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# Succession and diversity of microorganisms and their association with physicochemical properties during green waste thermophilic composting

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## ABSTRACT

A comprehensive characterization of the bacterial diversity associated to thermophilic stages of green waste composting was achieved. In this study, eight different treatments (T1–T8) and three replicated lab-scale green waste composting were carried out to compare the effect of the cellulase (i.e. 0, 2%), microbial inoculum (i.e. 0, 2 and 4%) and particle size (i.e. 2 and 5 mm) on bacterial community structure. Physicochemical properties and bacterial communities of T1–T8 composts were observed, and the bacterial structure and diversity were examined by high-throughput sequencing via a MiSeq platform. The results showed that the most abundant phyla among the treatments were the *Firmicutes*, *Chloroflexi* and *Proteobacteria*. The shannon index and non-metric multidimensional scaling (NMDS) showed higher bacterial abundance and diversity at the metaphase of composting. Comparing with 5-mm treatments, particle size of 2-mm had a richer diversity of bacterial communities. The addition of cellulase and a microbial inoculum could promote the fermentation temperature, reduce the compost pH and C/N ratio and result in higher GI index. The humic substance (HS) and humic acid (HA) contents for 2-mm particle size treatments were higher than those of 5-mm treatments. Canonical correspondence analysis suggested that differences in bacterial abundance and diversity significantly correlated with HA, E<sub>4</sub>/E<sub>6</sub> and temperature, and the relationship between bacterial diversity and environmental parameters was affected by composting stages. Based on these results, the application of cellulase to promote green waste composting was feasible, and particle size was identified as a potential control of composting physicochemical properties and bacterial diversity.

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## 1. Introduction

The generation of green waste has become unavoidable with the development of urban afforestation and accounts for approximately 50% of the municipal solid waste in Beijing, China. Composting is effective for the sustainable management of organic waste and is widely practised for resource recycling (Neher et al., 2013). It is a biological process that is influenced by physicochemical conditions and nutritional properties of the materials being composted (López-González et al., 2015), which depends on the enzymatic activities of different indigenous microbes (Awasthi

et al., 2017). Therefore, a deeper understanding of the dynamics of bacterial communities is conducive to improving composting efficiency.

The composition and dynamics of bacterial communities during the composting process have been extensively analysed. Some studies have focused on the metabolic activity of bacteria and their interactions related to carbon, nitrogen conversion (Takaku et al., 2006) and organic matter composition (Wang et al., 2016). However, the effect of unique composting processes on bacterial communities needs further investigation. The proper management of process parameters, such as temperature, moisture, texture or oxygen, determines the sequence of microbes, thereby resulting in a high-quality compost (Partanen et al., 2010). It is widely recognized that the composting bacteria are significantly affected by temperature fluctuation, however, not all studies point to temperature as the selective force that drives microbial diversity in

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composting (López-González et al., 2015). In this sense, several studies assessed the influence of environmental factors different from thermal values on biodiversity in composting. Insam et al. (2010) have proved that bacterial community successions are affected by the evaluation of physicochemical components of composting substrates, such as the C/N ratio, pH and salinity. Xi et al. (2016) investigated that the diversity of the humic-reducing microorganisms were significantly related with dissolved organic carbon (DOC) and nitrogen (DON) and germination index (GI). In addition, adjusting the particle size could affect composting maturity and microbial community, wood materials with 15 mm or 5–25 cm particle size was found to be optimal for composting (Zhang and Sun, 2015; Barrington et al., 2003), but there is no uniform standard on particle size of composting raw material. In addition, considering the temperature accumulation, grinding cost and promotional value, information on how particle size affects the composting requires future studies.

Moreover, nutritional properties of the composting material are major determinants for identifying bacterial communities and their specific capabilities for transforming main organic substrates (Bareither et al., 2013). Xi et al. (2016) investigated the diversity of microorganisms composition in three organic wastes composting systems across the three main phases. Awasthi et al. (2017) studied the effects of biochar amendment on the structure and succession of bacterial diversity during sewage sludge composting. As lignocellulosic fractions are the most difficult to degrade and the number of microbial species capable of doing so is certainly low, some studies have identified and selected specific microorganisms involved in the composting of lignocellulosic materials, and exploit the microorganisms for diverse uses such as compost inoculants and composting bioaugmentation (Paradelo et al., 2013; Jurado et al., 2014; Jurado et al., 2015). Microbial dynamics involved in the composting of chestnut residues was investigated in a natural environment (Ventorino et al., 2016). However, the relationship between microbial communities and the degradation process in green waste composting is lack of in-depth research.

A traditional method for investigating bacterial diversity in composting (i.e. culture dependent method) has been widely used, this method could not completely elucidate community structure and diversity during different composting stages because of culture medium limitations (Chandna et al., 2013; Ogino et al., 2001). To develop a comprehensive and detailed knowledge of microbial community composition, culture-independent methods have been successfully used that can identify unknown sequences (Egert et al., 2004; Partanen et al., 2010). Recently, molecular tools based on 16S rRNA (16S rDNA) sequence technologies have greatly advanced, and they can identify a specific microorganism in various environments (Martinez et al., 2013; Wang et al., 2015). Much research has been devoted to understanding the microbial dynamics and total microbe abundance during various kinds of organic waste composting. For instance, López-González et al. (2015) investigated the mycobiota biodiversity of agricultural lignocellulosic waste composting, Awasthi et al. (2017) identified bacterial diversity at the thermophilic stage during sewage sludge composting and Wang et al. (2015) studied bacterial diversity and its relationship with environmental parameters during composting of

different raw materials. However, they focused on the relationship between microorganisms generation and population but few studies have examined the impact of changing the composting conditions on the microbial communities. Further efforts are needed to obtain information on the bacterial dynamics during the different stages of green waste composting.

The enzymatic saccharification is the most efficient method for lignocellulose breakdown to sugars, and cellulases have a wide spectrum of applications in various industries. Traditionally, they are applied in food and brewery production, animal feed processing detergent production and laundry textile processing and paper pulp manufacture (Juturu and Wu, 2014; Cerda et al., 2017). Cellulase may be a promising conditioner that can increase the cellulose degradation and promote formation of humic substances during green waste composting. Currently, there are few reports on cellulase as a conditioner in composting. Hence, the present investigation was undertaken for studying the application prospects of cellulase in composting industry. Based on the incubation experiment, the effect of cellulase and particle size on the microbial diversity and the maturity of the composts between control and treatment groups throughout the process were evaluated. The purpose of this study was to (i) investigate the evolution of abundance and diversity of bacterial community between different treatments across the three main composting phases, (ii) examine the influence of cellulase and particle size on the structure and functionality of microbial groups during composting in a laboratory scale, (iii) identify the relationship between bacterial community composition and physicochemical parameters. This process of composting incubation will provide the basic information and technical reference for the application of additives in large scale composting production.

## 2. Materials and methods

### 2.1. Composting procedure and sampling

Green waste raw materials were provided by the Beijing Shoufa Tianren Ecological Landscape Co., Ltd. Green waste composting raw material consisting mainly with fallen leaves and branch cuttings of trees and shrubs during the municipal greening and maintenance in Beijing, China. Microbial inoculants were purchased from the Beijing Jingpuyuan Biological Engineering Co., Ltd. The cellulase was purchased from the Imperial Jade Bio-Technology Co., Ltd., and enzyme activity reaches to 20,000 U/g, the optimum reaction condition for the enzyme was at 40–50 °C and pH 4.5–5.5. The sheep manure organic fertiliser was purchased from the Beijing Academy of Landscape Architecture. A detailed description of the composition and properties of the materials used are shown in Table 1. The composting process was carried out using urban green waste with the addition of sheep manure, which was act as the bulking agent to adjust the C/N ratio of the raw material with the added weight fraction of 35%. By mixing the green waste with sheep manure, the initial total organic carbon content of composting material was 216–220.3 g kg<sup>-1</sup>, the total nitrogen content was 11.6–12.6 g kg<sup>-1</sup> and the C/N was 27–29 (Table 1).

**Table 1**  
Basic properties of compost raw materials.

Material	pH	TOC/g kg <sup>-1</sup>	TN/g kg <sup>-1</sup>	C/N
Green waste	6.76 (0.17)	480.2 (10)	8.9 (1.2)	54 (5.8)
Sheep manure	7.41 (0.06)	261.2 (19)	13.9 (0.8)	19 (2.3)
2 mm green waste composting material	7.75 (0.35)	220.3 (5)	11.6 (0.4)	29 (2.8)
5 mm green waste composting material	7.66 (0.11)	216.0 (14)	12.6 (0.6)	27 (1.4)

Notes: Data are reported as Mean (SD) with n = 3.

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