



# The evolution of water extractable organic matter and its association with microbial community dynamics during municipal solid waste composting



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## ABSTRACT

The humification of water extractable organic matter (WEOM) by microorganisms is widely used for assessing compost maturity and quality. However, the effect of bacterial and fungal community dynamics on humification of WEOM was not yet explored fully. Here, we used canonical correspondence analysis (CCA) and redundancy analysis (RDA) to investigate the link between bacterial and fungal community dynamics and humification process of WEOM, respectively. Results showed that water-soluble carbon (WSC), humification degree, molecule weight and abundance of aromatic carbon were significantly related to bacterial community ( $p < 0.05$ ), while the protein-like materials were statistically influenced by fungal community ( $p < 0.05$ ). Both bacterial and fungal communities significantly affected the abundance of oxygen-containing functional groups and humic-like materials ( $p < 0.05$ ). These humification parameters were most likely to be influenced by some of bacterial and fungal species at different composting stages. *Lactobacillus*, *Aspergillus fumigatus* and *Galactomyces geotrichum* can enhance the degradation of WSC and protein-like materials at the early composting. *Bacteroidetes* and *Firmicutes* could promote the increase of aromatic carbon, oxygen-containing functional groups, humification degree and molecular weight of WEOM during the initial fermentation stage. *Cladosporium herbarum* and *Chaetomium globosum* could be the dominant controllers at the second fermentation for accelerating the formation of oxygen-containing functional groups and humic-like materials of WEOM, respectively. Our results suggested that regulation for the dynamics of these special bacterial and fungal species at different composting stages might be a potential way to accelerate humification of municipal solid waste composting.

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## 1. Introduction

The production of municipal solid waste (MSW) is an inevitable consequence of today's consumer society. Finding safe, sustainable and cost-effective alternatives to the disposal and usage of MSW has recently become a significant issue for solid waste management (Hong et al., 2010). Composting as an attractive waste management option is defined as the biological decomposition of organic matter under controlled conditions to form a stable,

humus-like end product (Fialho et al., 2010). Thus it can be seen that humification process is the primary mechanism during composting and can be successfully allowed to assess the stability and maturity of compost (Said-Pullicino et al., 2007; Shao et al., 2009). Water-extractable organic matter (WEOM) has been demonstrated to be more easily utilized by microbes versus solid-phase organic matter during composting (Chefetz et al., 1998; Said-Pullicino and Gigliotti, 2007). Water-soluble phase is also an important reaction interface for the transformation of solid-phase organic matter by microbial metabolism (Chefetz et al., 1998; He et al., 2014; Said-Pullicino et al., 2007). Moreover, characterization of the composition of WEOM has been regarded as a better indicator of the overall transformation of the organic matter than solid-phase (Chefetz et al., 1998).

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With the composting time, the content polysaccharide structure of WEOM decreased during MSW composting and a higher content of macromolecules that related to humic substances were formed in WEOM at the final stage of composting (Chefetz et al., 1998). Thus, WEOM characteristics measured by spectroscopy could be used to monitor the compost maturity during composting (Chefetz et al., 1998; Hsu and Lo, 1999; Tian et al., 2012). The humification of WEOM during composting is facilitated by a diverse community of microbes, whose community dynamics vary greatly temporally, and generally involves the development of thermophilic temperatures as a result of biologically produced heat (De Gannes et al., 2013a,b; Goyal et al., 2005; Ishii et al., 2000).

Many studies have focused on the influence of environmental factors on microbial community (Wang et al., 2015; Zhang et al., 2011). Nevertheless, the mechanism with respect to the links of bacterial and fungal community dynamics and the humification parameters of WEOM was also worth researching. Previous studies demonstrated that WEOM humification with increasing in molecular weight and aromatic and alkyl structures was driven by microbes decomposing the fulvic acids, protein and water soluble carbon (WSC) of WEOM during composting (Senesi et al., 1989). However, the heterogeneous role of bacterial and fungal community during composting was not clear.

This study will therefore illuminate the evolution of WEOM and its association with bacterial and fungal community dynamics during MSW composting. The different driving roles of bacterial and fungal communities on WEOM humification process are also investigated. The purpose of this study is to provide basic information for improvement of MSW composting for through regulating microbial community dynamics.

## 2. Materials and methods

### 2.1. Composting process and sample collection

Composting samples were obtained from Beijing Asuwei Composting Plant, China. The factory disposes 800–1000 t of organic solid waste every day. The composting pile was 1.6 m high, with  $2.5 \times 3$  m wide, containing more than 2 t of organic solid waste. The composting pile was turned every 2 d by forklift before 21 d. The curing stage took 30 d to complete, and then the pile turned mechanically every 7 d. To ensure the composting was successful, the initial water content was adjusted to 65–70%, the C/N ratio was maintained at approximately 25–35:1, and average particle diameter of all raw materials was between 1.5 and 3.0 cm. The inner temperature of the compost increased to 69 °C at the 4th day that followed by a cooling period of approximately 30 days. At the completion of the composting on day 90, the C/N of the compost was 17.9.

Each sample (1.5 kg) contain triplicate composite samples that were collected from top, center, and bottom of the composts at day 0, 7, 14, 21, 28, 51 and 90, respectively, and was labeled as C0, C7, C14, C21, C28, C51 and C90. Triplicate of mixtures of MSWs were divided into two parts. A part of them was air-dried, ground to pass through a 0.25 mm sieve, and stored in a desiccator to investigate the composition and evolution of WEOM, and the rest was stored at –20 °C to extract total DNA and study microbial community. The moisture content of the composted MSWs was determined by drying the fresh sample at 105 °C for 24 h.

### 2.2. WEOM extraction

WEOM was modified according to Chefetz et al. (1998). Briefly, compost samples were extracted with distilled water (solid to

water ratio of 1:10, w/v) for 24 h in a horizontal shaker at room temperature. The suspension was then centrifuged at 10,000 rpm for 30 min and then filtered through a 0.45 µm membrane filter. The dry basis of WSC of all samples was measured immediately after extraction or fraction with an Analytik Jena model Multi N/C 2100 TOC analyzer (Analytik Jena, Germany).

### 2.3. Fluorescence spectra analysis

The WSC content of all WEOM samples was adjusted to 6.0 mg L<sup>-1</sup>, and fluorescence spectroscopy (FS) was measured using a Hitachi model F-7000 luminescence spectrophotometer. Both emission and excitation slit widths were set to 10 nm, and the scan speed was 500 nm min<sup>-1</sup>. Synchronous-scan excitation spectra were obtained over a range of 250–595 nm with a constant offset ( $\Delta\lambda = 30$  nm), as proposed by Hur et al. (2009). Region from 250 to 308 nm referred to as the protein-like region (PLR) (dos Santos et al., 2010). Region 308–363 nm referred to as the fulvic-like region (FLR) (dos Santos et al., 2010; Peuravuori et al., 2002). Region 363–595 nm corresponding to polycyclic aromatic called humic-like region (HLR), is related to the presence of humic substances (Peuravuori et al., 2002). Fluorescence emission spectra were scanned from 260 to 550 nm with an excitation wavelength (Ex) of 254 nm, and 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 (Said-Pullicino and Gigliotti, 2007). This ratio was positively correlated with the humification degree (Milori et al., 2006).

### 2.4. UV-Vis spectroscopy analysis

Ultraviolet-visible (UV-Vis) spectroscopy was measured using a Shimadzu model UV-1700 PC spectrophotometer. Specific ultraviolet absorbance at 254 nm ( $SUVA_{254}$ ) was calculated to evaluate the abundance of aromatic carbon (Shao et al., 2009). Absorbance ratio between 253 and 203 nm ( $E_{253}/E_{203}$ ), which is related to oxygen-containing functional groups, was calculated as well (Korshin et al., 1997). Furthermore, the slopes between 275 and 295 nm ( $S_{275-295}$ ) and between 350 and 400 nm ( $S_{350-400}$ ) were calculated, and the slope ratio ( $S_R$ ) obtained by dividing the  $S_{275-295}$  value by the corresponding  $S_{350-400}$  has an inverse relationship with the molecule weight of WEOM (Helms et al., 2008).

### 2.5. DNA extraction, PCR-DGGE and sequencing

PCR-DGGE was applied to discern the succession rules of bacterial and fungal communities during the composting process. Genomic DNA of bacterial and fungal community was extracted by the E.Z.N.A.TM Soil DNA kit (Omega Bio-tek, Guangzhou, China). The extracted DNA was purified, and the 16S rDNA and 18S rDNA genes were amplified with bacterial universal primers 338F/518R and fungal universal primers NS1/Fung, respectively (Hoshino and Morimoto, 2008). The PCR mixture was prepared with 1 µL of extracted DNA, 1 µL of 20 µmol L<sup>-1</sup> each primer, Premix Taq 25 µL (TaKaRa BioX., Dalian, China) and adjusted to a final volume of 50 µL with sterile Milli-Q water.

The PCR program of 16S rDNA was performed as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturing at 94 °C for 45 s, annealing at 55 °C for 40 s and extension at 72 °C for 40 s, and single extension at 72 °C for 7 min, and end at 4 °C. The 18S rDNA PCR program was carried out with an initial denaturation step at 94 °C for 5 min, followed by 35 cycles consisting of denaturation at 94 °C for 45 s, annealing at 55 °C for 50 s and elongation at 72 °C for 1 min, followed by a final elongation step at 72 °C for 7 min, and end at 4 °C. DGGE was carried out using a Dcode Universal Mutation Detection System (Bio-Rad, USA). PCR samples (30 µL) containing approximately equal amounts of PCR

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