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Microbial community structure and diversity in a municipal solid waste landfill

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ABSTRACT

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. 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.

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 CO_2eq, respectively (NCCC, 2013; USEPA, 2016). Microbial activities in disposed waste play a crucial role in decomposing organic waste and greenhouse gas emissions (Slezak et al., 2015). Various microorganisms proliferate abundantly in landfills due to richness of the organic matter and substrate complexity; therefore, landfills have been considered microbial pools (Song et al., 2015a,b). Many laboratory studies have examined the microbial activities associated with landfills (e.g., in leachate treatment systems, Fernandes et al., 2013) and bioreactor landfills (Sang et al., 2012; Bareither et al., 2013; Lee et al., 2014). However, the site-specific microbial profiles in landfills could differ from those identified in laboratory studies. Only limited research on the microbial profiles in landfills has examined the identity, ecology, and diversity of the microbial community structures at different landfill sites (Gomez et al., 2011; Song et al., 2015a,b). However, none of these studies discussed the vertical distribution of microbial composition in landfills.

Vertical distance from the surface to the bottom of a landfill may reflect different levels of the moisture content and composition of stored waste, and, accordingly, change the abundance and distribution of the microbial community. For example, methanogens and acid-producing bacteria decompose organic matter and generate methane at the bottom of a landfill (Slezak et al., 2015; Wang et al., 2015). *Clostridium*, *Syntrophus*, and *Sporotomaculum* promote methane production by accelerating hydrocarbon degradation in landfills (Gieg et al., 2014). Additionally, methanotrophs (e.g., *Methylocystis* and *Methylocaldum*) are able to fix methane using an enzyme complex and methane monooxygenase under aerobic conditions at the surface of landfills (Stralis-Pavese et al., 2004). Therefore, a study of the vertical distribution of microbial communities is needed to determine the microbial niches at each depth in landfills (Dong et al., 2015).

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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 in different layers (0–150 cm) of the landfill by applying the MiSeq method at the phylum and generic levels. Our study generated metagenomic datasets for both microbial and environmental studies; the factors (electrical conductivity [EC], organic matter, moisture content, and disposal depth) that potentially influence the microbial structure and diversity in landfills are also discussed.

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 m^2. 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_ indicates 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 × 1.5-m area in each sampling site. The cover soil and stored waste in each layer were completed 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 $CaCl_2$ for 60 min. The extract was filtered, and the NH_4^+-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′-GTGCCAGC MGCCGCGG-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 H_2O.

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 (Axygen 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 mismatch 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%.

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