial communities exhibited similar clustering trends, suggesting that the 18 dominant genera were highly representative of the major microbes in the community samples. Fig. 6 reveals that microbial community structure varied in the samples from Site-1 and Site-2 but that the microbial community structures in the cover soil from the two study sites were similar. The microbial community structure in stored waste at depths of 90, 120, and 150 cm was homogeneous, whereas it was similar at depths of 30 and 60 cm.

To further estimate the effects of environmental factors on microorganisms, the 18 genera with an average relative abundance > 1% in all samples were analyzed by detrended correspondence analysis using Canoco 5.0. A CCA sequencing diagram was developed using EC, soil organic matter levels, moisture content, and soil depth as environmental factors (Fig. 7). The analysis demonstrated that the environmental factors were strongly correlated with the microbial structure (p < 0.01). As also shown in Fig. 7, 45.96% of the information corresponded to the first sequencing axis, and 24.6% corresponded to the second axis. The 18 genera were all located in the vicinity of coordinate origin, excluding Methylohalobiusa and Gracillimonas, because these two genera were present only in cover soil.
The arrow lengths in Fig. 7 indicate that EC and organic matter and water content had significant effects on the microbial community structure. Other studies have demonstrated that moisture content is a dominant factor affecting the microbial community structure in landfills (Townsend et al., 1996). Moisture content was positively correlated with organic matter and soil depth. Eight genera, including Halocella in the third quadrant, were strongly positively correlated with moisture content. Five genera, including Halanaerobium in the second quadrant and Pseudomonas in the first quadrant, were primarily positively affected by EC. *Methylohalobius* and *Gracillimonas* had no clear associations with any of the environmental factors.

4. Conclusions

MiSeq methodology was applied to provide a high-resolution profile of the microbial community in a landfill. Microbial richness and abundance were significantly higher in stored waste than in the cover soil of the landfill. The microbial diversity analysis revealed that more microbial richness was found in the top and bottom layers of stored waste; however, less richness was found lower in the middle layer. According to our survey, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* were the most dominant phyla, whereas *Halanaerobium*, *Lactococcus*, *Methylohalobius*, *Ignatzschineria*, *Syntrophomonas*, *Fastidiosipila*, and *Spirochaeta* were the dominant genera. *Halanaerobium* and *Ignatzschineria* were reported in a landfill for the first time. The microbial diversity and structure in the landfill were affected by the physicochemical properties as well as the storage depths of the waste. EC and organic matter and moisture contents were the dominant factors affecting microbial diversity and structure. The dominant genus, which primarily consisted of halotolerant bacteria, may be ascribed to the high salinity in the stored waste.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.wasman.2017.04.023.

References


