

# Assessing the Performance and the Microbial Dynamics of Co-composting System Using Recycled Cow Manure and Bedding Material Waste

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## Research Article

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# Abstract

Co-composting of recycled cow manure and waste bedding material has been used to convert both agricultural wastes to biofertilizers. This study explored the succession of microbial community, metabolic function and substances conversion capacities during 60 days' co-composting using high throughput sequencing technology. The study revealed that co-composting of cow manure and bedding material waste at a ratio of 1.32 (CM+B) had the highest efficiency among four treatments. The bacterial and fungal community diversity changed significantly during the co-composting of CM+B group, and the major phyla included Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria and Ascomycota. PICRUSt and FUNGuild analysis showed that carbohydrate, lipid metabolism and especially nitrogen fixation were enhanced in the thermophilic phase, while animal and plant pathogens were not detected after the co-composting. Wood saprotrophs became the dominant fungal group (89.1%) in the maturation phase. Canonical correlation analysis (CCA) and redundancy analysis (RDA) confirmed that temperature influenced bacterial community succession more than it influenced fungal community succession. *Ruminiclostridium* had a significantly positive relationship with temperature ( $p\_value < 0.05$ ), while pH and C/N had significant effect on the fungal ( $p\_value < 0.05$ ), and *Penicillium* and *Mortierella* were significantly related to moisture ( $p\_value < 0.05$ ). This work describes an efficient methodology to deal with co-composting systems that had been successfully applied in agricultural wastes treatment, enabling further understanding in mechanisms underlying the substance conversion and the involved microbial community succession in sophisticated composting system.

## 1. Introduction

Livestock production makes up approximately 40% of the global value of agricultural products and provides livelihoods for almost 1.3 billion people worldwide, while also results in concern of manure generation in substantial quantity (Organization, 2017). Currently, more than half of the total manure generated from all livestock is cow manure. In China, approximately 380 million tons of cow manure are produced each year, mostly from centralized large-scale farms (Wang et al., 2018a). Generally, cow manure is rich in nutrients that are essential for crops, including nitrogen (N), phosphorus (P), potassium (K), etc., and has been considered a good source of long-term biofertilizer (He et al., 2011; Shen et al., 2018). However, it also contains various hazardous components and pathogens and improper handling of cow manure could pose threats to soils, crops and human health as well as causing water and air pollution to the surrounding environment (Li et al., 2020; Safferman & Wallace, 2015; Wang et al., 2020b). Biomass bedding is another popular technology in livestock industry, especially cow dairy industry, to provide a comfortable lying surface for animals on farms (Black et al., 2013; Fournel et al., 2019; Husfeldt et al., 2012). A large amount of biomass materials, such as rice husks and sawdust, are often used to build the bedding layer (Atencio et al., 2010; Leso et al., 2020; Mierzwa-Hersztek et al., 2018). The consumed bedding materials would also need to be disposed typically annually or biennially (Wolfe et al., 2018), while limited efforts have been made to investigate the appropriate treatment method.

Composting is a microorganism-mediated, cost-efficient method to convert various unstable and complex organic matters into stable and humus-like substances. The major components of organic wastes include carbohydrates, including cellulose, proteins, lipids and lignin, and the biodegradation process of organic wastes is carried out by mesophilic and thermophilic microorganisms in sequence (Duan et al., 2019; Xu et al., 2019). The composting products can be further utilized to improve the physical properties of soil (Bello et al., 2020; Esmaeili et al., 2020; Guo et al., 2019). Dissolved organic matters (DOM), as the direct material and energy resource for microorganisms, reflect compost stability and maturity (Said-Pullicino et al., 2007). Notably, the composting process is dramatically affected by physiochemical properties of feedstock (e.g. moisture and C/N ratio) and environmental factors (e.g., temperature and pH) (Awasthi et al., 2018; Guo et al., 2012; Wang et al., 2018b). For this reason, fresh cow manure is unsuitable feedstock for composting as its moisture content is generally higher than 90%. However, studies have shown that when amending with co-compost materials such as wood shavings, sawdust, and cornstalks, the composting process of livestock manure was enhanced because those diverse additives altered the physiochemical properties of feedstock to a more appropriate level (Chang et al., 2019; Jeong et al., 2017; Kebibeche et al., 2019; Ren et al., 2010). The dewatering treatment of fresh cow manure can also effectively reduce the moisture content and obtain the recycled cow manure. Due to the significant difference in physicochemical properties between recycled cow manure and bedding material waste, it is proposed that co-composting using the mixture of both wastes shall be able to promote biotransformation efficiency comparing to the composing of recycled cow manure alone.

In this study, an efficient method for bedding material waste was developed to serve as additives for composting of recycled cow manure. The effects of bedding material waste supplementation were investigated systematically and dynamically regarding physiochemical properties of compost samples and degradation of organic matters. To obtain a comprehensive understanding of the co-composting process, the microbial community of composting system (included both bacterial and fungal community) and metabolic function profiles were analyzed. The correlations among bacterial and fungal communities, physicochemical properties, and metabolic function distribution were explored. The degradation and humification of organic matters were also analyzed to evaluate the potential application value in agricultural and landscape industry.

## 2. Materials And Methods

### 2.1. Composting process and sampling

Recycled cow manure (obtained after fresh cow manure dewatering) and waste bedding material were collected from the research farm at Jixiang Livestock Co., Ltd., Fujian, China and used as feedstock for co-composting. The basic features of these two materials were shown in Table 1. Four different treatments were performed, including pure cow manure (CM), cow manure with bedding material waste (CM + B; weight ratio = 1.32:1), cow manure with bedding material waste and urea (CM + B + N; weight ratio = 1:1; nitrogen loading = 80 kg), and cow manure with bedding material waste, urea and sheeting (CM + B + N + S; weight ratio = 1:1; nitrogen loading = 80 kg). Urea was used as an inorganic nitrogen

source (Awasthi et al., 2018) and sheeting with small pores was used to maintain temperature of compost. The co-composting piles were built after thoroughly mixing raw materials using a mechanized mixer, in a pyramid-shape of approximately 3 m × 3 m × 1.5 m (length × width × height). Each pile was turned by forklift every 5 day to facilitate oxygen supplementation and avoid anaerobic reactions. The co-composting process lasted for 60 days. For each pile, samples were taken from five different positions of the middle layers (30–50 cm deep) on day 0, 3, 7, 10, 15, 20, 30, 40 and 60, and mixed homogeneously as a representative sample at each time point before each turning. Each sample was about approximately 1kg and used for analysis of physicochemical parameters in triplicate. According to the temperature change, mixed samples from three replicates on day 0, 7, 20, 40 and 60 were used for microbial community analysis.

Table 1  
Physicochemical properties of selected materials used in the present experiments (dry weight basis).

| Materials              | Moisture content (%) | pH         | TOC (%)    | TKN (%)    | C/N ratio  |
|------------------------|----------------------|------------|------------|------------|------------|
| Cow manure             | 78.6 ± 0.1           | 6.92 ± 0.1 | 39.9 ± 0.3 | 0.90 ± 0.2 | 44.5 ± 0.3 |
| Waste bedding material | 54.4 ± 0.1           | 8.84 ± 0.1 | 49.3 ± 0.4 | 2.21 ± 0.1 | 22.3 ± 0.2 |
| Origin CM + B          | 70.8 ± 0.1           | 7.84 ± 0.1 | 46.6 ± 0.1 | 1.59 ± 0.1 | 29.3 ± 0.4 |
| Origin CM + B + N      | 71.2 ± 0.9           | 7.98 ± 0.1 | 45.5 ± 0.5 | 1.50 ± 0.1 | 30.3 ± 0.3 |

<sup>1</sup>CM+B: cow manure with bedding material (at waste weight ratio = 1.32:1); CM + B + N: cow manure with bedding material waste and urea (weight ratio = 1:1; nitrogen loading = 80 kg);

<sup>2</sup>TOC: Total organic carbon; TKN: Total Kjeldahl nitrogen; C/N ratio: Carbon: nitrogen ratio

## 2.2. Physicochemical analysis

The moisture content was determined as the weight loss after drying samples at 105°C for 24 h in an electric oven in the laboratory. The dried samples were then crushed to pass through a 0.25 mm sieve. Measurements of pH and electrical conductivity (EC) were conducted by portable pH and EC meters after mixing each sample with distilled water at a ratio of 1:10 (w/w). The total organic carbon (TOC) and total Kjeldahl nitrogen (TKN) were measured using the Kjeldahl method and the C/N ratio was calculated accordingly. The germination index (GI) was tested according to the methods in previous report (Alwaneen, 2016; Li et al., 2019).

## 2.3. Spectroscopy analysis of DOM

The three-dimensional fluorescence EEM spectra, characterized by the excitation/emission (Ex/Em) wavelength pairs and the specific fluorescence intensity, were able to qualitatively reveal the DOM composition. Five grams of dried cow manure from each sample was extracted with 50 mL deionized water for 24 h at 25°C, and the aqueous extract was harvested by centrifugation at 10,000 rpm for 10 min and filtration through a 0.45 mm membrane filter. A fraction of the aqueous extract of the samples was freeze-dried to obtain solid DOM for spectroscopy analysis. The chemical changes in the DOM were

quantitatively assessed by spectral methods, including excitation-emission matrix (EEM) fluorescence (Wang et al., 2013). The fluorescence measurement was conducted on the aqueous extract using an FP-6500 fluorescent spectrometer equipped with a xenon excitation source. To obtain the fluorescence spectra of the EEM, the excitation wavelengths were increased from 220 to 450 nm at 5 nm steps, and the emission wavelengths were detected from 280 to 550 nm at 2 nm steps.

## 2.4. DNA extraction and high-throughput sequencing

Genomic DNA was extracted from 0.5 g of each sample (dry weight) by using a PowerSoil Kit (MO BIO Laboratories, Carlsbad, CA, USA). The concentration of extracted DNA was determined by using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). The DNA extract was pooled and kept at -80°C until use. The variable V3-V4 region of the 16S rRNA gene was selected for the construction of the bacterial community library for MiSeq sequencing. ITS1-F (5'-C TTGGTCATTAGAGGAAGTAA-3') and ITS2-R (5'-GCTGCGTTCTTCATCGATGC-3') were used to analyse the fungal community. The PCRs were as described in (Song et al., 2016). The PCR products of the samples were sent to Majorbio Biopharm Bio-technology Co., Ltd. (Shanghai, China) for sequencing using Illumina PE 300 (Illumina Inc., San Diego, CA).

## 2.5. Bioinformatic analysis

Raw gene sequence data generated from the Illumina sequencing were imported into Trimmomatic and FLASH software for data modification. The sample sequence is flattened by the minimum sample sequence number. Usearch 7.1 (<http://qiime.org/>) was used to cluster the sequences into operational taxonomic units (OTUs) with 97% pairwise identity in QIIME. The Ribosomal Database Project classifier (Release 11.1 <http://rdp.cme.msu.edu/>) was used for taxonomic classification of the representative sequences of bacteria against the Greengenes 16S rRNA database (Release 13.5 <http://greengenes.secondgenome.com/>) and the Silva database (release 115, <http://www.arb-silva.de>) (del Carmen Portillo & Mas, 2016). The fungal taxonomic classification was based on the fungal UNITE ITS 12\_11 database (release 5.0, <http://unite.ut.ee/index.php>). The taxonomic information for the 35 most abundant OTUs was uploaded to the FUNGuild database (<http://www.stbates.org/guilds/app.php>) for functional prediction, and their sequences were aligned using PhyDE (Phylogenetic Data Editor); manual adjustments were made to the alignment where necessary (Nguyen et al., 2016). The neighbor-joining trees were constructed using MEGA version 6.0 (<http://megasoftware.net>) with 1200 bootstrap replicates. The bacterial OTUs were imported into PICRUSt, which demonstrated that the genome prediction accuracy was > 0.80. The metabolic pathways were analyzed by using the Kyoto Encyclopedia of Genes and Genomes (KEGG) module after normalizing the OTU table (Langille et al., 2013). CANOCO 5 software was used to assess the correlations between microbial communities and environmental factors by canonical correlation analysis (CCA) and redundancy analysis (RDA).

## 3. Results And Discussion

### **3.1. Changes in physicochemical characteristics during co-composting**

The main physicochemical parameters of the co-composting piles are shown in Fig. 1. Compost temperature is used as an indicator of microbial activity throughout the composting process, and also an indicator of the maturity and stability of the compost product (Zhang et al., 2020). Among the four treatments, CM + B group arrived the highest temperature very fast (Fig. 1a). There were four phases in CM + B composting, mesophilic phase (< 55°C, day 0–7), thermophilic phase (55–62°C, day 7–32), cooling phase (40–55°C, day 32–41), mature phase (< 40°C, day 41–60). The temperature rose rapidly to 55°C on day 7 and reached 62°C after 20 days, reflecting active microbial degradation during the mesophilic and thermophilic phase. Diverse labile substances (e.g., carbohydrates and proteins) were decomposed rapidly, which released a large amount of heat (Zhou et al., 2018). The thermophilic phase (> 55°C) lasted for approximately 25 days, followed by a cooling phase and a maturation phase during which the temperature gradually declined to approximately 36°C upon the end of the composting. The temperature of the other groups arrived the highest on day 30–45, prolonging the composting period. Water content in all the treatments decreased because of the increasing temperature and ventilation in the composting process (Fig. 1b). The water content of CM + B group dropped the fastest from 70–80–55% during the thermophilic phase because of the sharp temperature change, and gradually stabilized to about 50% in the cooling and mature stage. Compared with CM + B group, the variation of moisture content in other treatments was less dramatic because the temperature increase was slow. Similar to temperature, the pH showed an increasing trend possibly due to the release of NH<sub>3</sub> from microbial metabolism (Reyes-Torres et al., 2018), and then fell gradually (Fig. 1c). The highest pH of CM + B, CM + B + N, CM + B + N + S was around 8.99–9.20, which reflected a balance of acid production and ammonia accumulation in the composting piles. The final EC value was observed to be higher than the original value in all treatments (Fig. 1d), which was consistent with the previous studies (Liu et al., 2018). They also fell in the desired range for a mature compost product and unlikely to be phytotoxic (Wang et al., 2020a). Treatment of CM + B also exhibited the highest EC value, increasing from initially 0.55 mS cm<sup>-1</sup> to 2.8 mS cm<sup>-1</sup>. As Fig. 1e and 1f, the contents of TOC and TKN in all treatments declined in the mesophilic phase because of the aerobic degradation of macromolecules and the rapid loss of CO<sub>2</sub> and NH<sub>3</sub>. Then an increase of TOC and TKN was observed in CM and CM + B during thermophilic phase, which may be caused by decomposition of organic matter and N<sub>2</sub> fixation (Zhou et al., 2018). Consequently, C/N ratio declined rapidly in the mesophilic stage of composting and stabilized in the cooling and maturation phase (Fig. 1g). GI value greater than 80% is generally considered an indicator that compost is mature and phytotoxicity-free (Yu et al., 2011; Zeng et al., 2009; Zhang et al., 2018). During composting process, GI in CM and CM + B groups kept increasing and met the standard of mature compost product, while GI in CM + B + N and CM + B + N + S was too low to be satisfactory maybe because of the addition of different nitrogen source compared to the other two groups (Fig. 1h).

### **3.2. Analysis of humic substances**

The fluorescence of organic matter would be influenced by the presence of condensed aromatic rings and/or unsaturated aliphatic carbon chains (Yu et al., 2019). Previous studies revealed that the redshift in the maximum fluorescence intensity could be attributed to an increase in aromatic group condensation in these molecules (Lv et al., 2013; Said-Pullicino et al., 2007). The contours of DOM from the four composting piles (CM, CM + B, CM + B + N, CM + B + N + S) showed five peaks marked as A, B, C, D and E (Fig. 2). Peak D and E ( $\text{Em} < 380 \text{ nm}$ ,  $\text{Ex} < 250 \text{ nm}$ ) in the early composting process represents aromatic proteins. The peak C ( $\text{Em} > 380 \text{ nm}$ ,  $\text{Ex} < 250 \text{ nm}$ ), peak B ( $\text{Em} < 380 \text{ nm}$ ,  $\text{Ex} > 250 \text{ nm}$ ) and peak A ( $\text{Em} > 380 \text{ nm}$ ,  $\text{Ex} > 250 \text{ nm}$ ) are attributed to fulvic acid, water-soluble microbial metabolites and humic acid, respectively. The fluorescence intensity of peak B decreased from thermophilic stage followed by an increase. The fluorescence intensity of peak D and peak E showed a downtrend while the fluorescence intensity of peak A and peak C showed an uptrend. That suggested during the composting process, the primary reactions were the transformation of protein and water-soluble microbial metabolites to fulvic acid and humic acid, especially during mesophilic phase. After that, the tendency gradually shifted to the stabilization of the newly formed humic acid-like and fulvic acid-like organic materials during the curing and mature phases. Compared with the other treatments, CM + B treatment exhibited an extremely higher fluorescence intensity of peak A. That was an indicator of high DOM conversion efficiency during composting process, possibly due to the appropriate nutrient ratio and fast temperature rise in CM + B. The efficient transformation of DOM was also consistent with GI data because humic substances benefit the seed germination.

### 3.3. Similarity and diversity of microbial communities

A total of 31,081 bacterial sequences and 36,761 fungal sequences from all samples were analyzed after quality filtering. Venn diagrams was used to analyze the similarity of the microbial communities in the samples of the four treatments (Fig. 3a&b). Specially, in CM + B there were 1826 bacterial OTUs and 434 fungal OTUs identified respectively, of which 346 and 168 were unique. That indicated the highest diversity of microbial community in CM + B and significant differences from other treatments. A dramatic variation of bacterial community was also in CM + B observed suggested by the sobs index (Fig. S1A), while change of fungal community was expected to be similar in CM + B, CM + B + N and CM + B + N + S (Fig. S1B). Based on the above results, the treatment CM + B was selected for dynamic analysis of microbial community in compost samples.

The similarity of samples at different time points of CM + B composting process was also analyzed by Venn diagram and PCoA. Bacterial diversity increased significantly in the mesophilic phase and gradually decreased in the cooling phase, while fungal diversity decreased continuously as composting progressed (Fig. 3c&d). Samples of day 7 and day 20 exhibited the most unique bacterial OTUs of 544 and 269 respectively. The fungal taxonomic richness was found to be much lower than the bacteria. Only 6 fungal OTUs were identified from fungal OTU libraries, including *Pseudeurotium*, *Mycothermus*, and some other *unclassified\_k\_Fungi*. According to PCoA, the close distance between day 40 and day 60 for both bacteria and fungi indicated the stable status of the microbial community after the cooling phase. The short distance between day 7 and day 20 in the bacterial analysis indicated that the bacterial community had a

significant change in the mesophilic phase (Fig. 3e), while there was an obvious change in the fungal community on day 20 according to the PCoA (Fig. 3f), indicating that the fungal community was significantly influenced by thermophilic phase in the co-composting process.

### 3.4. Bacterial community succession and predicted bacterial functions

At phylum level, a closer observation of the bacterial community revealed eight dominant groups in the four composting systems (Fig. 4a). A similar profile of bacterial communities was observed in CM + B + N and CM + B + N + S group, indicating the insignificant effects of sheeting. During mesophilic phase, CM showed no obvious change of bacterial community, while a dramatic change was observed in CM + B possibly due to the fast elevation of temperature. Firmicutes was the most richness phyla in all these compost samples, which plays a major role in the degradation to lignocellulose similar to previous report (Li et al., 2019). Further observation of microbial community analysis at genus level showed the bacterial community structure in CM + B changed dramatically during co-composting process. *Acinetobacter* (42.5%), *Pseudomonas* (25.57%), and *Psychrobacillus* (8.86%) were the most abundant genera in the original co-compost mixture (Fig. 4b). *Acinetobacter* are ubiquitous in nature and have been found to exist in various environments, including activated sludge, sewage treatment plants and raw wastewater, by different research groups in Australia, Portugal, Korea and Pakistan (Al Atrouni et al., 2016).

*Pseudomonas aeruginosa* is the third most common nosocomial pathogen that is associated with chronic, eventually fatal lung disease in cystic fibrosis (CF) patients, while *Pseudomonas syringae* and *pathovars* are prominent plant pathogens (Goldberg et al., 2008). After 7 days of co-composting, those genera disappeared and *Bacteroides* (19.09%), *Paenibacillus* (5.71%), *Corynebacterium* (5.15%), and *Romboutsia* (4.70%) became the main genera. The relative abundances of *Symbiobacterium*, *Bacillus*, and *Thermoflavimicrobium* increased by 98.7%, 92.1%, and 94.9% from day 7 to day 20. In contrast, *Bacteroides* and *Corynebacterium* declined by 98.7% and 99.2%, respectively, in the thermophilic phase. From day 40 to day 60, *Lysinibacillus* (13.97–16.61%), *Solibacillus* (10.42–13.90%), and *Acinetobacter* (12.12–13.75%) were dominant genera. *Lysinibacillus* can be used as a potential compost inoculant as it can mediate the variation of C/N ratio, change the activities of carbon- and nitrogen-cycling enzymes, and accelerate the composting process (Ganguly & Chakraborty, 2018). It can also act as an indicator of mature product as its dominant abundance only appeared in the maturation phase. *Solibacillus* has a high capacity to degrade lignocelluloses and lignin (Huang et al., 2019). *Acinetobacter harbinensis*, a heterotrophic nitrifying bacterium, has the ability to remove ammonium (Qin et al., 2017). The bacterial community structure of other composting groups in genera level was showed in Fig. S2. A-C.

The metabolic potential of bacterial communities during CM + B composting was evaluated by PICRUSt based on the Clusters of Orthologous Groups (COG) database. As predicted, there were three main functional groups, including metabolism, information storage and processing, and cellular processes and signaling (Fig. 4c). The metabolism group (49.87–52.76%) was the largest of the three groups during co-composting. We noticed that the proportion of the lipid and carbohydrate metabolism group generally increased from day 0 to day 60, which may be related to the high biodegradability of those substrates.

However, amino acid metabolism experienced a significant decline during the first week of composting and then increased in the cooling phase and mature phase. Amino acid metabolism is known to enhance microbial growth and activity since amino acids can serve as sources of both carbon and energy for microbes during composting (Bello et al., 2020). Therefore, the energy production and conversion subsystem were further analysed by PICRUSt based on the KEGG database (Fig. S3A). The oxidative phosphorylation metabolism and carbon fixation pathway were two main categories during the composting of CM + B. The relative abundances of genes involved in carbon fixation exhibited an increase during the thermophilic phase. As for nitrogen metabolism, the functional enzymes that showed significant change were associated with nitrification, denitrification and nitrogen fixation (Fig. S3B). The relative abundance of nitrogenase (*nif*) increased during composting and became the dominant functional enzyme in the thermophilic phase.

### **3.5. Fungal community succession and predicted fungal functions**

For fungal community, six main phyla were detected during co-composting (Fig. 5a). The similarities of fungal community between CM + B and CM + B + N groups indicated that the ratio of cow manure and bedding material possibly have a minor impact at least at phylum level. During the composting of CM + B, Ascomycota made up the greatest proportion of the classified OTUs, and its relative abundance increased from initial value of 47.04–88.05% on day 40 and 90.07% on day 60. At the genus level, the relative abundances of the 20 most abundant classified fungal genera showed obvious variation over the 60-day composting period (Fig. 5b). *Orpinomyces*, which was detected only in the original cow manure, includes anaerobic fungi that inhabit the gastrointestinal tract of mammalian herbivores and cannot survive in the aerobic compost matrix (Zavrel et al., 2013). The thermophilic genus *Mycothermus* was dominant during the composting process, especially on day 40 (97.32%) and day 60 (98.77%). The microbial consortium consisting of the thermophilic fungus *Mycothermus thermophilus* (*Scytalidium thermophilum*) and a range of thermophilic *Proteobacteria* and *Actinobacteria* was primarily responsible to biodegradation of feedstock and release of ammonia (Kertesz & Thai, 2018a). *Candida* and *Aspergillus* were detected in all samples, indicating their high adaptability toward diverse environmental conditions (e.g. temperature, moisture and pH). Both of their relative abundance reached the highest value during thermophilic phase, consistent with their thermophilic features. Under relatively high temperature, they were able to degrade various components of lignocelluloses, promote the formation of precursor substances and thus accelerate the synthesis of humic substances (Huang et al., 2019). The fungal community structure of other composting groups was showed in Fig. S2D-F. The fungi in the co-composting of CM + B system, including the 35 most abundant OTUs, were classified by ecological guild and by trophic mode (Fig. 5c). A neighbor-joining phylogenetic tree was constructed to demonstrate the phylogenetic relationships among the main fungal communities in the CM + B co-composting system which showed that pathotrophs and saprotrophs were the dominant fungal trophic modes in this co-composting system and saprotrophic fungi were the most commonly detected taxa, with 13 OTUs belonging to 11 known genera of Ascomycota (e.g., *Aspergillus* and *Candida*) and Basidiomycota (e.g., *Wallemia*). The wood saprotrophs and many undefined saprotrophs were the groups that showed the most significant changes during the

co-composting. Notably, the relative abundances of the OTUs of wood saprotrophs started at only 3.5% on day 0 but increased quickly, and they became the dominant fungal group (89.1%) in the mature phase. These data confirmed the efficient conversion of cellulose and hemicellulose during the co-composting of CM + B. Animal and plant pathogens were not detected after 60 days of co-composting in this experiment, indicating that the CM + B co-composting of CM + B improved the safety of raw materials as an agricultural fertilizer. High temperature was reported to be the most important factor in the elimination of pathogens during aerobic composting (Duan et al., 2019). Compared to bacteria, fungi was more advantageous on lignocellulose degradation because of their mycelial structure (Wang et al., 2018c). Ascomycota and Basidiomycota are the dominant fungal phyla for lignocellulose degradation, and high abundances of these phyla promote the degradation of organic waste during composting (Jiang et al., 2019). *Mycothermus*, *Penicillium*, and *Aspergillus* were the core functional genera to produce lignocellulose-degrading enzymes during composting (Kertesz & Thai, 2018b). Specifically, *Mycothermus* has been reported to produce thermostable cellulases, hemicellulases and xylanase (Basotra et al., 2016; EFSA Panel on Food Contact Materials et al., 2019; Ma et al., 2017), and act as a key organism in the decomposition of plant materials and plant-derived compounds (Cannon & Kirk, 2007). The thermophilic microbe *Aspergillus* has a high capacity to degrade lignocelluloses and lignin (Huang et al., 2019), and can use malodorous sulfur compounds to reduce odour pollution in composting systems (Liu et al., 2013).

### 3.6. Relationships of bacterial/fungal communities to physicochemical characteristics and microbial metabolism

CCA, RDA and heatmaps were used to describe the relationship between microbial community during CM + B composting process and environmental factors, including temperature, moisture, pH, and C/N (Fig. 6 &S4). All the *p*\_value data was showed in tables in supplementary file (Table. S1-4). CCA showed the dynamics of the genera data for the top 20 bacterial genera in the different phases of co-composting process (Fig. 6a). *Romboutsia*, *Paenibacillus* and *Symbiobacterium* were observed mainly on days 7–20 and were positively correlated with temperature (Fig. S4A). Entering thermophilic phase, *Ruminiclostridium* turned to be the dominant genus and also had a significantly positive relationship with temperature (*p*\_value < 0.05). *Bacteroides*, *Solibacillus* and *bacillus* showed significantly positive relationships with moisture (*p*\_value < 0.05). In contrast, *Lysinibacillus*, *Solibacillus*, and *Thermobacillus* showed negative correlations with moisture and C/N. *Ruminiclostridium* and *Symbiobacterium* had a significantly positive relationship with pH (*p*\_value < 0.05). In addition, the five most abundant genera, including *Acinetobacter* and *Bacillus*, were all positively correlated with pH at 40 and 60 days. Interestingly, the RDA revealed that only four fungal genera showed positive correlations with temperature not significantly (Fig. 6b and Fig. S4B), including *Candida* which was dominant on day 20, and *Pyrenophaetopsis* which was detected only on day 20 (Fig. S4B). Both genera *Penicillium* and *Mortierella* detected mainly in the early phase of co-composting, were significantly related to moisture (*p*\_value < 0.05). *Unclassified\_f\_Neocallimastigaceae* and *unclassified\_p\_Ascomycota* were significantly related to the C/N. In addition, *Aspergillus*, and *Ascochyta* were negatively correlated with pH and temperature.

These results indicated that temperature had less influence on the succession of fungal communities than on the succession of bacterial communities. CCA and RDA revealed the dominant bacterial/fungal genera in the original cow manure co-compost mixture was affected mainly by moisture and C/N. Thermophilic genera became dominant producers with increasing temperature. PH was a key factor affecting the bacterial community structure during the curing and mature phases of cow manure co-composting.

## 4. Conclusion

This work designed a co-composting system to deal with diverse kinds of agricultural waste simultaneously. Recycled cow manure and bedding material waste were used as feedstock at an optimal mass ratio of 1.32:1. With the addition of bedding material waste, the temperature of composting pile rose quickly and achieved efficient degradation of organic matters. The EEM spectroscopy analysis indicated that co-composting helped convert degradable organic components in raw materials into humus-like substances, which increased the productivity of compost products and enhanced the value for agricultural and landscaping applications. Bacterial and fungal community diversity changed significantly during co-composting. High-throughput 16S rRNA/ITS gene sequencing indicated that the dominant phyla included Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria of bacteria and Ascomycota of fungal. *Lysinibacillus* and *Mycothermus* were the indicators of bacterial and fungal community in the maturation phase, respectively. The carbohydrate and lipid utilization capacity as well as the metabolic diversity within the bacterial community displayed an increase in this co-composting process, and animal and plant pathogens were not detected after co-composting. *Ruminiclostridium* had a significantly positive relationship with temperature ( $p\_value < 0.05$ ), while pH and C/N had significant effect on the fungal ( $p\_value < 0.05$ ). The relative abundances of the OTUs of wood saprotrophs increased quickly and became the dominant fungal group (89.1%) in the mature phase. The results proved the effectiveness of co-composting as a method for treatment of diverse agricultural and livestock industry waste.

## Declarations

### Acknowledgments

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### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing Interests

The authors declare that they have no competing interests.

### Authorship contribution

Conceptualization, JS., B.Z. X.L. and H.D.; methodology, H.D; software, H.D.; validation, H.D, J.S., and B.Z.; formal analysis, H.D., M.J., A.C.; investigation, M.J., J.S., Y.X., and A.C.; resources, J.S. and H.D.; data curation, H.D.; writing-original draft preparation, H.D.; writing-review and editing, J.S., B.Z. and X.L.; visualization, J.S.; supervision, J.S. and B.Z.; project administration, J.S. and H.D.; funding acquisition, J.S., B.Z., and J.S. All authors have read and agreed to the published version of the manuscript.

## References

1. Al Atrouni A, Joly-Guillou M-L, Hamze M, Kempf M (2016) Reservoirs of non-baumannii Acinetobacter species. *Front. Microbiol* 7: 49.
2. Alwaneen WS (2016) Cow Manure Composting by Microbial Treatment for Using as Potting Material: An Overview. *Pak J Biol Sci* 19(1): 1-10.
3. Atencio JL, Fernandez JA, Gernat AG, Murillo JG (2010) Effect of Pine Wood Shavings, Rice Hulls and River Bed Sand on Broiler Productivity When Used as a Litter Sources. *Int J Poult Sci* 9(3): 240-243.
4. Awasthi MK, Chen H, Wang Q, Liu T, Duan Y, Awasthi SK, Ren X, Tu Z, Li J, Zhao J, Zhang, Z (2018) Succession of bacteria diversity in the poultry manure composted mixed with clay: Studies upon its dynamics and associations with physicochemical and gaseous parameters. *Bioresour Technol* 267: 618-625.
5. Basotra N, Kaur B, Di Falco M, Tsang A, Chadha BS (2016) *Mycothermus thermophilus* (Syn. *Scytalidium thermophilum*): Repertoire of a diverse array of efficient cellulases and hemicellulases in the secretome revealed. *Bioresour Technol* 222: 413-421.
6. Bello A, Han Y, Zhu H, Deng L, Yang W, Meng Q, Sun Y, Egbeagu UU, Sheng S, Wu X, Jiang X, Xu X (2020) Microbial community composition, co-occurrence network pattern and nitrogen transformation genera response to biochar addition in cattle manure-maize straw composting. *Sci Total Environ* 721: 137759.
7. Black RA, Taraba JL, Day GB, Damasceno FA, Bewley JM (2013) Compost bedded pack dairy barn management, performance, and producer satisfaction. *J Dairy Sci* 96(12): 8060-8074.
8. Cannon PF, Kirk PM (2007) Fungal families of the world. Cabi.

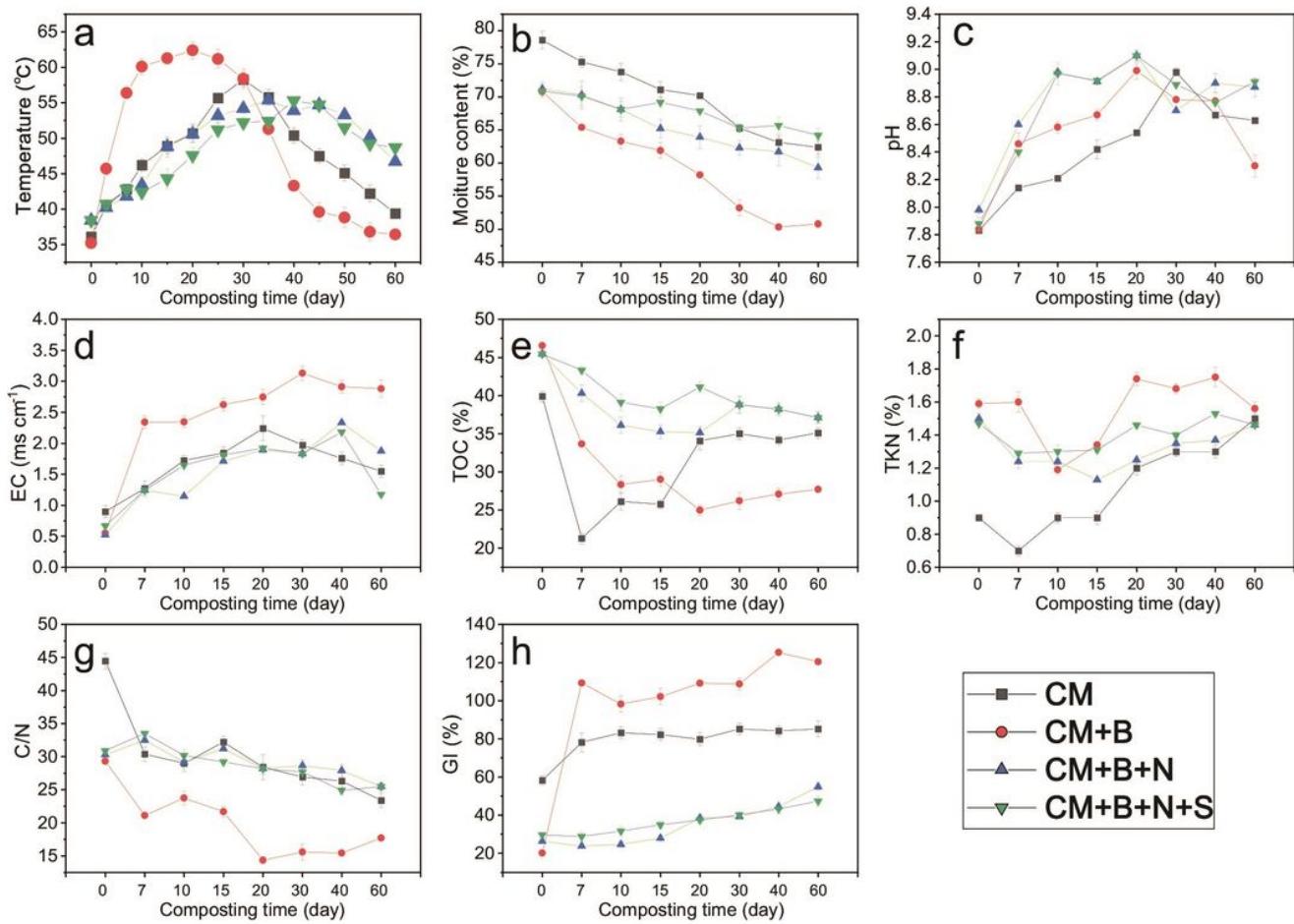
9. Chang R, Yao Y, Cao W, Wang J, Wang X, Chen Q (2019) Effects of composting and carbon based materials on carbon and nitrogen loss in the arable land utilization of cow manure and corn stalks. *J Environ Manage* 233: 283-290.
10. del Carmen Portillo M, Mas A (2016) Analysis of microbial diversity and dynamics during wine fermentation of Grenache grape variety by high-throughput barcoding sequencing. *LWT-Food Science and Technology* 72: 317-321.
11. Duan Y, Awasthi SK, Liu T, Chen H, Zhang Z, Wang Q, Ren X, Tu Z, Awasthi MK, Taherzadeh MJ (2019) Dynamics of fungal diversity and interactions with environmental elements in response to wheat straw biochar amended poultry manure composting. *Bioresour Technol* 274: 410-417.
12. EFSA Panel on Food Contact Materials, E, Aids P, Silano V, Barat Baviera JM, Bolognesi C, Brüschweiler BJ, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E (2019) Safety evaluation of the food enzyme  $\beta$ -glucanase, xylanase and cellulase from *Mycothermus thermophiloides* (strain NZYM-ST). *EFSA J* 17(3): e05631.
13. Esmaeili A, Khoram MR, Gholami M, Eslami H (2020) Pistachio waste management using combined composting-vermicomposting technique: Physico-chemical changes and worm growth analysis. *J. Clean Prod*: 242.
14. Fournel S, Godbout S, Ruel P, Fortin A, Duquette-Lozeau K, Letourneau V, Genereux M, Lemieux J, Potvin D, Cote C, Duchaine C, Pellerin D (2019) Production of recycled manure solids for use as bedding in Canadian dairy farms: II. Composting methods. *J Dairy Sci* 102(2): 1847-1865.
15. Ganguly RK, Chakraborty SK (2018) Assessment of microbial roles in the bioconversion of paper mill sludge through vermicomposting. *J Environ Health Sci Eng* 16(2): 205-212.
16. Goldberg JB, Hancock RE, Parales RE, Loper J, Cornelis P (2008) *Pseudomonas* 2007. *J Bacteriol* 190(8): 2649-2662.
17. Guo A, Zhao Z, Zhang P, Yang Q, Li Y, Wang G (2019) Linkage between soil nutrient and microbial characteristic in an opencast mine, China. *Sci Total Environ* 671: 905-913.
18. Guo R, Li G, Jiang T, Schuchardt F, Chen T, Zhao Y, Shen Y (2012) Effect of aeration rate, C/N ratio and moisture content on the stability and maturity of compost. *Bioresour Technol* 112: 171-178.
19. He X, Xi B, Wei Z, Guo X, Li M, An D, Liu H (2011) Spectroscopic characterization of water extractable organic matter during composting of municipal solid waste. *Chemosphere* 82(4): 541-548.
20. Huang Y, L D, Shah GM, Chen W, Wang W, Xu Y, Huang H (2019) Hyperthermophilic pretreatment composting significantly accelerates humic substances formation by regulating precursors production and microbial communities. *Waste Manag* 92: 89-96.
21. Husfeldt AW, Endres MI, Salfer JA, Janni KA (2012) Management and characteristics of recycled manure solids used for bedding in Midwest freestall dairy herds. *J Dairy Sci* 95(4): 2195-2203.
22. Jeong KH, Kim JK, Ravindran B, Lee DJ, Wong JW, Selvam A, Karthikeyan OP, Kwag JH (2017) Evaluation of pilot-scale in-vessel composting for Hanwoo manure management. *Bioresour Technol* 245(Pt A): 201-206.

23. Jiang Z, Lu Y, Xu J, Li M, Shan G, Li Q (2019) Exploring the characteristics of dissolved organic matter and succession of bacterial community during composting. *Bioresour Technol* 292: 121942.
24. Kebibeche H, Khelil O, Kacem M, Kaid Harche M (2019) Addition of wood sawdust during the co-composting of sewage sludge and wheat straw influences seeds germination. *Ecotoxicol Environ Saf* 168: 423-430.
25. Kertesz MA, Thai M (2018a) Compost bacteria and fungi that influence growth and development of *Agaricus bisporus* and other commercial mushrooms. *Appl. Microbiol Biotechnol* 102(4): 1639-1650.
26. Kertesz MA, Thai M (2018a) Compost bacteria and fungi that influence growth and development of *Agaricus bisporus* and other commercial mushrooms. *Appl. Microbiol Biotechnol* 102(4): 1639-1650.
27. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, Beiko RG, Huttenhower C (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol* 31(9): 814-821.
28. Leso L, Barbari M, Lopes MA, Damasceno FA, Galama P, Taraba JL, Kuipers A (2020) Invited review: Compost-bedded pack barns for dairy cows. *J Dairy Sci* 103(2): 1072-1099.
29. Li J, Xing W, Bao H, Wang J, Tong X, Zhang H, Luo W, Wu F (2019) Impact of pine leaf biochar amendment on bacterial dynamics and correlation of environmental factors during pig manure composting. *Bioresour Technol* 293: 122031.
30. Li Y, Zhao J, Achinas S, Zhang Z, Krooneman J, Euverink GJW (2020) The biomethanation of cow manure in a continuous anaerobic digester can be boosted via a bioaugmentation culture containing Bathyarchaeota. *Sci Total Environ* 745: 141042.
31. Liu C, Liu J, Li J, He H, Peng S, Li C, Chen Y (2013) Removal of H<sub>2</sub>S by co-immobilized bacteria and fungi biocatalysts in a bio-trickling filter. *Process Saf. Environ. Prot* 91(1-2): 145-152.
32. Liu L, Wang S, Guo X, Zhao T, Zhang B (2018) Succession and diversity of microorganisms and their association with physicochemical properties during green waste thermophilic composting. *Waste Manag* 73: 101-112.
33. Lv B, Xing M, Yang J, Qi W, Lu Y (2013) Chemical and spectroscopic characterization of water extractable organic matter during vermicomposting of cattle dung. *Bioresour Technol* 132: 320-326.
34. Ma R, Bai Y, Huang H, Luo H, Chen S, Fan Y, Cai L, Yao B (2017) Utility of thermostable xylanases of *Mycothermus thermophilus* in generating prebiotic xylooligosaccharides. *J. Agric. Food Chemistry* 65(6): 1139-1145.
35. Mierzwa-Hersztek M, Glen-Karolczyk K, Gondek K (2018) Fungistatic activity of composts with the addition of polymers obtained from thermoplastic corn starch and polyethylene - An innovative cleaner production alternative. *Sci Total Environ* 635: 1063-1075.
36. Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG (2016) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol* 20: 241-248.
37. Organization, WH (2017) WHO report on the global tobacco epidemic, 2017: monitoring tobacco use and prevention policies. World Health Organization.

38. Qin W, Li WG, Gong XJ, Huang XF, Fan WB, Zhang D, Yao P, Wang XJ, Song Y (2017) Seasonal-related effects on ammonium removal in activated carbon filter biologically enhanced by heterotrophic nitrifying bacteria for drinking water treatment. *Environ Sci Pollut Res Int* 24(24): 19569-19582.
39. Ren L, Schuchardt F, Shen Y, Li G, Li C (2010) Impact of struvite crystallization on nitrogen losses during composting of pig manure and cornstalk. *Waste Manag* 30(5): 885-892.
40. Reyes-Torres M, Oviedo-Ocana ER, Dominguez I, Komilis D, Sanchez A (2018) A systematic review on the composting of green waste: Feedstock quality and optimization strategies. *Waste Manag* 77: 486-499.
41. Safferman SI, Wallace JM (2015) Cow Manure: Waste or Resource? *IEEE Potentials* 34(1): 25-29.
42. Said-Pullicino D, Erriquens FG, Gigliotti G (2007) Changes in the chemical characteristics of water-extractable organic matter during composting and their influence on compost stability and maturity. *Bioresour Technol* 98(9): 1822-31.
43. Shen J, Treu R, Wang J, Thorman R, Nicholson F, Bhogal A (2018) Modeling nitrous oxide emissions from three United Kingdom farms following application of farmyard manure and green compost. *Sci Total Environ* 637-638: 1566-1577.
44. Song SM, Mu J, Hu WL, Xiao PG (2016) Unearthing microbial diversity of *Taxus* rhizosphere via MiSeq high-throughput amplicon sequencing and isolate characterization. *Sci Rep* 6: 22006.
45. Wang K, Chu C, Li X, Wang W, Ren N (2018a) Succession of bacterial community function in cow manure composting. *Bioresour Technol* 267: 63-70.
46. Wang K, Li W, Gong X, Li Y, Wu C, Ren N (2013) Spectral study of dissolved organic matter in biosolid during the composting process using inorganic bulking agent: UV-vis, GPC, FTIR and EEM. *Int. Biodeterior. & Biodegr* 85: 617-623.
47. Wang K, Mao H, Li X (2018b) Functional characteristics and influence factors of microbial community in sewage sludge composting with inorganic bulking agent. *Bioresour Technol* 249: 527-535.
48. Wang K, Yin X, Mao H, Chu C, Tian Y (2018c) Changes in structure and function of fungal community in cow manure composting. *Bioresour Technol* 255: 123-130.
49. Wang W, Zhang L, Sun X (2020a) Improvement of two-stage composting of green waste by addition of eggshell waste and rice husks. *Bioresour Technol* 320(Pt B): 124388.
50. Wang Y, Gong J, Li J, Xin Y, Hao Z, Chen C, Li H, Wang B, Ding M, Li W, Zhang Z, Xu P, Xu T, Ding GC, Li J (2020b) Insights into bacterial diversity in compost: Core microbiome and prevalence of potential pathogenic bacteria. *Sci Total Environ* 718: 137304.
51. Wolfe T, Vasseur E, DeVries TJ, Bergeron R (2018) Effects of alternative deep bedding options on dairy cow preference, lying behavior, cleanliness, and teat end contamination. *J Dairy Sci* 101(1): 530-536.
52. Xu J, Jiang Z, Li M, Li Q (2019) A compost-derived thermophilic microbial consortium enhances the humification process and alters the microbial diversity during composting. *J Environ Manage* 243: 240-249.

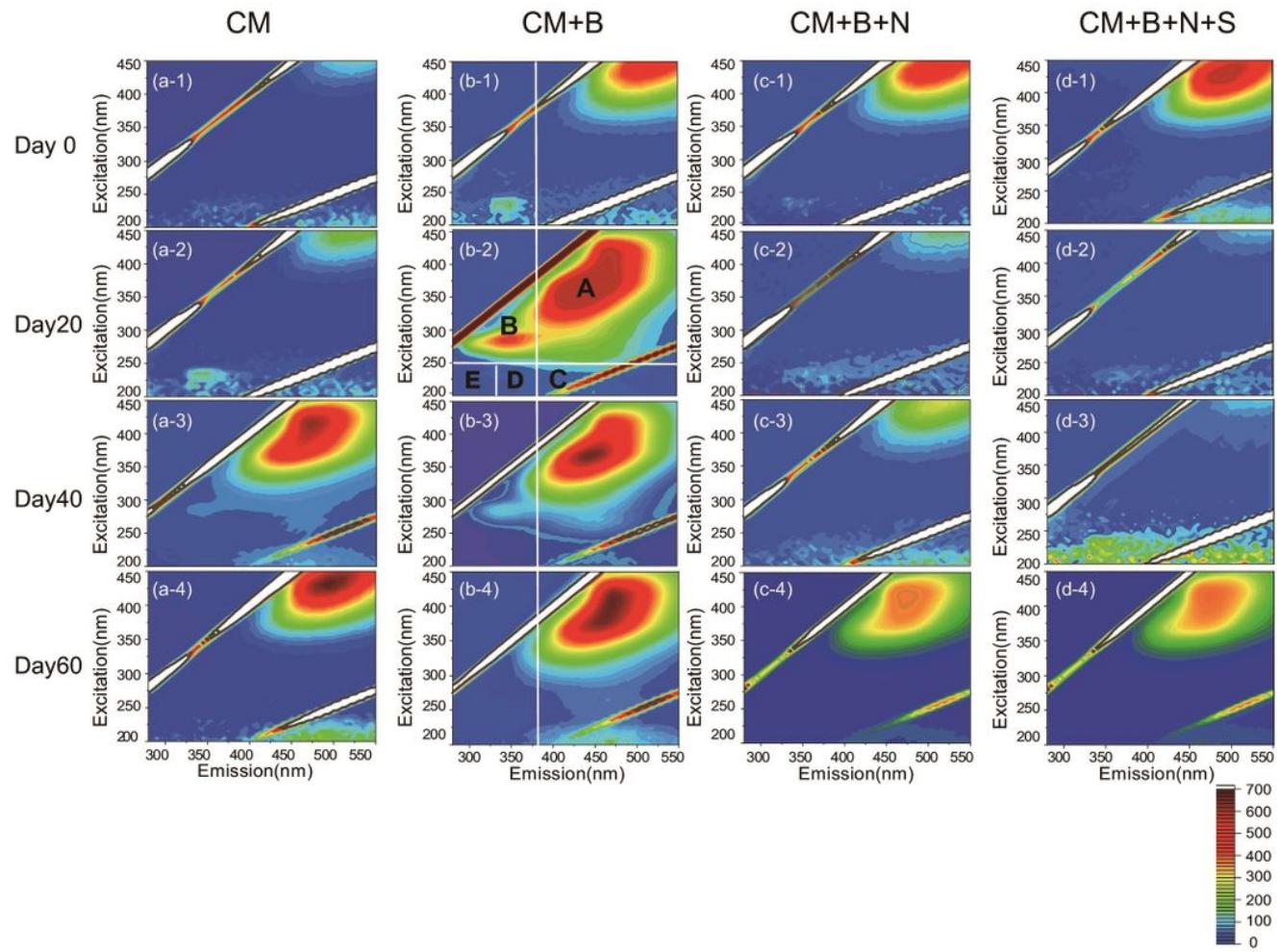
53. Yu Z, Liu X, Zhao M, Zhao W, Liu J, Tang J, Liao H, Chen Z, Zhou S (2019) Hyperthermophilic composting accelerates the humification process of sewage sludge: molecular characterization of dissolved organic matter using EEM–PARAFAC and two-dimensional correlation spectroscopy. *Bioresour Technol* 274: 198-206.
54. Yu Z, Zeng G-M, Chen Y-N, Zhang J-C, Yu Y, Li H, Liu Z-F, Tang L (2011) Effects of inoculation with *Phanerochaete chrysosporium* on remediation of pentachlorophenol-contaminated soil waste by composting. *Process Biochem* 46: 1285-1291.
55. Zavrel M, Hoot SJ, White TC (2013) Comparison of sterol import under aerobic and anaerobic conditions in three fungal species, *Candida albicans*, *Candida glabrata*, and *Saccharomyces cerevisiae*. *Eukaryot Cell* 12(5): 725-38.
56. Zeng GM, Huang HL, Huang DL, Yuan XZ, Jiang RQ, Yu M, Yu HY, Zhang JC, Wang RY, Liu XL (2009). Effect of inoculating white-rot fungus during different phases on the compost maturity of agricultural wastes *Process Biochem* 44(4): 396-400.
57. Zhang C, Gao Z, Shi W, Li L, Tian R, Huang J, Lin R, Wang B, Zhou B (2020) Material conversion, microbial community composition and metabolic functional succession during green soybean hull composting. *Bioresour Technol* 316: 123823.
58. Zhang D, Luo W, Li Y, Wang G, Li G (2018) Performance of co-composting sewage sludge and organic fraction of municipal solid waste at different proportions. *Bioresour Technol* 250: 853-859.
59. Zhou H, Zhao Y, Yang H, Zhu L, Cai B, Luo S, Cao J, Wei Z (2018) Transformation of organic nitrogen fractions with different molecular weights during different organic wastes composting. *Bioresour Technol* 262: 221-228.

## Figures



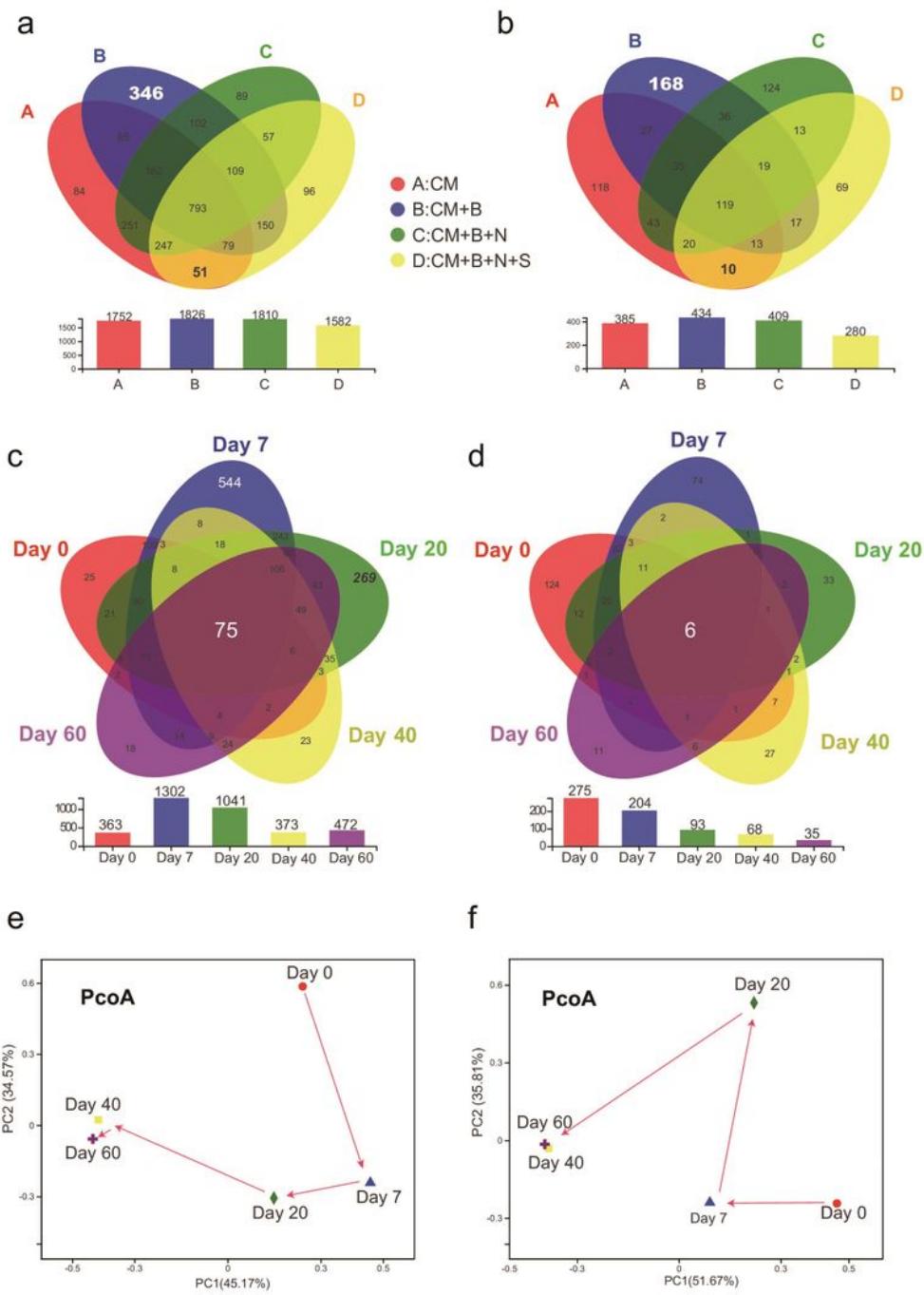
**Figure 1**

Changes in physicochemical parameters during the composting process of four treatments. (a) Temperature, (b) Moisture, (c) pH, (d) electrical conductivity (EC), (e) Total organic carbon (TOC) concentration, (f) Total Kjeldahl nitrogen (TKN) concentration, (g) carbon nitrogen ratio (C/N), and (h) germination index (GI).



**Figure 2**

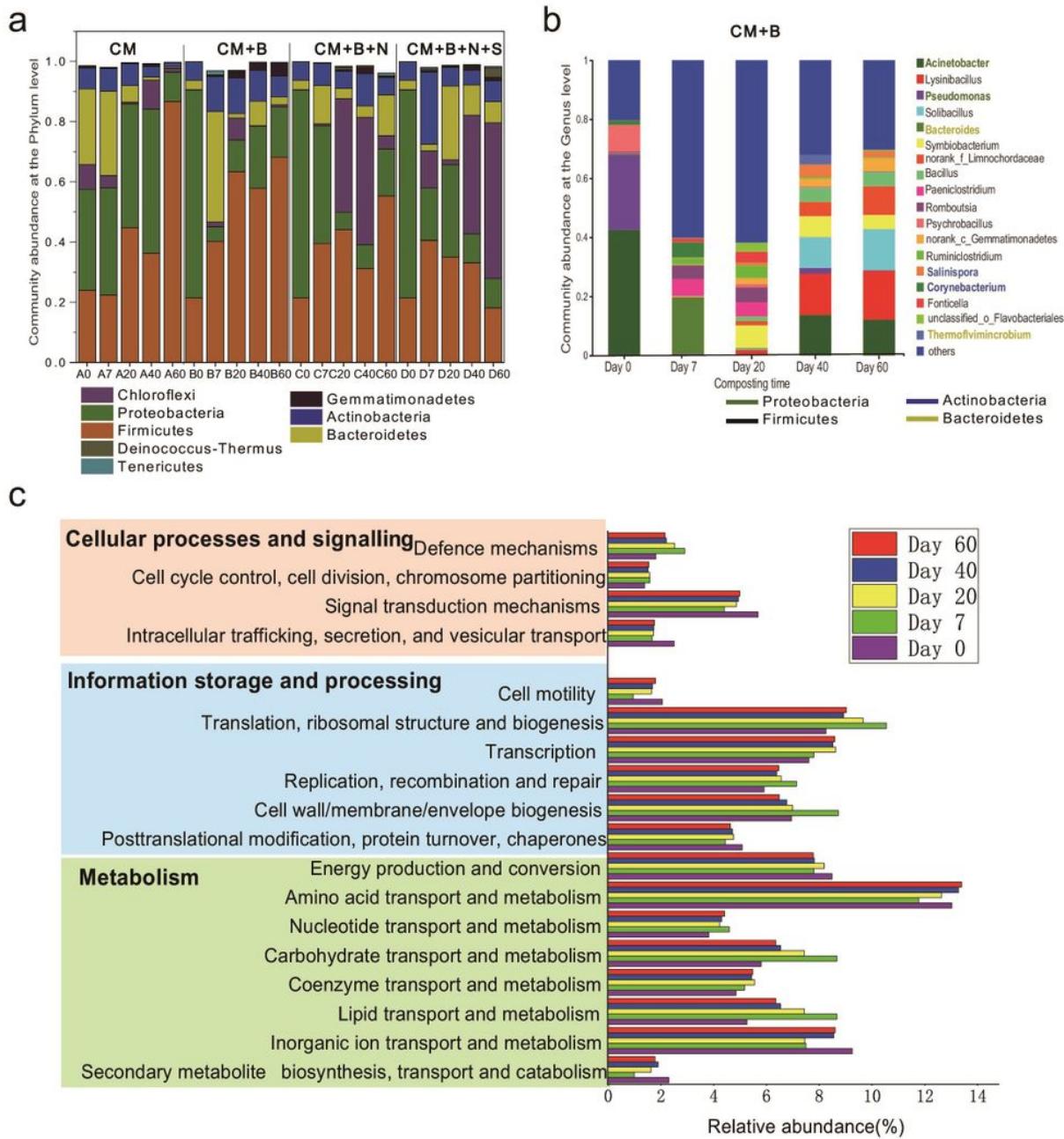
Excitation-emission matrix (EEM) fluorescence spectra of DOM extracted from biosolids in the composting process of four treatments. Region A: humic acid-like substances. Region B: soluble microbial by-products; Region C: fulvic acid-like substances; Regions D and E: simple aromatic proteins.



**Figure 3**

Similarity analysis of microbial communities in the co-composting of cow manure at the different periods: (a) Venn diagram of bacteria OTUs; (b) Venn diagram of fungal OTUs. (c) PcoA similarity analysis of bacteria community; (d) PcoA similarity analysis of fungal community. Similarity analysis of microbial communities in the different treatments of cow manure. A. Venn diagram of bacteria OTUs of four treatments; B. Venn diagram of fungal OTUs of four treatments; C. Venn diagram of bacteria OTUs in

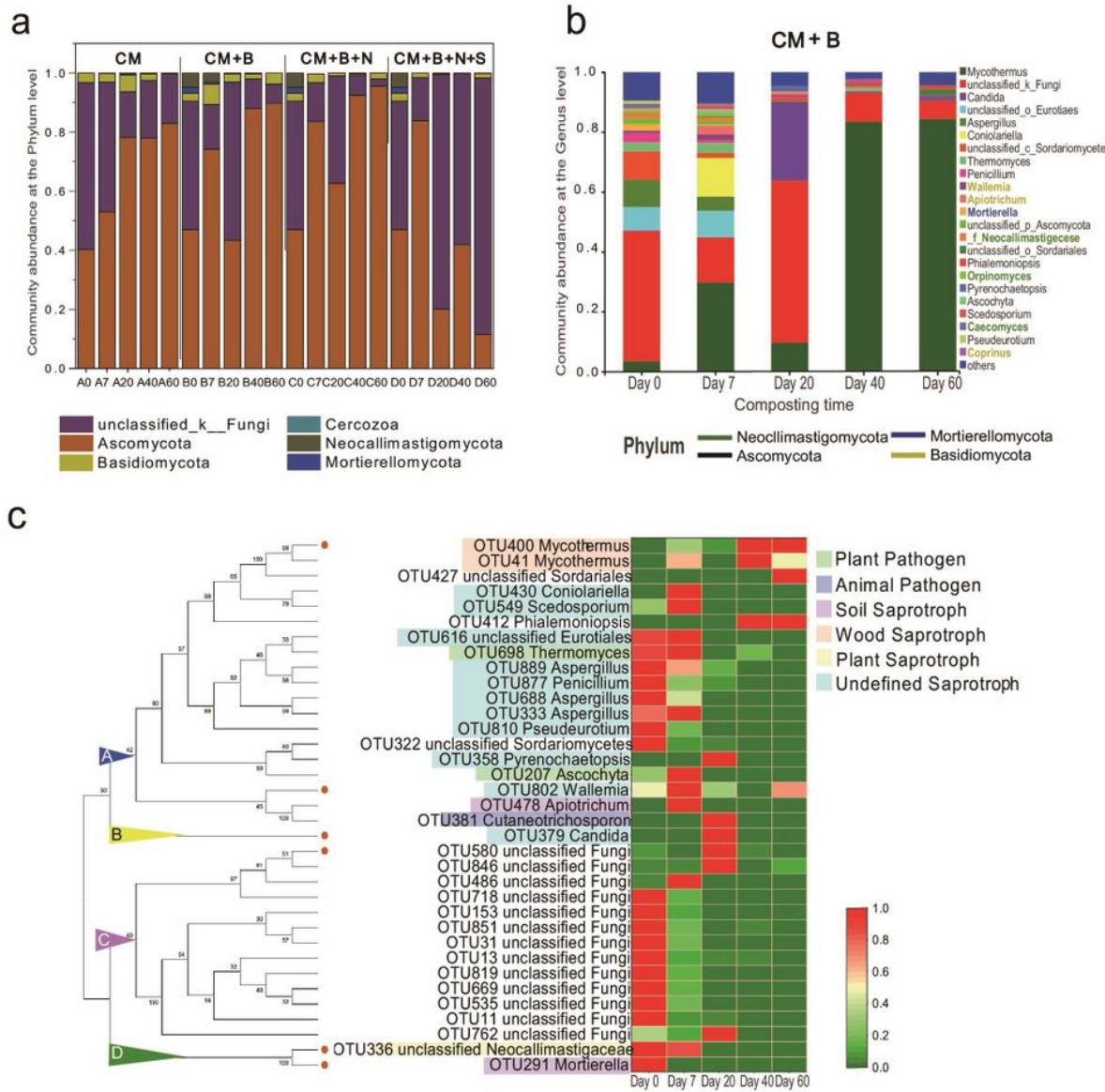
the co-composting of CM+B; D. Venn diagram of fungal OTUs in the co-composting of CM+B; (e) PcoA similarity analysis of bacteria community in the co-composting of CM+B; (f) PcoA similarity analysis of fungal community in the co-composting of CM+B.



**Figure 4**

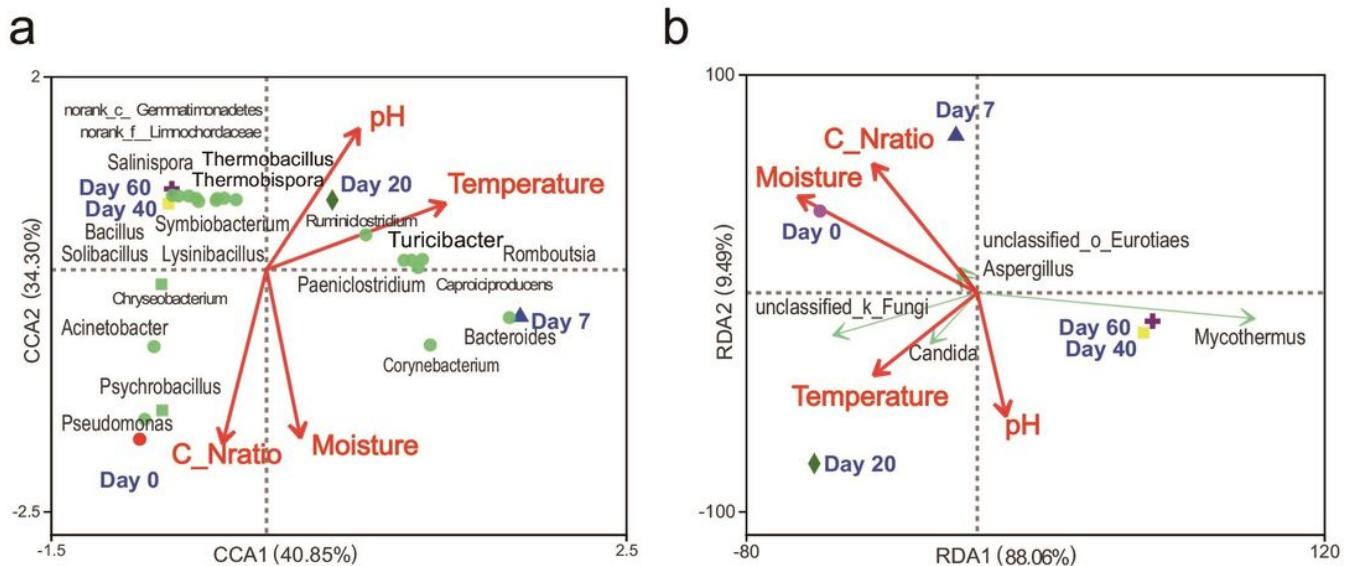
Succession of bacterial community composition in four treatments of cow manure. (a) Change of bacteria community composition at phyla level in four treatments; (b) Change of bacteria community composition at genera level in the co-composting of cow manure and bedding materials (CM+B); (c)

Predicted COG database using PICRUSt method in the co-composting of cow manure and bedding materials (CM+B).



**Figure 5**

Succession of fungal community composition in four treatments of cow manure. (a) Change of fungal community composition at phyla level in four treatments; (b) Change of fungal community composition at genera level in the co-composting of cow manure and bedding materials (CM+B); (c) Phylogram and fungal guilds of top 35 fungal species in the co-composting of cow manure and bedding materials (CM+B).



**Figure 6**

Relationship between microbial community composition and environmental factors in the co-composting of cow manure and bedding materials at ratio (CM+B). (a) Canonical Correlation Analysis of environment factors, composting periods and the top 20 bacterial genera in (CM+B) co-composting periods; (b) Redundancy Analysis of environment factors and the top 5 fungal genera in (CM+B) co-composting periods.

## Supplementary Files

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