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Succession and diversity of microbial communities in landfills with depths and ages and its association with dissolved organic matter and heavy metals

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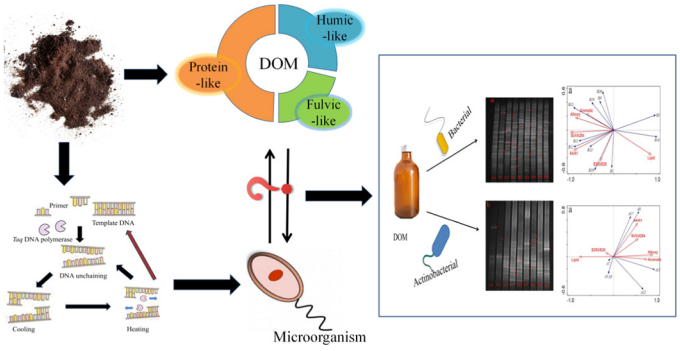
HIGHLIGHTS

- The abundance of microbes in the upper and lower layers of landfills was high.
- *Firmicutes*, *Proteobacteria*, and *Actinobacteria* were the dominant phyla in landfill.
- *Firmicutes* played an important role in the synthesis of humic-like matter.
- *Proteobacteria* facilitated to increase the content of aromatic compounds.
- *Actinobacteria* could oxidize fatty chains into oxygen-containing functional groups.

GRAPHICAL ABSTRACT



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ABSTRACT

Landfill is an important method for the treatment of municipal solid wastes. Microbes play a central role in the biodegradation and stabilization of organic matter during landfill; however, the succession of microbial communities in landfills and their association with organic matter still remain unclear. This study investigated the succession and diversity of microorganisms in landfill depending on different depths and ages as well as its association with dissolved organic matter (DOM) and heavy metals. The results showed that the actinobacterial diversity and richness were high compared to bacteria in young landfill cells. The diversity and richness of bacteria and actinobacterial were the highest in the middle layer in the intermediate and old landfill cells. *Firmicutes*, *Proteobacteria*, and *Actinobacteria* were the most dominant phyla. *Firmicutes* were mainly affected by the humification degree, and the aromatic and protein-like substance content of the landfill-derived DOM. The phylum *Proteobacteria* was greatly affected by the lipid and humic-like substances content of the landfill-derived DOM, while the distribution of *Actinobacteria* was regulated by both aromatic and humic-like substances. The effect of dissolved heavy metals on the microbial distribution in landfill differed for the metals Cr, Ni, Pb, Mn, Cu, Zn, and Cd. Siderophile elements (Cr, Ni, and Pb) were necessary trace elements for *Proteobacteria* and *Actinobacteria*, and promoted their growth. Oxyphilic element (Mn) was an important factor promoting the growth of *Actinobacteria*. However, no apparent relationship was found between sulfurophile elements (Cu, Zn, and Cd) and microorganisms.

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1. Introduction

A remarkable increase in municipal solid waste (MSW) generation has been observed in China in response to the rapid industrialization and urbanization. Effective solid waste management became a major social and environmental concern (Calli et al., 2006). There are many methods to handle and dispose of solid wastes from municipal and industrial activities (e.g., incineration, composting, landfill, and material recovery), and landfilling is the most commonly used disposal alternative in most countries (Semrau, 2011). The biodegradable components in solid wastes can be decomposed through a series of complex biochemical reactions when the solid wastes are landfilled. All types of microorganisms compete or cooperate to complete the process of garbage degradation (Barlaz et al., 1989). It has been reported that organic matter can be utilized by microorganisms only if it is dissolved in water during the landfill process, as the biodegradation of organic matter mainly occurs within a liquid film on the surface of particles. Therefore, the change of dissolved organic matter (DOM) can better indicate the microbial degradation process of landfill organic matter (He et al., 2006).

The microorganisms that are responsible for the degradation of organic wastes play a key role in landfill (Slezak et al., 2015). Various microorganisms proliferate in landfills due to the richness of the organic matter and the prevailing substrate complexity, and therefore, landfills have been considered as microbial pools (Song et al., 2015a, 2015b). The distribution of microorganisms at specific sites in the landfill may differ from the date generated in laboratory studies. Few studies focus on the microbial community structure using laboratory-scale landfills, and these studies generally do not discuss the vertical distribution of the microbial composition in real landfills (Gomez et al., 2011). In general, wastes are landfilled from the bottom to the top of the landfill cell; therefore, the wastes at the bottom are old while those at the top are young. This may influence the abundance and distribution of microbial communities. In addition, the leachates are generated and assembled at the bottom of a landfill cell during the landfill process, and consequently, the moisture level is high at the bottom, which facilitates microbial growth. The oxygen content of the top layer is generally high compared to the middle and bottom layer, thus altering both the abundance and diversity of microbial communities. Therefore, it is necessary to study the vertical distribution of the microbial community structure in landfills to determine the depth of the microbial niche in landfills (Dong et al., 2015; Zhang et al., 2015).

To study the succession and diversity of microbial communities in this situation, many molecular techniques, including polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE), single strand conformational polymorphism (SSCP), and terminal restriction fragment length polymorphism (t-PFLP), have been used (Egert et al., 2004). PCR-DGGE has been used to study the spatial change of the microbial community in landfill, indicating it was a feasible tool for the study of microbial diversity in environmental samples (Kjellin et al., 2007). 16S rDNA sequence analysis can also be employed to identify specific microorganisms, which may be important (Muyzer and Ramsing, 1995). Significant research has been devoted to the understanding of the structure and diversity of microbes in this specific circumstance. Xiao et al. (2010) investigated the SBBR biofilm community dynamics using the PCR-DGGE method. Wu et al. (2016) used PCR-DGGE to analyze the bacterial quantity and diversity distribution characteristics along landfill depth and its relationship with environmental factors in the Beijing Beishenshu municipal solid waste landfill. However, few reports addressed the characteristics and changes of vertically distributed microbial diversity in landfills.

Microbial communities are often affected by physicochemical parameters, and it is difficult to precisely identify the environmental factors that affect the microorganisms. Recently, both redundancy analysis (RDA) and canonical correspondence analysis (CCA) have been introduced to correlate microbial communities with environmental factors. These methods have also been combined with genetic fingerprinting further to identify the main factors that affect microbial communities (Gilbride et al., 2006).

For the present case study, a typical landfill, located in Beijing, was selected. The objectives of this study were to (1) investigate the dynamics, diversity and vertical distribution characteristics of the microbial community in the landfill; (2) to analyze the main factors affecting the microbial structure and its diversity in the landfill. These factors may provide a solid scientific basis for the effective control of the microbial community management and ecological restoration in landfills.

2. Materials and methods

2.1. Sampling method

Landfill samples were collected in 2009 from the Asuwei landfill, which is a typical MSW landfill located in the Changping District, Beijing, North China. To study the vertical distribution of microbial communities in landfill waste, the landfill was sampled at intervals of 2 m, at a maximum the deepest sampling depth of 12 m. The samples, which were collected from the depth of 0–2, 2–4, and 4–6 m of the landfill unit operated in the year of 2007 to 2009, were labeled as A1, A2, and A3, respectively. Another six samples, which were obtained from the depths of 0–2, 2–4, 4–6, 6–8, 8–10, and 10–12 m of the landfill cell operated in the years of 1996 to 2003, were selected and numbered as B1, B2, B3, B4, B5, and B6, respectively. Wastes such as metal, plastic, wood blocks and stones were removed by hand, and typical samples of specific quality were collected via the quarter-method. Samples were then mixed evenly and packed into self-sealed bag and brought back to the laboratory within 24 h. After natural air drying, the collected landfill samples were ground through a 100 mesh sieve and stored at −20 °C.

2.2. DOM extraction

The samples were extracted using ultrapure water (1:10 ratio), and then shaken for 24 h in a horizontal shaker at room temperature. The extracts were centrifuged at 12000 rpm for 20 min and then filtered through a 0.45-μm membrane filter. The total organic carbon (TOC) of the DOM was determined via TOC analyzer (Shimadzu TOC-5000, Japan).

2.3. Spectroscopic analysis

2.3.1. UV–vis spectroscopy analysis

Prior to spectral analysis, the DOC concentrations of all samples were diluted to 10 mg/L to avoid the inner-filtration effect (IFE). Ultraviolet–visible (UV–vis) spectroscopy was conducted using a Shimadzu model UV-2802 PC spectrophotometer. The absorbance values of landfill samples under 254, 253 and 203 nm were measured, and specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) was calculated to evaluate the aromatic carbon abundance (Shao et al., 2009). The calculated absorbance ratio ranged between 253 and 203 nm (E_{253}/E_{203}), which is related to oxygen-containing functional groups (Korshin et al., 1997).

2.3.2. Fluorescence spectroscopy analysis

Fluorescence spectroscopy was measured using a Hitachi model F-7000 luminescence spectrophotometer. Both emission and excitation slit widths were set to 5 nm, and the scan speed was 240 nm min^{-1}. Fluorescence emission spectra were scanned from 260 to 550 nm at an excitation wavelength (Ex) of 254 nm. The area ratio (A_4/A_1) of the region from 435 to 480 nm (A_4) to that from 300 to 345 nm (A_1) was calculated (Li et al., 2008). This ratio was positively correlated with the humification degree (Xiao et al., 2017).

For the determination of three-dimensional excitation emission matrix fluorescence spectrum, the slit widths of both fixed excitation and emission wavelength were 5 nm; the excitation wavelength range