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Changes in structure and function of fungal community in cow manure composting

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ABSTRACT

In this study, dynamic changes in fungal communities, trophic modes and effect factors in 60 days composting of cow manure were analyzed by using high throughput sequencing, FUNGuild and Biolog FF MicroPlate, respectively. *Orpinomyces* (relative abundance > 10.85%) predominated in feedstock, and *Mycothermus* became the dominating genus (relative abundance > 75%) during the active phase. Aerobic composting treatment had a significant effect on fungal trophic modes with pathogenic fungi fading away and wood saprotrophs increasing over composting time. Fungal communities had the higher carbon sources utilization capabilities at the thermophilic phase and mature phase than those in the other periods. Oxidation reduction potential (ORP) significantly increased from −180 to 180 mV during the treatment. Redundancy analysis showed that the succession of fungal community during composting had a significant association with ORP ($p < .05$). This indicated that aerobic composting treatment not only influenced fungal community structure, but also changed fungal trophic modes and metabolic characteristics.

1. Introduction

In China, the number of dairy cows has passed 15 million, which produce a large quantity of cow manure, above 17.3 billion tons per year (Huang et al., 2013). Cow manure, containing a large amount of nitrogen, phosphorus and potassium, has become a major environmental pollutant and endangers human health due to insufficient utilization. Aerobic composting could stabilize organics in livestock manure and minimize their negative effects by bacteria and fungi (Juan et al., 2015; Neher et al., 2013; López-González et al., 2015). Composting represents an important solution for converting livestock manure into resource for agricultural and horticultural applications.

Microbial activities play key roles in successful bio-transformation of organic substrates in composting treatment. Although the bacterial communities in the composting system have been studied by many researchers (Tian et al., 2013; Green et al., 2004; Piceno et al., 2017), the role of fungal communities cannot be neglected. Some fungi are able to tolerate high temperature(Ryckeboer et al., 2003). For instance, *Aspergillus*, *Corynascus*, *Trichoderma*, *Penicillium*, *Phanerochaete* and *Pseudallescheria* were found in the thermophilic period of composting (Sebők et al., 2015; Langarica-Fuentes et al., 2014). *Ascomycota* and *Basidiomycota* represented the main fungal decomposers in livestock manure and agricultural waste composting (Neher et al., 2013; Yu et al., 2015). The low level of fungal diversity was determined in the previous studies because of the limitation of classical culture-based methods and DGGE analysis. Although high throughput sequencing (HTS) has been adopted to analyze microbial community composition in environmental sample, the knowledge on metabolism function of fungal community in a composting system is very limited.

The metabolic function of microbial communities is essential to understand organics transformation and stabilization process during composting. The substrate utilization capacities of fungal community directly reflect their metabolism characteristics. The assessment of fungal biodegradability previously focused on selected fungi to utilize simple carbon resources, such as sugars, cellulose or lignin (Lindahl & Finlay, 2006; Rinkes et al., 2011; Koukol & Baldrian, 2012). Ming & Kirk (1983) found that white-rot fungus *Phanerochaete chrysosporium* (Hymenomycete) had the ability to degrade lignin. Filamentous fungi cultivated on wet-oxidised wheat straw generated cellulose and hemicellulose-degrading enzymes (Thygesen et al., 2003). However, the assessment of metabolism diversity of fungal community was limited by the traditional method, because the most of fungi are uncultivable in the compost system. Therefore, little literature is available on the relationship between fungal community and substrates utilization diversity in cow manure composting.

Fungal trophic mode reflects the safety of fungi communities in compost product. Many researchers has reported composting is an effective method for the eradication of bacterial pathogens (Boulter et al.,

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2002; Hoitink et al., 1997), while some species such as *Clostridium perfringens*, were able to survive a peak compost temperature over 60 °C (Jones & Martin, 2003). However, fungal pathogens cannot be ignored, because some fungi are harmful for plant and animal health. Therefore, it is particularly necessary to detect the fungal trophic mode in the composting system. FUNGuild is a novel tool to comprehensively examine the fungal community from an ecological perspective (Nguyen et al., 2016). The fungal OTUs from high-throughput sequencing could be partitioned into 3 trophic modes and 12 guilds based on this database. Therefore, the FUNGuild could explore fungal trophic type and metabolic function characteristics rather than taxonomic identity.

The environmental factors (temperature, moisture, aeration rate, pH, C/N ratio, etc.) dramatically change during composting process, which could influence microbial community and metabolism activity. Zhang et al. (2011) found that the temperature and water soluble carbon presented a predominant effect on bacterial community composition. Liang et al. (2003) showed that the moisture content had a greater influence on microbial activity than the temperature in sludge composting. The pH and C/N ratio of feedstock also had an impact on microbial community dynamics (Yu et al., 2015; Wang et al., 2015a,b). In addition, various bulking agents not only impact the C/N ratio of feedstock but also the porosity in the composting matrix (Wang et al., 2013; Yanez et al., 2009). Although the effect factors on bacterial community represented the different results in the previous literature, the information on the relationship between environmental factors and fungal community in the composting system is limited.

In accordance with the above mentioned, the main objective of the present study was to (1) examine the dynamic change of fungal community in cow manure composting process by using high-throughput sequencing; (2) determine the association between environmental factors and fungal community by redundancy analysis (RDA), to identify the critical factor affecting the succession of fungal species, and (3) evaluate the functional groups and metabolic characteristics of fungal community by using FUNGuild database and Biolog FF microplates. Besides, this study also attempted to show the effect of environmental factors and microbial community structure on fungal metabolic function at different composting periods.

2. Materials and methods

2.1. Composting process and sample collection

Cow manure was obtained from a livestock farm in Harbin, northeast China. Temperature, moisture, pH, ORP, the ratio of total carbon and total nitrogen (C/N), volatile solid (VS) and ash contents of the cow manure were determined during 60 days composting (Table 1). The data in Table 1 were calculated based on the dry weight of the samples. Wood chips collecting from birch were mixed with the fresh cow manure at a ratio of 2.5:1 (volume ratio) before aerobic composting. The average size of the wood chips was 3.5–4.0 cm long, 1.5–2.0 cm wide and 0.2–0.5 mm thick. The mixture was put into a cylindrical plexiglass composting reactor with a total volume of 40 L (40 cm diameter × 100 cm height). The circumjacent temperature was controlled by a water bath to prevent excessive heat loss from cow manure composting system (Wang et al., 2014). There were three temperature sensors were used to detect the temperature of the inner compost (T1), closed to the reactor wall (T2) and in the bath (T3). The detected value of three sensors was modulated nearly equal (± 2 °C) to the interior of the compost by a temperature control system (Wang et al., 2018). Continuous air (2.5 L/min) was charged into the reactor by a pipe at the bottom to remain an aerobic condition in the composting process.

Samples were collected at Day_0, Day_10, Day_30 and Day_60 of composting representing raw cow manure, thermophilic phase, cooling phase and mature phase (production). The samples of equivalent quality were collected randomly at 9 different sites in three different depths at each time point: 3 surface samples (10 cm below the surface of the pile), 3 middle samples (30 cm below the surface of the pile), and 3 bottom samples (bottom of the pile). The nine samples were mixed thoroughly and separated equally into three parts (Hao et al., 2016).

2.2. Chemical and physical analyses

The moisture content of the sample was analyzed based on the initial weight and the final weight after drying at 105 °C to a constant weight. Then the dried samples were put into the oven at 550 °C for 4 h to measure the VS content. The temperature of the composting system was recorded by a digital thermometer every day during the composting process. 10 g of fresh sample was extracted with 100 ml ultrapure water (resistivity > 18 MΩ·cm) by shaking for 2 h on a horizontal shaker at 25 °C. The extracts were centrifuged at 200 rpm for 60 min. The pH and ORP of the solution were recorded by a pH/ORP meter (PHSJ4F, China) (Wang et al., 2015a,b). The dried samples were ground and screened through a 200 mesh screen, which were used to detect TN and C/N by elemental analyzer (Vario EL, German).

2.3. DNA extraction and PCR amplification

Genomic DNA was extracted from samples, using MoBio Power Soil DNA Isolation Kit (MoBio Laboratory, Carlsbad, CA, USA), following the manufacture instruction. The extracted DNA was dissolved in 50 lITE buffer, quantified using a spectrophotometer, and stored at −80 °C until further use. Polymerase chain reactions (PCR) amplification of internal transcribed spacer (ITS) region of ribosomal DNA followed those of Holman et al. (2016) and Kataoka et al. (2008). PCR were performed in a 50 μl reaction mixture, including 25 μl Premix Tap and 25 μl Milli-Q water. The samples were put through 30 cycles of the following: extension at 72 °C for 1 min, degeneration at 95 °C for 30 s, annealing at 50 °C for 30 s, followed by extension at 72 °C for 45 s. Finally, primer extension occurred at 72 °C for 7 min. Amplicons were separated on 1.5% (w/v) agarose gels, stained with ethidium bromide (1 mg/L) for 30 min, and visualized under UV light transilluminator to check success in PCR amplification. HTS was carried out at the Centre for Genomic Research, Majorbio Bio-Pharm Technology Co., Ltd, China.

2.4. Taxonomic assignment of sequence reads and sequence analysis

The ITS sequences were analyzed 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. The sequences shorter than 150 base pairs or containing any unresolved nucleotides were removed from the data set. Usearch 7.1 (http://qiime.org/) was used to cluster the

Table 1
Physical–chemical characteristics of composting materials at different composting periods.

Samples	Temperature(°C)	Moisture (%)	ORP (mV)	pH	C/N	VS/Ash (g/g)
Day-0	25 ± 0.4	85.2 ± 0.3	-180 ± 2	7.6 ± 0.1	20.7 ± 0.2	5.62 ± 0.03
Day-10	61 ± 0.3	81.8 ± 0.2	50 ± 3	8.6 ± 0.1	24.9 ± 0.1	4.52 ± 0.04
Day-30	30 ± 0.4	73.4 ± 0.5	150 ± 4	8.1 ± 0.1	13.9 ± 0.3	3.72 ± 0.04
Day-60	27 ± 0.7	61.1 ± 0.6	180 ± 3	7.8 ± 0.1	12.9 ± 0.2	2.75 ± 0.06