



How do fungal communities and their interaction with bacterial communities influence dissolved organic matter on the stability and safety of sludge compost?

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Abstract

This study was conducted to assess the effect of fungal communities and their interaction with bacterial communities on the dissolved organic matter (DOM) transformation for the stability and safety of sludge composting. The results showed that fungal community had strong shifts in diverse stages of sludge composting along with the changes of temperature. Correlation analysis demonstrated that fungal communities had significant connections with bacterial communities during composting but were not directly related to the indicators of phytotoxicity and maturity. Variance partitioning analysis suggested that the interactions of fungal and bacterial communities had the biggest contribution (49.75%) to composting stability and safety. Based on structural equation modeling, the possible way of fungal community participated in the transformation of DOM components and the formation of humic-like substances of DOM by interacting with bacterial community was proposed, which will provide important information for understanding the biotic interaction in composting and improving composting fermentation process.

Keywords Composting · Fungal community · Bacterial community · Stability · Dissolved organic matter

Introduction

More than 600 wastewater treatment plants had been established by the year 2005 in China, producing around 5 billion kilograms of sewage sludge every year (Su et al.

2015). Sewage sludge contains a high proportion of biodegradable organic compounds and a large amount of toxic substances and pathogens, which may pose a serious threat to the environment and cause loss of useful nutrients if not disposed properly (Villar et al. 2016). Composting is a widely used technology for the biotransformation of organic wastes into relatively stabilized and nontoxic products that can be used as organic fertilizer or soil amendment, which is suitable for recycling of sewage sludge (Yang et al. 2015). The process of composting exhibited rich microbial diversity with different enzymatic capabilities, which played important roles on the decomposition and stabilization of organic substrates (Zhang et al. 2011). With bacteria being more influential due to their metabolic versatility, substantial research efforts have been carried out to evaluate the impacts of bacterial community on composting performance (López-González et al. 2015). The roles of fungi in composting are usually considered to be minor in comparison to those of bacteria because of their relatively lower abundance during composting and pathogenic fungi from composts amended with sewage sludge additions as well as their preference of a limited range of physico-chemical conditions (Bonito et al. 2010; Tuomela et al. 2000). However, some researches believed that the effect of

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fungal community on composting may be underestimated, supporting that the dominance of fungi in the maturation or mesophilic phase could benefit the increase in the degree of polycondensation and the cross-linking of organic compounds for the stability and maturity of sludge composting (Amir et al. 2008). Therefore, the understanding of the dynamic changes of fungal communities during sludge composting is essential for evaluating the role of fungi in material transformation from organic wastes into stable and safe final products. There are many different factors that affect composting, mainly depending on the complex interactions among microorganisms, compost substrate, and environmental parameters (e.g., pH, temperature, and moisture content) (Lu et al. 2018). Meanwhile, considering the vital roles of microbes as the driver of organics transformation, the complex interactions among different microorganisms, that is, bacteria and fungi (the integral part of composting microbial community), may affect composting nutrients' utilization and organic matter structure as their contributions in degraded soils (Rashid et al. 2016). So far, many studies focused on evaluating the relationship between environmental parameters and the bacterial or fungal composition changes and the selection of relatively important composting environmental factors (Wu et al. 2017; Zhang et al. 2011). Nevertheless, less is reported on what extent of the interaction of fungal and bacterial communities influence sludge composting.

Dissolved organic matters (DOMs) contain a wide range of chemical compounds and a variety of decomposed and synthesized products (Wang et al. 2015). Generally, the aromatic and alkyl compounds in DOM increase during composting, resulting in an increased degree of humification and stability in DOM. Therefore, the characteristics of DOM could be an important indicator to understand composting organics transformation progress and compost stability (Gómez-Brandón et al. 2008; Wei et al. 2014b). DOMs are also more easily utilized substrates by microorganisms, as the decomposition by microorganisms occurs predominantly in the thin liquid films (biofilms) on the surface of the organic particles (He et al. 2014; Ryckeboer et al. 2003), but the DOM with high degree of humification in manure compost is more resistant to biodegradation. Therefore, the changes of DOM are significantly related to the growth and community composition of microbes (bacteria and fungi) (Zhang et al. 2011). Different microbial communities that could be adapted to a particular environment and more competitive for substrates predominate in different stages of composting, and metabolites produced by primary decomposers can be utilized by secondary organisms, creating the dynamic interactions within a microbial web (He et al. 2011). Despite this knowledge and an increasing use of microbial community analysis in composting, our understanding of the role of fungal community on DOM biotransformation and how they potentially interact with complex composting bacterial communities remains scant.

In the present work, the dynamic changes of fungal community during sludge composting were investigated to understand how the fungal community and their interaction with the bacterial community influence DOM transformation for composting stability and safety, which will provide important information for understanding the biotic interaction in composting and improving composting fermentation process.

Materials and methods

Composting experimental design and preliminary data

The composting experiments using sewage sludge and rice hulls at a ratio of 10:3 on a wet basis were performed in 34-L laboratory reactors for 42 days with three replicates. Homogeneous samples of the compost were collected using the multipoint sampling method at days 0, 3, 6, 12, 18, 26, 34, and 42, then were air-dried for physical-chemical analysis or stored at $-20\text{ }^{\circ}\text{C}$ for DNA extraction. The composting properties including temperature, pH, organic matter (OM), the ratio of carbon to nitrogen (C/N), and germination index (GI) are shown in Table S1 in the supplementary materials. The bacterial community composition and diversity were detected by denaturing gradient gel electrophoresis (DGGE), and the sequences were compared with the compilation of 16S rDNA genes in the Genbank database (Figs. S1 and S2 in the supplementary materials). The details of DOM extraction, UV-Vis spectroscopy, and fluorescence spectroscopy measurements of DOM as well as PARAFAC can be accessed in another published study (Zhao et al. 2018). Previous studies in our team have identified four components from the DOM samples during composting using PARAFAC analysis by MATLAB 2013a (Mathworks, Natick, MA) according to the procedure described by Stedmon et al. (2003), and the concentration scores of the PARAFAC components were expressed as maximum fluorescence intensity (F_{max}) (R.U.) for each modeled component. The DOM components (C1, C2, C3, C4) as well as the DOM characteristic parameters including SUVA_{254} , SUVA_{280} , $E_{253/203}$, $A_{240-400}$, and S_{R} are shown in Table S2 in the supplementary materials. C1 and C2 were protein-like fluorescent compounds. C3 and C4 were characterized as fulvic acid fluorophore groups and humic-like materials, respectively.

Fungal PCR-DGGE analysis

Genomic DNA was extracted by soil DNA kit (Omega Biotek, Inc.). To analyze the fungal community, 18S rDNA genes were amplified using polymerase chain reaction (PCR) by the primers NS1/GCfung according to Xi et al. (2015). DGGE was carried out using the PCR product loaded into

polyacrylamide (6%) gels with gradients of 20–40% of denaturants (urea/formamide). Electrophoresis was performed for 16 h at 60 °C and 60 V. After electrophoresis, gels were stained in 3 × GelRed™ nuclear acid gel stain (Biotium, USA) and photographed with a UVP Imaging System (UVP Inc., USA). Representatives of bands were excised from DGGE gels and sequenced as described by Lu et al. (2018). The sequences were compared with the GenBank database by BLAST.

Data analysis

DGGE banding profiles were digitized and band numbers were counted using Quantity One software (version 4.5, Bio-Rad, USA). The evenness (Shannon-Wiener index, H') and dominance (Simpson index, D) were calculated as follows: $H' = -\sum p_i \log_2 p_i$ and $D = \sum (p_i)^2$, where p_i represents N_i/N (N_i being the relative intensity of a specific band i and $N = \sum N_i$). Nonmetric multidimensional scaling (NMDS) ordination and redundancy analysis (RDA) were carried out by Canoco 5.0. The matrix correlations were analyzed by Mantel tests using Primer 5.0 and NTSYS version 2.0. Variance partitioning was used to assess the variation in the parameters of composting stability explained by bacterial and fungal communities according to Qiu et al. (2016). Structural equation modeling (SEM) was constructed by SPSS AMOS 23.0 as described by Liang et al. (2017).

Results and discussion

Changes in fungal community during composting

The DGGE diagram illustrated that the fungal community had strong shifts in different sampling days (Fig. 1a and Fig. S3 in the supplementary materials), suggesting that fungal communities were very dynamic during composting. Thirty-one different 18S rDNA bands were detected in the DGGE profiles, mainly belonging to Ascomycota and Basidiomycota (Fig. S4 in the supplementary materials). Most of those bands, especially band 9, 15, and 27, were ubiquitous but different in abundance in the bio-oxidative process including mesophilic phase, thermophilic phase, and cooling phase (Bernal et al. 2009). Two biodiversity indices were employed to demonstrate the dynamics of fungal diversity in sludge composting (Fig. 1b). H' of the fungal community ranged from 2.3 to 3.0 but there is no significant difference between days 18, 26, 34, and 42. D showed an inverse trend because it represented the probability that two random individuals taken from a sample belong to the same species (López-González et al. 2015), whose maximum values were found at the thermophilic and cooling stages, demonstrating that there is a gradual adaptation of dominant fungal community in the cooling stage (Amir et al. 2008). NMDS showed that the samples were dispersed during composting, suggesting that the community of fungi was different at each composting stage (Fig. 1c). The fungal communities of diverse days were divided into three categories: (1) fungi in heating stage, (2) fungi in thermophilic stage,

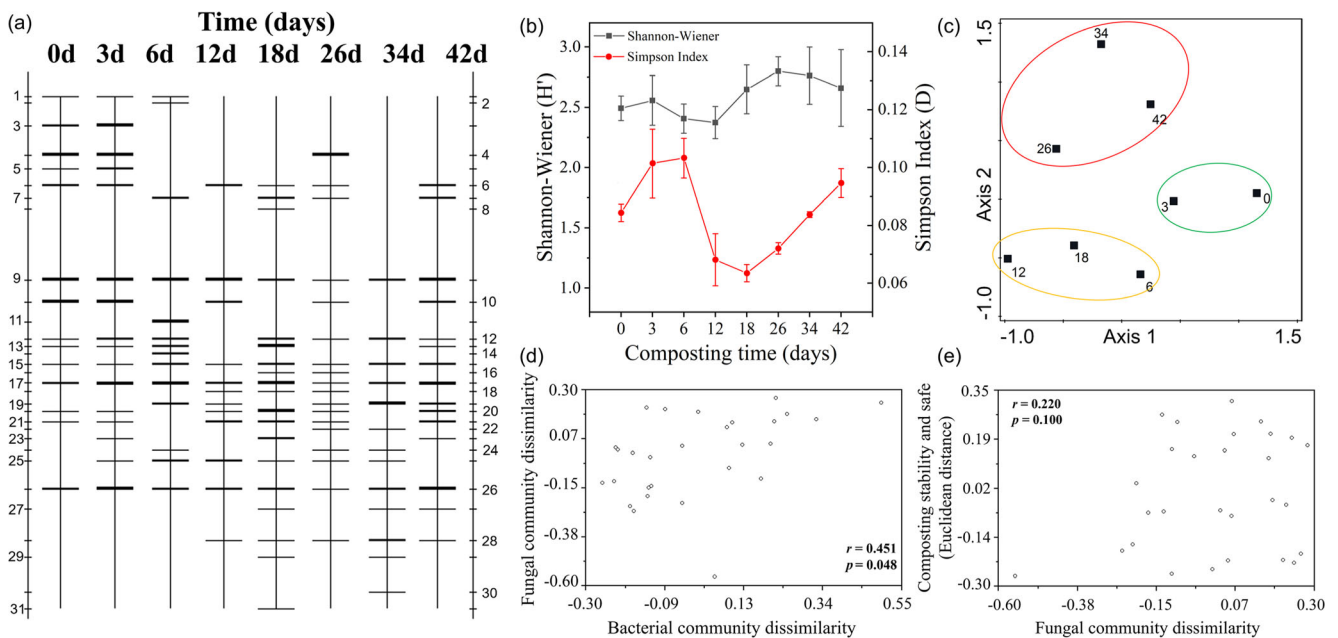


Fig. 1 a The DGGE fingerprint diagram of fungal community based on 18S rDNA fragments. b The Shannon-Weaner and Simpson indices of fungi throughout the composting process. c NMDS ordination of fungal community based on DGGE profile dataset. d The relationship between

Bray-Curtis dissimilarity of fungal community and Bray-Curtis dissimilarity of bacterial community during composting. e The relationship between fungal community dissimilarity and composting stability property

and (3) fungi in cooling stage. This result demonstrates that the trend of fungal community succession was similar to that of bacteria but had slower changes compared to the succession of bacteria, suggesting that temperature is crucial to change the composition of both bacterial and fungal communities (López-González et al. 2015). Samples from the cooling phase clustered more dispersedly for fungi than for bacteria, suggesting that the fungal community may have better adaptability to the changed environmental conditions.

Correlations between fungal community and composting stability and safety

It was certified that the bacterial community with the ability to degrade tartaric acid played important roles in regulating the transformation of DOM components, which was helpful for the stability and safety of the compost (Zhao et al. 2018). According to the results of Mantel test, a close correlation was detected between the fungal community dissimilarity matrix and the bacterial community dissimilarity matrix during composting (Fig. 1d), which suggested that the composition of bacterial community was correlated with heterogeneous fungal species by interspecific cooperation or dissymmetry

mutualism. Interestingly, there is no significant correlation of fungal community dissimilarity matrix with composting stability and safe Euclidean distance matrix (Fig. 1e). The results indicated that fungal community might affect the function and succession of bacterial strains under the influence of environmental factors, but not directly participate in or have less contribution to converting different organic fractions to form humic substance for composting stability (Wu et al. 2017).

The interaction of fungal and bacterial communities affects DOM for composting stability and safety

In order to determine how the activity of the fungal community influences the stability and safety of sludge compost, it is necessary to consider completely the biotic factors including the composition and diversity of bacterial and fungal communities during composting. The indicators of GI, C/N, SUVA₂₅₄, SUVA₂₈₀, $E_{253/203}$, $A_{240-400}$, and S_R were chosen to assess the compost maturity and safety (Cui et al. 2017; He et al. 2011). RDA was performed to analyze the effect of microbial community on the key environment factors reflecting the safe and stable composts (Fig. 2a). Monte Carlo tests for the first and all canonical axes were highly significant ($P = 0.002$). GI

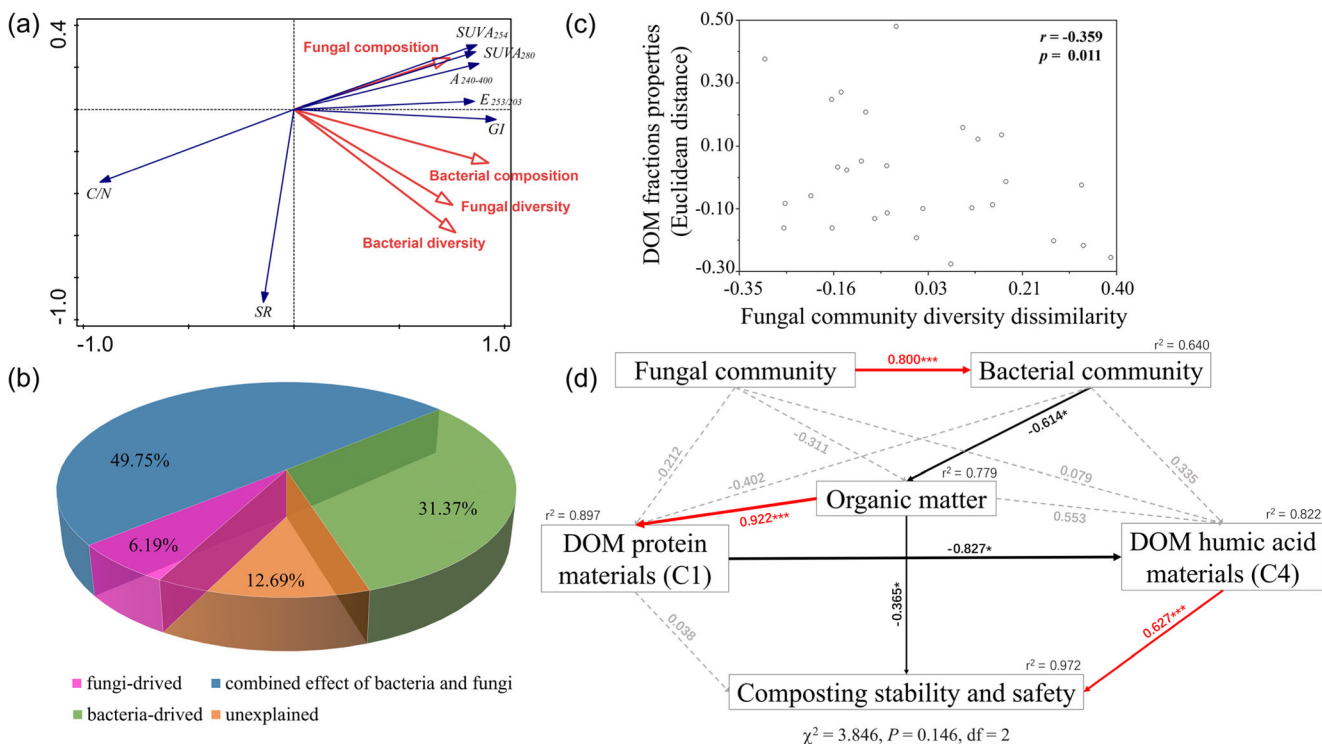


Fig. 2 **a** RDA of the correlation between bacterial and fungal communities as well as composting stability indices. **b** The Venn diagram showing the variation in composting stability indices explained by bacteria and fungi. **c** Relationship between Bray-Curtis dissimilarity of fungal community diversity and DOM fractions' properties during composting. **d** Structural equation models showing the hypothesized causal relationships of fungi, bacteria, composting organic matter

property, and DOM fractions on composting stability and safety. Arrows depict causal relationships: red lines indicate positive effects, and black lines indicate negative effects. Continuous and dashed arrows indicate significant and nonsignificant relationships, respectively. Arrow widths are proportional to r values. Significance levels are indicated: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

is an important indicator of the safety level of composting materials; meanwhile, $SUVA_{254}$, $SUVA_{280}$, $E_{253/203}$, and $A_{240-400}$ represent the parameters of composting DOM humification and stability (Cui et al. 2017). The included angle of two factors in RDA was an indicator for correlation; that is, an acute angle represented a positive correlation and a negative correlation was showed by an obtuse angle (Wang et al. 2015). Positive correlations were found between GI and the bacterial composition ($R = 0.899$, $P = 0.002$) or fungal composition ($R = 0.698$, $P = 0.044$). Meanwhile, the bacterial and fungal compositions were also positively correlated with GI, $SUVA_{254}$, $SUVA_{280}$, $E_{253/203}$, and $A_{240-400}$. The sum of all canonical eigenvalues indicated the total variation explained by bacterial and fungal communities, accounting for 87.3% statistically (Fig. 2b). A substantially larger contribution (31.4%) of composting stability and safety was attributed to bacterial community and the 6.2% of variation may be associated with fungal activities, which may lead to the insignificant relationship as the above results. It is noteworthy that the interactions between bacterial and fungal communities contributed to a variation of 50.0%, which is much higher than that explained by sole factor. These results indicated that the special fungal community might promote the growth of functional bacterial strains with multiple biochemical capabilities by collaborative symbiosis, e.g., two or multiple symbionts (Currie et al. 1999), which were mostly responsible for improving the stability and safety level of composts.

The result based on Mantel test gripped the attention as demonstrated in Fig. 2c that the correlation coefficient between DOM fractions' property matrix and fungal community diversity dissimilarity was -0.359 ($P = 0.011$). It is known that DOM is typically composed of low-molecular-weight organic acids, aromatic acid polysaccharides, and numerous other organic compounds (Guppy et al. 2005; Wei et al. 2014a). Biodegradability of DOM may influence microbial community structure, and a part of DOM could act as a regulator of composting microenvironment to modify the microbial diversity and functional redundancy during composting (Docherty et al. 2006; Wei et al. 2018). Considering the important characteristics of the DOM formed during composting that were related to the microbiologic activities that occurred in solid-liquid interface and the transformation progress of composting materials (Wei et al. 2014b), DOM may act as a "bridge" between fungal and bacterial communities as well as composting stability and safety. Therefore, SEM was conducted to detect the casual relationships among the microbial community information, DOM components, and composting stability as well as safety (Fig. 2d). Squared multiple correlations showed that over 90% of the variance of composting stability and safety could be explained by the SEMs. In sludge composting, the fungal community assists the activity of functional bacterial species, which mainly participated in the degradation of OM. Strongly negative effects of C1 component

on C4 component were also observed, which have an indirect effect on the changes of composting stability and safety. Therefore, C1 component of DOM (protein materials) may be consumed as energy and carbon source of microbial metabolism and transformed to C4 component of DOM (humic acid materials) as proposed by He et al. (2011). The greater the amount of C1 component reduced and C4 component produced, the better the promoting effect on composting stability and safety by microbial community. Interestingly, the previously similar SEM without fungal community data suggested that the bacterial community significantly promotes the formation of C4 component by transforming C3 component. Both models indicated that the transformation of DOM components as the precursors of humic-like substances directly influenced the stability and safety of composting materials. Nevertheless, the participation of different microbial communities would affect the dominant ways in forming humic-like substances during composting. Further study on selecting the key fungi and strengthening their beneficial interaction with key bacteria would lead to increased understanding of DOM biotransformation during composting and optimizing composting products.

Conclusion

This study showed that fungal community had strong shifts in diverse stages of sludge composting along with the changes of temperature. There was a strong correlation between fungal community and bacterial community during composting, and variance partitioning analysis suggested that the interactions of fungal and bacterial communities had the biggest contribution (49.75%) to composting stability and safety, but single fungal community was not significant. Based on the comparison of SEM, the role of fungal community on the stability and safety of compost was proposed, which was to affect different DOM components' transformation for the formation of humic-like substances by interacting with bacterial community.

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