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# Fungi diversity from different depths and times in chicken manure waste static aerobic composting

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## HIGHLIGHTS

- Fungi diversity research in the aerobic composting was studied with illumina sequencing.
- Time and depth are examined to reveal the fungi diversity variation in this study.
- Dirichlet multinomial mixtures mode was applied for illumina sequencing data analysis.

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## ABSTRACT

The Dirichlet multinomial mixtures mode was used to analyse illumina sequencing data to reveal both temporal and spatial variations of the fungi community present in the aerobic composting. Results showed that 670 operational taxonomic units (OTUs) were detected, and the dominant phylum was *Ascomycota*. There were four types of samples fungi communities during the composting process. Samples from the early composting stage were mainly grouped into type I and *Saccharomycetales* sp. was dominant. Fungi community in the medium composting stage were fallen into type II and III, *Sordariales* sp. and *Acremonium alcalophilum*, *Saccharomycetales* sp. and *Scedosporium minutisporum* were the dominant OTUs respectively. Samples from the late composting stage were mainly grouped into type IV and *Scedosporium minutisporum* was the dominant OTU; *Scedosporium minutisporum* was significantly affected by depth ($P < 0.05$). Results indicate that time and depth both are factors that influence fungi distribution and variation in c waste during static aerobic composting.



## 1. Introduction

Poultry and livestock manure, kitchen waste, crops straw and other organic wastes have become a primary environmental pollutant, endangering human health. At the same time, these wastes contain N, P, K and other nutrition elements. Aerobic composting can help realize organic wastes through harmless treatments and effective resource utilization (Lim et al., 2016; Wei et al., 2014; Wu et al., 2017). Aerobic composting is an organic matter biodegradation process that uses the microbes' action to convert organic matter into stable humus (Juan et al., 2015; Neher et al., 2013; Baddi et al., 2004; Guo et al., 2007). The decomposition process is closely related to the microbial activities. In aerobic composting, microbial communities rapidly adapt to environmental changes, and heat is released during organic matter decomposition (Tian et al., 2013).

Bacterial and fungi species are important microbial components during the organic waste composting process (MacCready et al., 2013; Neher et al., 2013). Many studies have focused on bacterial species due to their large surface area, fast breeding and resistance to high temperatures (Vidya et al., 2013; Tian et al., 2013; Germán et al., 2017; Wu et al., 2017). Bacterial properties such as quick reproduction and the use of organic matter, such as starch and proteins during metabolism, ensure that complete biodegradation occurs. One study showed that fungi play an important role in lignin and cellulose degradation (Floudas et al., 2012; Zhang et al., 2015). Fungi not only effect cellulose material degradation but also produce a variety of extracellular enzymes which degrade organic matter in raw materials (De Gannes et al., 2013; Juan et al., 2015). Some fungi can tolerate high temperatures within the composting environment. In fact, fungi play an important role in the aerobic composting process, yet there are few reports on the role of fungus

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versus that of bacteria. Previous studies have grouped compost samples artificially in accordance with temperature or time. However, the composting reaction is a complex process. Environmental conditions, raw material properties and process parameters affect compost microbial distribution, activity and population change. Importantly, fungi community composition at varying depths can be different during aerobic static composting. In fact, researchers have previously reported differences in the composition of bacterial communities within varying areas during the composting process (Koki et al., 2010; Guo et al., 2012, 2015). Hence, it is difficult to accurately predict fungi community variation by simply sampling at different times or temperatures. In this study, sample collection was carried out according to different depth and time.

The Dirichlet multinomial mixtures (DMM) was applied for the probabilistic modelling of fungi metagenomics data. The data could be described as a matrix providing the number of times each taxa is observed in each sample. From high-throughput sequencing data, we observed that samples have different sizes. Sub-sampling methods can be used to reduce all samples to the same size, but this inevitably discards large amounts of meaningful data. In addition, the microbe's communities are diverse and skewed to rare taxa. Most methods used previously to classify or cluster samples have ignored these features. However, the DMM could describe each community by a vector of taxa probabilities. These vectors are generated from one of a finite number of Dirichlet mixture components, each with different hyperparameters. The mixture components cluster communities into distinct type and groups of samples with a similar composition. The model can also deduce the impact of a treatment and be used for classification (Ian et al., 2012). In this study, the DMM was used for analysing the Illumina Miseq sequencing data to reveal the fungi community variation at different times and depths during static aerobic composting.

## 2. Materials and methods

### 2.1. Composting materials

Mushroom residues (Guangdong Dongguan Xinghe Biotech Company Fungus Factory) and chicken manure (Zhongluotan town poultry farm in Guangzhou city Baiyun district) were used as materials in static aerobic composting. Select characteristics of the materials are shown in Table 1.

### 2.2. Aerobic composting reactor

A pilot-scale compost system that was modified from a reactor was used. The compost reactors consisted of stainless steel cylinders with 100 L effective volumes (Fig. 1). There was a temperature probe inside the reactor for temperature monitoring, and ventilation devices were installed at the same time that could be automatically controlled with ventilation rate (dry matter): 0.2 L (kg min)$^{-1}$ and ventilation frequency: ventilation 10 min interval 6 h.

### 2.3. Experiment design and sampling

Mushroom residues and chicken manure were mixed at a 1:1 (w:w) ratio, and moisture was adjusted to approximately 50%. The static aerobic compost lasted 21 days, and samples were collected from three different depths [20 cm (D1), 40 cm (D2), 60 cm (D3)] and seven different time [ day 1 (T1), day 3 (T2), day 5 (T3), day 7 (T4), day 10 (T5), day 14 (T6), day 21 (T7)]. Triplicate samples were collected with 63 samples in total. At each time point, we collected approximately 500 g from 5 different areas (centre and 4 corners) at the same depth, which were then combined for analysis. Fresh samples were prepared for seed germination index (GI) tests and DNA extraction. Parts of samples were stored after dying, smashing and sieving with 100 mesh. Samples were stored at −20 °C.

### 2.4. DNA extraction, PCR amplification and sequencing

The Sequencing process was applied by Shanghai Personal Biotechnology Co., Ltd. Genomic DNA was extracted from samples, using MoBio PowerSoil® DNA Isolation Kit, following the manufacture instructions. The ITS region was amplified using barcode primers ITS5F (5′-GGAAGEAAAAGECGEAACAAGG-3′) and ITS1R (5′-GCTGCGTTCTTCATCGATGC-3′). The reaction mixture (25 μl) contained: 0.25 μl Q5 high-fidelity DNA polymerase, 5 μl Reaction Buffer, 5 μl High GC buffer, 0.5 μl dNTP (10 mM), 1 μl DNA template and 1 μl each primer. The amplification programme was as follows: 98 °C for 30 s, 27 cycles at (98 °C for 15 s and 50 °C for 30 s), 72 °C for 30 s, 72 °C for 5 min and 4 °C forever. Amplified PCR products were checked by gel electrophoresis on 2% agarose gel. We used Quant-It PicoGreen dsDNA Assay Kit to treat PCR products on a microplate reader (Biotek Flx800) and then mixed. The DNA library was built by TruSeq Nano DNA LT Library Prep Kit, and 1 μl DNA library was checked by Agilent High Sensitivity DNA Kit on Agilent Bioanalyser machine. Sequencing was performed using Miseq Reagent Kit V3 (600 cycles).

### 2.5. Taxonomic assignment of sequence reads

The ITS raw sequences were analysed by QIIME software (Quantitative Insights Into Microbial Ecology, v 1.8.0) (Caporaso et al., 2010). Sequences were trimmed for primers, quality filtered and assigned to DNA libraries according to their tags. Sequences shorter than 150 base pairs or containing any unresolved nucleotides were removed from the data set. Chimeras were identified and removed from the dataset using USEARCH (v5.2.236 http://www.drive5.com/usearch). Using QIIME software to call the UCLUST sequence alignment tool (Edgar, 2010), we merged the qualified sequence into OTU according to the sequence similarity 97%. The representative sequence was that which was the most abundant in each OTU. Using default parameters in QIIME software, we obtained OTU taxonomic information by aligning the representative sequence with UNITE ITS database (Release 5.0, https://unite.ut.ee/) (Koljalg et al., 2013).

### 2.6. Seed germination indices

Seed germination indices (GI) were determined as previously described (Zucconi and De Bertoldi, 1987). Distilled water (50 ml) was added to 5 g fresh compost, which was mechanically shaken for 1 h and then filtered. A 5 ml compost extract was added to culture dishes (9 cm diameter) containing two pieces of filter paper. A total of 20 radish seeds were evenly distributed on the surface of

**Table 1**
Physical-Chemical properties of composting material.

| Raw material | Moisture content (%) | C/N ratio | Organic matter (%) | Total N (%) | Total P (%) | Total K (%) |
|---|---|---|---|---|---|---|
| Chicken manure | 34.76 | 7.10 | 48.58 | 3.97 | 2.81 | 4.04 |
| Mushroom residue | 10.76 | 30.17 | 82.28 | 1.62 | 1.87 | 1.18 |