

Mechanism That Allows Manno-Oligosaccharide To Promote Cellulose Degradation By Microbes And Composting of Cow Manure With Straw

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Abstract

New sugar source, manno-oligosaccharide, can regulate the structure of microbes. The effects of adding manno-oligosaccharide at four different levels (0, 0.1%, 0.5%, and 1% w/w compost) to cow manure–straw compost on the composting process, degradation of lignocellulose, and bacterial community in this study was investigated. The results showed that adding 0.5% manno-oligosaccharide had the greatest effects on accelerating the composting process, reducing the toxicity, and improving the stability of the product. After composting for 25 days, 0.5% oligosaccharide treatment can decrease the hemicellulose, cellulose, and lignin contents to 2.25%, 11.25%, and 7.07% respectively, compared with CK. Manno-oligosaccharide affected the distribution of the bacterial community, inhibited pathogenic bacteria, and improved the abundance of functional genes. Finally, adding 0.5% manno-oligosaccharide mainly affected the degradation of lignocellulose by enhancing the C/N value and the abundances of *Streptomyces* and the secretion system during composting was demonstrated by redundancy analysis.

Introduction

The manure is an inevitable by-product of the domestic animal breeding industry, the cow manure output, by-product of cow farming in the line with the report, is 13.8 billion tons in China every year (Ge et al., 2020). Considering the probable risk of cow manure bring, the aerobic composting technology is urgently needed to control the pollution efficiently, and the composting product can be regarded as a soil amendment with ecological benefits (Zhao et al., 2017; Kulcu and Yaldiz, 2004). However, cattle manure is not suitable for composting alone because of its high nitrogen content and poor air permeability, and thus a carbon conditioner must be added to improve the C/N ratio and air permeability. Common conditioners such as wheat straw have advantages such as a high carbon content and low cost; however, wheat straw mainly comprises lignocellulose (hemicellulose, cellulose, and lignin), which has a complex structure. Lignin is an essential component of the cell wall that can resist microbial degradation, thereby requiring a long composting cycle and low degree of humification (Kluczek-Turpeinen et al., 2003). Microorganisms are mainly responsible for biodegradation of organic matter (Zhao et al., 2017). Therefore, it is important to improve the structure of the microbial community, speed up the degradation of lignocellulose and shortening the period of composting.

The degradation of compost materials is mainly driven by microorganisms (bacteria, fungi) and their metabolism (Wei et al., 2019; Gao et al., 2019). Bacteria are highly adaptable in terms of their metabolism and the lignocellulose-degrading enzymes secreted by bacteria are resistant to high temperatures (Harindintwali et al., 2020). The research found some aerobic bacteria species can degraded cellulose such as *Pseudomonas*, *Klebsiella*, and *Bacillus* are the important decomposers (Rainey et al., 1994; Bredholt et al., 2009; Lynd et al., 2002). Therefore, many scholars improve the bacterial community structure by inoculating bacterial strains. However, inoculating specific strains will inevitably lead to competition between the inoculated and indigenous bacteria to disrupt the benign structure of the microbial community in the compost, which is not conducive to the preservation of nutrients and improving the quality of the compost product. In addition, chemical additives can have positive effects on

remodeling the structure of the bacterial community. For example, nanohematite can increase the abundances of bacteria to 1.24–1.58 times that in the control. Surfactants (rhamnolipid and sucrose esters) can also improve the abundances of bacteria and the capacity to metabolize carbohydrates or amino acids, thereby accelerating the bacterial degradation of cellulose and lignin (Zhang et al., 2019; Yin et al., 2019; Li et al., 2020). However, determining changes in the bacterial community is not sufficient to understand the organic matter degradation process during composting, where bacterial functional metabolism fundamentally affects the properties of compost (Duan et al., 2020). Therefore, identifying a suitable additive that can reshape the bacterial community structure and strengthen the biodegradation functions of bacteria is a feasible method for improving the aerobic composting of cow manure–straw.

Sugars are excellent sources of carbon and energy for microbes. Adding sugars or carbon sources, such as sugarcane molasses and sugar beet pulp, could improve the microbial population richness (Zhu et al., 2020b; Zhang et al., 2018). In particular, oligosaccharides are new sugar sources that are widely used in food, medicine, agriculture, and other fields. Different from common sugar sources, the oligosaccharides can significantly increase the numbers of beneficial bacteria in intestinal, inhibit the spread of harmful bacteria (Ma et al., 2020), and play the role of water retention because of their highly hydrophilic properties (Song et al., 2018). Manno-oligosaccharide (MO) is a kind of functional oligosaccharides. that are readily utilized by *Bacillus subtilis* and *Bifidobacterium*. MO and its hydrolytic derivatives can selectively regulate the structure of beneficial microorganisms, such as *Bacillus subtilis* and *Bifidobacterium* (Fu et al., 2020), and effectively inhibit pathogen growth in intestinal (Ismail et al., 2019). In addition, MO can improve the metabolic pathways of *Clostridium butyricum*, and then reduce the pH during the fermentation of manure (Wei et al., 2020), and produce laccases that can degrade lignin (Zang et al., 2015). However, the possible roles of MO as regulators of microbial flora and their effects on the microbial community structure and microbial functional diversity during composting have not been reported previously.

The present study investigated the effects of adding MO to cow manure–straw during aerobic composting on the composting process and the degradation of cellulose. The mechanism that might allow MO to affect the bacterial community structure and functional metabolism during composting was investigated, and the optimal amount of added MO was determined. The aim of this study was to provide a theoretical basis and technical support for agricultural waste treatment and sustainable development of the ecological environment.

Materials And Methods

2.1. Experimental design and sample collection

The fresh cow manure and straw in raw compost materials were collected from Shaanxi Qinbao Animal Husbandry Co. Ltd and a local farm in Yangling, China respectively. The cow manure and straw were determined after air drying and crushing, and the basic characteristics were shown in Table 1. The MO applied in the experiment was purchased from China Wanbang Industrial Co. Ltd.

Table 1
Basic characteristics of raw materials for aerobic composting.

Materials	TOC (g/kg)	TN (g/kg)	pH	MC (%)	CC (%)	HC (%)	LC (%)
cow manure	292	16.7	8.41	7.52	33.9	26.8	10.5
straw	414	6.9	7.61	5.88	38.0	28.5	0.4

Note: TOC: Total organic carbon. TN: Total nitrogen. MC: Moisture content. CC: Cellulose content. HC: Hemicellulose content. LC: Lignin content

The composting experiment was carried out in the State Key Laboratory of Eco-hydraulics in Northwest Arid Region of China, Xi'an University of Technology from July 11, 2020 to August 6, 2020. In the composting, each treatment was mixed into cow manure (4kg), straw (2kg) and deionized water (3.9L) to give the C: N = 25:1 and the moisture content = 65%. The experiment consists of four treatment. Without MO (CK), inoculated 0.1% (dry weight of compost) MO (O1), inoculated 0.5% (dry weight of compost) MO (O2), inoculated 1.0% (dry weight of compost) MO (O3). Raw materials of O1, O2 and O3 treatment were uniformly mixed with MO respectively. Aerobic composting tests were carried out in four insulated foam boxes (0.55m × 0.50 m base with 0.45 m high, 0.03m thickness), which had two circular holes (0.022 × 0.02 m) in the top, bottom, and four walls of the foam boxes to ensure ventilation and the supply of oxygen.

According to the temperature change, the whole composting experiment lasted for 25 days. Representative samples of each treatments were taken on days 0, 1, 2, 6, 13, 25, including 6 stages: initial phase, mesophilic phase, thermophilic phase, cooling phase, primary maturation phase and secondary maturation phase. Each sample (900g) is a mixture of three subsamples (300g) at different depths of the compost heap and was divided in two parts. A portion of the sample was air dried to analyze its physicochemical properties, and another portion of the sample was stored at – 80°C for experiment pH, GI (seed germination index), TOC (total organic carbon), TN (total nitrogen), WSC (water-soluble carbon) and extract DNA.

2.2. Physicochemical properties

The temperature of surrounding environment and composting at three points were measured at 9:00 and 18:00 daily. The moisture content of the composting sample was determined according to the weight loss after drying in a 105°C oven for 24h. The pH, GI, TOC, WSC and C/N were determined according to the previous research methods (Duan et al., 2020). The lignocellulose contents, including cellulose, hemicellulose and lignin, were determined using the method described by **Van Soest. (1991)** with a cellulose tester (Fibertec 2010, FOSS, Denmark). The hemicellulose, cellulose, and lignin contents were expressed as percentages of the initial total matrix.

2.3. DNA extraction and prediction of functional gene profiles

DNA was extracted according to the manufacturer's instructions (MP Biomedical, USA). PCR amplification of bacterial 16S rRNA genes V4 hypervariable region was performed using the universal primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT). Then the hypervariable region was sequenced and the raw data obtained by sequencing were spliced and filtered to obtain clean data. The high-quality sequences were clustered into Operational Taxonomic Units (OTUs) at 97% sequence identity by UPARSE software. The sequences with the highest occurrence frequency in OTUs were selected as the representative sequences of OTUs, and species annotation was made for each OTU sequence to obtain the corresponding species information and specie-based abundance distribution. Qiime software (Version 1.9.1) was used to calculate the index of Observed-otus, Chao1, Shannon, Simpson, ace, Goods-coverage, PD whole tree.

2.4. Data analysis

Microsoft Excel 2018 was used to conduct statistical analyses of the data. SPSS 22.0 was employed to calculate the standard errors and to test for significance differences at $P < 0.05$. Sigma Plot 14.0 and TB tools were used to draw the graphics. Redundancy analysis (RDA) by Canoco 5.0.

Results And Discussion

3.1. Physicochemical properties of compost

Table 2 shows the changes in the physicochemical properties of the compost during different stages. The temperatures in all treatments increased to their highest levels ($> 60^{\circ}\text{C}$) on day 2. The reason for this result might be that microorganisms prioritized to use the easily degradable organic components. The thermophilic phase in O2 treatment had the highest temperature, which was consistent with **Zhu (2020b)** on the impact of sugarcane molasses on the composting temperature, indicating that nutrient-regulating substances could effectively increase the temperature in thermophilic phase. However, the duration of high temperature for all treatments in this study was relatively short, which could be related to the size of the compost (Meng et al., 2020). The pH increased at the beginning of the composting and then decreased. The increase in the pH might have related to the mineralization of nitrogen-containing organic matter. And a large amount of nitrogen was released from the compost in the form of ammonium nitrogen (Yang et al., 2019). As the composting process continued, ammonia volatilization and nitrification increased, and inorganic acids and organic acids were produced, and thus the presence of H^+ made the pH decrease gradually before it then tended to stabilize (Zhang et al., 2017). At the end of the composting process, the pH value decreased most significantly in the O2 treatment compared with the other treatments ($P < 0.05$).

Table 2

Changes in physicochemical properties (temperature, pH, C/N ratio (total organic carbon/total nitrogen), and seed germination index (GI)) under different treatments during composting (n = 3).

Treatment		Temperature (°C)	pH	C/N	GI (%)
Day 0	CK	30.0 ± 1.2a	7.79 ± 0.01a	24.7	23.3 ± 3.1a
Day 1	CK	43.7 ± 1.5c	8.52 ± 0.00c	24.4	31.0 ± 3.9bc
	O1	52.0 ± 2.0b	8.66 ± 0.02a	24.7	35.0 ± 0.3b
	O2	51.5 ± 0.9b	8.56 ± 0.01b	24.4	40.0 ± 2.2a
	O3	57.8 ± 1.9a	8.32 ± 0.02d	24.5	33.5 ± 12.1bc
Day 2	CK	57.4 ± 1.2b	8.72 ± 0.01a	24.5	41.6 ± 2.5ab
	O1	62.8 ± 1.3a	8.55 ± 0.02b	24.2	39.1 ± 0.7b
	O2	63.2 ± 1.2a	8.41 ± 0.01c	23.8	44.8 ± 7.9a
	O3	62.0 ± 1.2a	8.39 ± 0.00c	23.8	42.7 ± 1.4ab
Day 6	CK	42.7 ± 0.6c	8.39 ± 0.01a	23.1	57.3 ± 2.6b
	O1	46.7 ± 0.5a	8.18 ± 0.00b	22.8	66.7 ± 4.4a
	O2	46.3 ± 1.2a	7.97 ± 0.00c	23.3	64.4 ± 4.7ab
	O3	44.7 ± 1.0b	7.85 ± 0.01d	23.1	62.0 ± 1.7ab
Day 13	CK	43.5 ± 1.3a	8.28 ± 0.01a	21.9	79.4 ± 4.1b
	O1	43.3 ± 0.5a	8.00 ± 0.01b	20.2	82.4 ± 6.0ab
	O2	42.7 ± 0.6a	7.57 ± 0.00d	20.1	84.7 ± 6.8a
	O3	39.2 ± 0.5b	7.74 ± 0.04c	21.3	69.2 ± 2.0c
Day 25	CK	34.0 ± 0.9a	8.14 ± 0.01a	20.8	93.9 ± 21.1c
	O1	32.0 ± 0.5ab	8.06 ± 0.03b	20.0	91.6 ± 19.3c
	O2	31.0 ± 0.6ab	7.68 ± 0.02d	19.5	109.4 ± 34.7a
	O3	30.5 ± 0.5b	7.76 ± 0.02c	21.6	100.8 ± 11.2b

Note: The C/N values were calculated based on the average total carbon and total nitrogen contents, and they did not differ significantly. The a, b, c, d means difference between groups, and the same superscripts denoted not significant difference ($P > 0.05$), difference superscripts denoted significant difference ($P < 0.05$).

The GI value in the four treatment increased in general trend. The initial GI, 23%, increased to 93% in CK, 92% in O1, 109% in O2, and 101% in O3 in the end compliance with the compost maturity standard proposed by **Bernal (2009)** of GI > 80%. The GI value was relatively high with the O2 treatment, thereby indicating that adding 0.5% MO had an important effect on reducing the toxicity of the compost. Thus,

adding MO at a specific concentration may have effectively inhibited the growth of harmful bacteria and promoted the reproduction of beneficial bacteria to reduce the toxicity of the compost and increase the GI value to some extent (Ismail et al., 2019). Similar to the findings obtained in other studies, the C/N ratio decline in trend with the composting time (Zhao et al., 2017), and the C/N ratio decreased significantly with the O2 treatment, possibly due to the carbon loss caused by mineralization.

3.2. Lignocellulose contents

Plant cell walls mainly comprise cellulose, hemicellulose, and lignin. Figure 1a shows that the hemicellulose contents stabilized in the four treatments, after the initial decreased rapidly in mesophilic phase with the composting time, as also found by Wang et al. (2017). Because hemicellulose is an active component of lignocellulose and it can be utilized as a carbon and energy source by the microorganisms in compost (Xu et al., 2019). Microorganisms require large amounts of energy to reproduce and grow during the mesophilic and thermophilic phases of composting which made hemicellulose contents decreased rapidly. The hemicellulose contents descend with the time and tend to be stable because of the consumed by microorganisms. At the end of the composting process, the hemicellulose contents were 3.12% in CK, 2.95% in O1, 2.25% in O2, and 2.65% in O3. Figure 1b shows that the changes in the cellulose contents were similar to those in the hemicellulose contents, but the hysteresis was observed clearly, possibly due to the enzymatic degradation of cellulose being inhibited by the formation of the lignin–hemicellulose complex (Xu et al., 2019). At the end of composting process, the cellulose contents were 13.99% in CK, 12.76% in O1, 11.25% in O2, and 11.83% in O3. Thus, the final proportions of hemicellulose and cellulose was lowest in O2, thereby indicating that inoculation with 0.5% manno-oligosaccharide effectively improved the enzymatic reaction with lignocellulose to reduce the hemicellulose and cellulose contents.

Because of its special structure, lignin is not easy to be degraded by microorganisms (Kluczek-Turpeinen et al., 2003). The lignin contents decreased slowly in initial (Fig. 1c), it was due to the preferential use of simple organic matter (polysaccharides, proteins, hemicellulose, etc.) by microorganisms. After the cooling period, the microorganisms associated with lignin degradation gradually became the dominant population, and thus the lignin content decreased more rapidly in the middle and late composting periods. At the end of the composting process, the final lignin contents were 7.76% in CK, 7.50% in O1, 7.07% in O2, and 7.95% in O3. The lignin content decreased significantly in O2 in the later stage of composting, thereby indicating that adding 0.5% MO could effectively adjust the microbial community structure in the compost and reduce the lignin content in this stage.

Composting usually takes a long time. Cellulase can promote the biotransformation of organic matter and it has an important role in the degradation of lignocellulose and fasten compost maturation process. Previous studies have shown that the activity of cellulase depends on the abundances and structural composition of microorganisms involved in cellulose decomposition (Goyal et al., 2005). Figure 1d shows that the cellulase activity increased initially, and peaked in the cooling stage because microorganisms could store sufficient energy to release cellulase and degrade cellulose (Du et al., 2019). Compared with the other treatments, the cellulase activity of O2 treatment was significantly higher in maturation phase,

indicating that adding 0.5% MO enhanced the microbial activity in the late stage to promote the secretion of cellulose-degrading enzymes and accelerate the degradation of cellulose.

3.3. Carbon contents

It is noteworthy that Fig. 2a shows TOC contents first decreased and gradually stabilized because microorganisms can degrade organic matter and the carbon will be emitted as the form of CO₂ and CH₄ (Pisa et al., 2020). However, with the humification strengthened and mineralization weakened during composting process, the rate of carbon loss decreased, and thus the TOC contents gradually stabilized. The carbon degradation rates of CK, O1, O2 and O3 in the thermophilic phases (day 25) were 9.7%, 10.8%, 11.2% and 9.0%, respectively. The total TOC content in each treatment on day 26 was ranked as follows: O3 (255.4 mg/g) > CK (248.9 mg/g) > O1 (241.5 mg/g) > O2 (238 mg/g). The results indicated that a small number of MO could effectively improve the carbon degradation of compost, and excessive MO had an inhibitory effect. The carbon degradation rate in O3 treatment was the lowest, which could be explained by two reasons. The first reason was related to the high content of MO, the existence of MO resulted in the high TOC content. The second reason was that microorganisms preferentially use MO, which led to the organic matter in O3 treatment could not be fully degraded in the initial stage of compost.

WSC comprises active carbon components and the WSC content can reflect the maturity and stability of compost (Bai et al., 2020). As shown in Fig. 2b, the WSC contents inside composting gradually decreased due to the presence of abundant degradable substances with high water solubility in the raw materials. As the degree of humification increased, large amounts of stable substances such as humus were formed and the water solubility of the materials decreased. Therefore, the WSC contents were low in the mature compost (Zhu et al., 2020a; Duan et al., 2020). After 25 days, the WSC contents were ranked in the order: CK (mg/g) > O1 (9.07 mg/g) > O3 (6.37 mg/g) > O2 (5.53 mg/g), and all were less than the compost maturity index (WSC < 17 mg/g) proposed by **Bernai (1998)**, thereby indicating that the compost products were fully mature.

3.4. Changes in the bacterial community

According to a previous study, compared with O2, O3 treatments, the values and contents of pH, GI, hemicellulose, cellulose and WSC in O1 treatment were closer to CK treatment. So, the Illumina HiSeq PE250 platform was used to conduct high-throughput sequencing of the variable V4 region of the 16S rRNA gene for CK, O2, and O3. The sequencing results are shown in Fig. 3. The four bacterial phyla with the highest abundances in all treatments were Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes, which accounted for more than 80% of the total, and the similar results was obtained in previous studies of compost (Wei et al., 2018; Akyol et al., 2019). In all of the composting stages, the highest relative bacterial abundances occurred in the thermophilic phase. The dominant phylum in this stage were Firmicutes and Actinobacteria. The addition of MO significantly changed the bacterial composition during composting, where the relative abundance of Firmicutes increased most in O2 and this phylum accounted for more than 55% of the total bacteria. The similar results were obtained in a

previous study (Zhang et al., 2016). The high simple organic matter content (hemicellulose and cellulose) in the early stage could have been utilized directly by Firmicutes to facilitate its rapid reproduction. The simple organic matter content decreased in the cooling and maturation phase, and the relative abundance of Firmicutes decreased. The dominant phylum in the maturation phase was Proteobacteria. Correlation analysis also showed that the abundance of Proteobacteria had a strong negative correlation with the lignocellulose content (Table 3), possibly because the members of Proteobacteria remained in a dormant state during the thermophilic phase before reactivating during the cooling phase (Tkachuk et al., 2014), and the lignocellulose content decreased gradually with the composting time. In addition, Actinobacteria was the main phylum involved in cellulose degradation (DeAngelis et al., 2011) and this phylum was mainly detected in the maturation phase. Actinobacteria can regulate the activities of key enzymes to degrade lignocellulose (Wei et al., 2019). The abundance of Actinobacteria had strong positive correlations with the cellulose and lignin contents, thereby indicating that Actinobacteria made an important contribution to the degradation of lignocellulose during the composting process. Figure 3a shows that the relative abundance of Actinobacteria was highest in O2, which explains the low cellulose and lignin contents in this treatment.

Table 3
Correlations between lignocellulose contents and bacterial phyla.

	Proteobacteria	Firmicutes	Actinobacteria	Bacteroidetes
Cellulose	-0.736**	0.859**	0.679*	-0.715**
Hemicellulose	-0.690**	0.883**	0.830**	-0.754**
Lignin	-0.647*	0.817**	0.766**	-0.683*

Note: * represents a significant correlation ($P < 0.05$), and ** represents a highly significant correlation ($P < 0.01$).

At the genus level, *Thermobifida* (Actinobacteria) was the most abundant in all treatments, followed by *Thermopolyspora* (Actinobacteria), *Geobacillus* (Firmicutes), *Planifilum* (Firmicutes), and *Streptomyces* (Actinobacteria). The dominant bacteria differed among the various periods and treatments. *Streptomyces*, *Romboutsia*, and *Planococcus* were the main genera in the early stage of composting, and *Thermobifida*, *Bacillus*, *Thermopolyspora*, and *Planifilum* were highly abundant in the thermophilic phase. Previous studies have shown that members of the genus *Thermobifida* in Actinobacteria can degrade cellulose (Zhang et al., 2020), which might explain the rapid decline in the cellulose contents during the thermophilic phase. The abundance of *Thermobifida* decreased during the composting process under all treatments, but a high abundance of *Thermobifida* was observed in O2 during the maturation phase, and especially in the early maturation phase. *Bacillus* was an abundant genus in the phylum Firmicutes. According to Gannes (2013), members of *Bacillus* can tolerate high temperatures and degrade lignocellulose. The abundances of *Luteimonas* (Proteobacteria) and *Chelativorans* (Proteobacteria) were high in the cooling stage, and previous studies have shown that these bacteria can degrade lignocellulose and xylan (Zhang et al., 2011). *Chryseolinea*, *Altererythrobacter*, *Taibaiella*, and *Cellvibrio* were the

dominant bacterial genera in the maturation phase. *Chryseolinea* is present in the soil rhizosphere and it can improve the soil structure and crop growth (Visioli et al., 2018).

3.5. Microbial functional genes

During composting, the transcription and expression of the corresponding functional genes were giving the microbial functions and the secreted enzyme. The classification information for the microbial community is usually insufficient to assess its impact on the composting process (Manoharan et al., 2017; Chen et al., 2020). Therefore, it is more appropriate to analyze the effects of microorganisms during the composting process by quantifying the diversity of the microbial functional genes. In this study, PICRUSt was used to investigate the microbial functional diversity and to determine the effects of MO on the microbial functions. The level 1 KEGG prediction results (Fig. 4a) identified seven functions, which “Metabolism” accounted for 49.7–51.7% and the largest proportion of all the functional genes, the following 15.3–16.2%, 12.6–15.6%, 12.8–13.8%, 2.9–4%, 0.8–1.2%, 0.8–0.9% were in genetic information processing, environmental information processing, unclassified, cellular processes, human diseases, and others, respectively. The abundances of functional genes increased in all treatments with the composting time.

The relative abundances of the first 30 functional genes among the level 3 KEGG prediction results are shown in Fig. 4b. There were some differences in the efficiencies of the bacterial functions during composting. The relative abundances of pyruvate metabolism, glycolysis/gluconeogenesis, amino sugar and nucleotide sugar metabolism genes related to carbohydrate metabolism increased during the thermophilic phase, especially O2 treatment. Similarly, **Wang (2018)** found that the relative abundances of genes related to the biosynthesis and metabolism of sugars and ketones increased during the thermophilic stage, and the carbon transformation was positively correlated with carbohydrate degradation genes (Hartman et al., 2017). It suggested that the rich carbohydrate metabolism genes at this stage were the main reason for the rapid degradation of lignocellulose and carbon. Membrane transcription and nucleotide metabolism also accounted for a large proportion in thermophilic stage, which was related to the rapid reproduction of microorganisms. Obviously, the relative abundance of these functional genes in O2 treatment was higher. The relative abundances of genes associated with arginine and proline metabolism, glycine, serine and threonine metabolism, and valine, leucine, and isoleucine degradation enhanced gradually in the cooling and maturation phase. This was because the carbohydrate degrading enzymes secreted by bacteria decomposed the proteins, cellulose and hemicellulose in thermophilic phases (Goh et al., 2013). A previous study also found that more sequences were annotated with roles in amino acid metabolism in this stage to significantly enhance the synthesis of humus (Wu et al., 2017). Replication, transcription, and translation are the main pathways related to bacterial enzyme secretion, and the abundances of genes associated with ribosome, chromosome, and ribosome biogenesis increased with the composting time, thereby indicating that bacteria continued to produce secretions to participate in lignocellulose metabolism. In addition, some functions were processed by genes associated with “Environmental information,” such as “Secretion system,” which also demonstrates that the secretion of related enzymes increased with the composting time. The propanoate

and butanoate contents are closely related to the pH, and the increased of butanoate metabolism and propanoate metabolism mainly explained the decrease and stabilization in the pH during the composting process (Duan et al., 2020). Figure 4b shows that the relative abundance of tuberculosis (human diseases) in O₂ treatment was lowest in all treatments, which indicated that 0.5% of MO had a certain inhibitory effect on pathogenic bacteria.

3.6. Correlations between physicochemical properties, microbial community composition, functional genes, and lignocellulose contents

The bacterial community and its functions were affected by the physicochemical properties of compost, which affecting lignocellulose content. Thus, RDA was conducted to assess the correlations between the composting environment, microbes, and changes in the lignocellulose contents. Figure 5 shows the effects of the temperature, pH, C/N, microbial community, and metabolic functions on the lignocellulose, TOC, and WSC contents. RDA1 and RDA2 accounted for 98.8% and 0.2% of the variation, respectively (Fig. 5a). Among the eight factors were considered, C/N explained 84.5% of the total variation in the lignocellulose, TOC, and WSC contents ($P = 0.002$), *Streptomyces* explained 5.5% of the total variation ($P = 0.012$), temperature explained 4.9% of the total variation ($P = 0.008$), *Luteimonas* explained 1.7% of the total variation ($P = 0.094$), pH explained 1.5% of the total variation ($P = 0.206$), and *Chryseolinea* explained 0.8% of the total variation ($P = 0.202$). Thus, the C/N ratio had the greatest influence on the lignocellulose content because lignocellulose is rich in carbon. Carbon loss was inevitable due to the continuous degradation of lignocellulose, and thus the C/N ratio explained much of the total variance. The temperature was also positively correlated with the lignocellulose and carbon contents because the temperature decreased gradually with the compost matured, and the lignocellulose and carbon contents decreased due to microbial degradation. *Streptomyces* was the dominant genus in the early stage and it could tolerate the weak alkali environment. Studies have shown that *Streptomyces* can utilize lignocellulose as a sole carbon source to support growth, as well as effectively degrading hemicellulose, cellulose, and lignin (Feng et al., 2021). *Luteimonas* and *Chryseolinea* were mainly detected in the maturation phase and their abundances were negatively correlated with temperature, and thus these two bacterias were not resistant to high temperature but they were involved with the degradation of organic matter in the later stage.

As shown in Fig. 5b, RDA1 and RDA2 explained 99.1% and 0.3% of the variation in the cellulose contents, respectively. Among the eight factors were considered, C/N still explained most of the changes in the cellulose contents during composting, where it explained 83.6% of the total variation ($P = 0.002$), while the secretion system, pH, amino sugar and nucleotide sugar metabolism, purine metabolism, and temperature explained 4.6% ($P = 0.084$), 3.3% ($P = 0.116$), 1.5% ($P = 0.43$), 2.3% ($P = 0.188$), and 1.6% ($P = 0.166$), respectively. All of the functional genes were mainly distributed in the maturation stage and they had no significant negative correlations with the lignocellulose and carbon contents, mainly because the degradation of lignocellulose was more rapid in the early stage and bacterial functional genes were more abundant in the maturation stage. Compared with the other treatments, the O₂ treatment had a stronger

correlation with functional genes in the maturation phase. The results showed that adding 0.5% MO could effectively improve the abundance of functional genes and degrade lignocellulose. Furthermore, the pH was positively correlated with the lignocellulose and carbon contents, but negatively correlated with butanoate metabolism. It is well known that the pH is mainly affected by the organic acids produced via the degradation of complex organic matter (e.g., lignocellulose) (Duan et al., 2020). Therefore, organic acids accumulated and the pH decreased as the lignocellulose contents decreased. In addition, butanoate metabolism increased and butanoate was consumed in large quantities to inhibit the accumulation of organic acids.

Conclusion

The addition of MO increased the temperature in the thermophilic phase. Compared with the other treatments, O₂ reduced the toxicity, promoted the degradation of lignocellulose and enzymatic reactions, and improved the stability of the final compost product. MO reshaped the bacterial community structure, reduced the abundances of pathogenic bacteria, and changed the abundances of functional genes. RDA also indicated that C/N, temperature, and *Streptomyces* were the main factors related to lignocellulose degradation. Thus, adding 0.5% MO to cow manure–straw during aerobic composting could effectively regulate the composting properties, enhance the bacterial community structure and functional genes, and promote the degradation of lignocellulose.

Declarations

Statement:

1. Ethics approval and consent to participate: Not applicable.
2. Consent for publication: All authors of the above article agree to submit their contributions to the ***Environmental Science and Pollution Research***.
3. Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.
4. Competing interests: The authors declare that they have no competing interests.
5. Funding: All the funding from the teacher project support.
6. Authors' contributions:

Yuhua Zhang: Formal analysis; Investigation; Methodology; Writing - original draft

Manli Duan: Funding acquisition; Project administration; Supervision; Writing - review & editing

Beibei Zhou: Funding acquisition; Supervision; Writing - review & editing

Quanju Wang: Funding acquisition; Supervision

Zhenshi Zhang: Supervision

Lijun Su: Data curation; Investigation; Methodology

Qingjun Bai: Supervision

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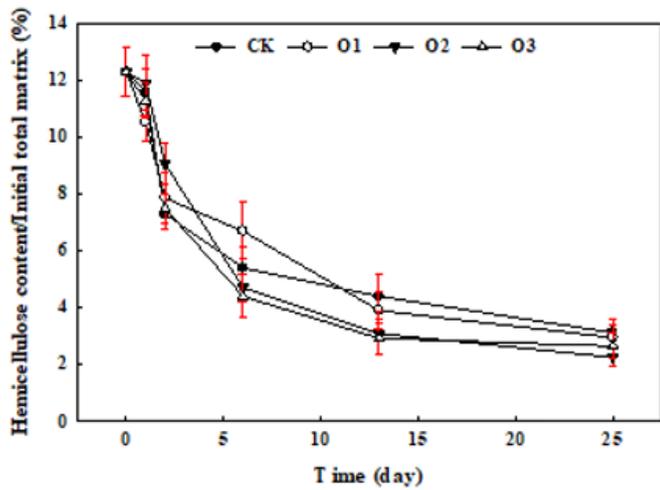
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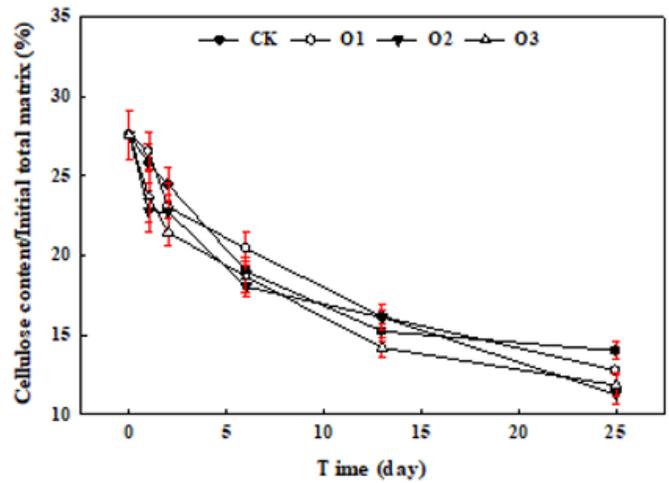
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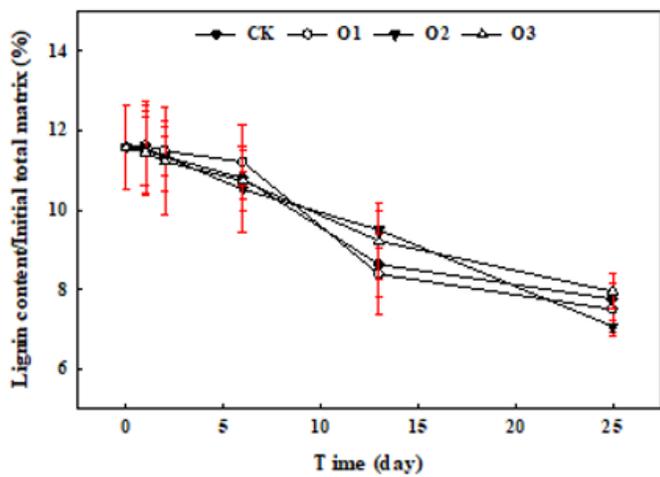
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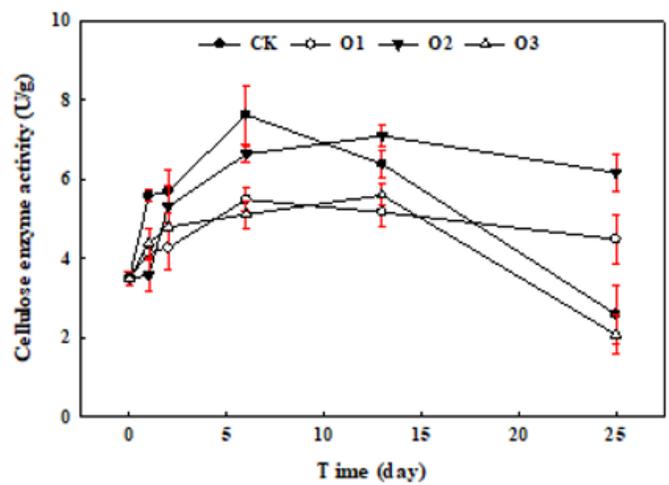
(a)



(b)



(c)



(d)

Figure 1

Changes in hemicellulose, cellulose, and lignin contents, and cellulose enzyme activity during composting: (a) hemicellulose; (b) cellulose; (c) lignin; and (d) cellulose enzyme activity. The hemicellulose, cellulose, and lignin content represent the percentages relative to the initial values in the total compost matrix (n = 3).

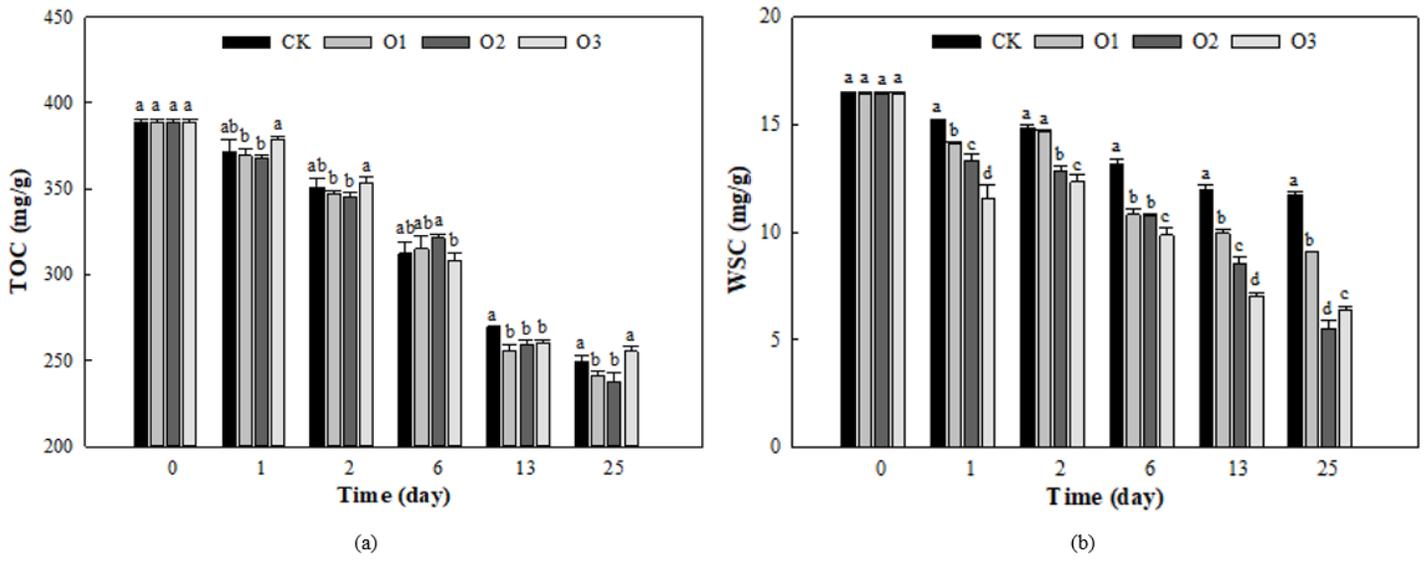


Figure 2

Changes in total organic carbon (TOC) and water-soluble carbon (WSC) contents during composting: (a) TOC, and (b) WSC. The bars represent standard deviations (n = 3). Note: The a, b, c, d means difference between groups, and the same superscripts denoted not significant difference (P > 0.05), difference superscripts denoted significant difference (P < 0.05).

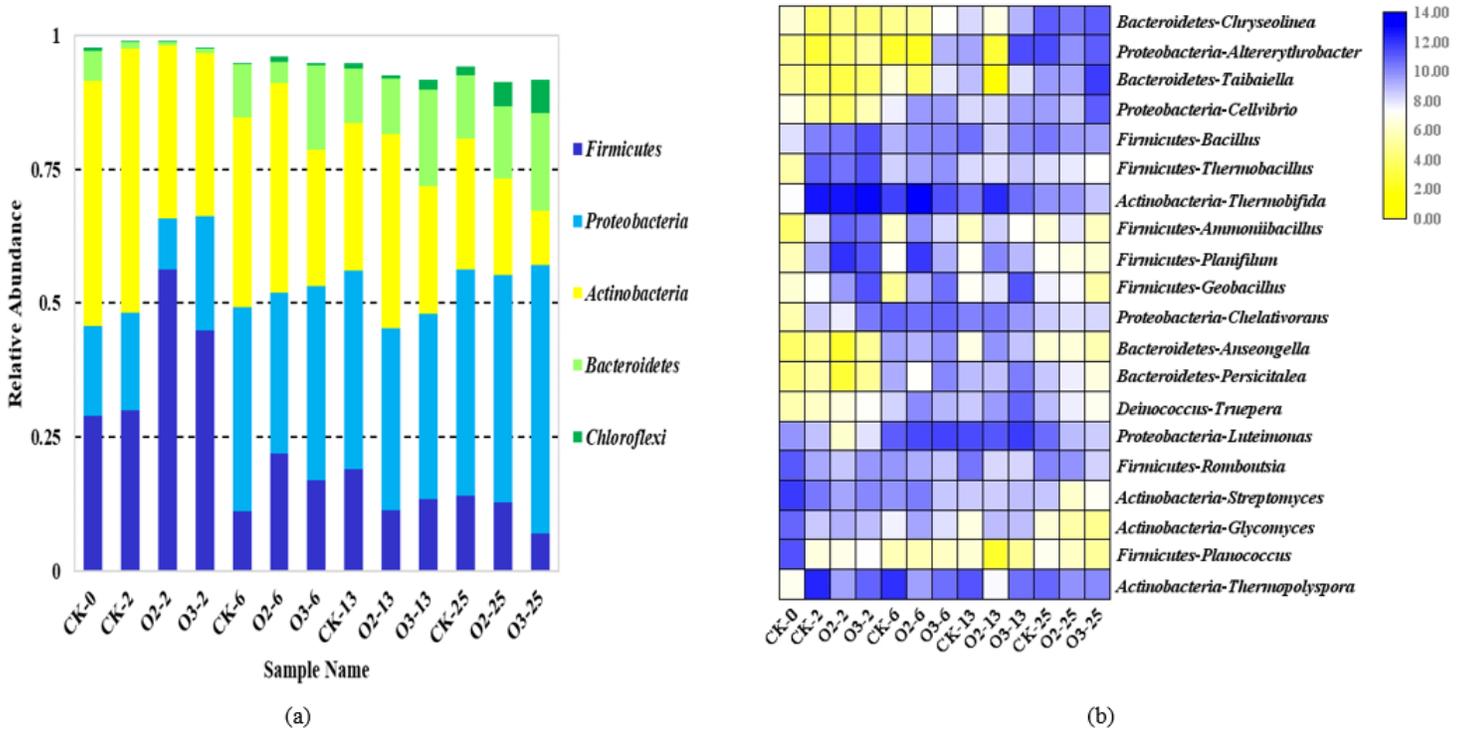
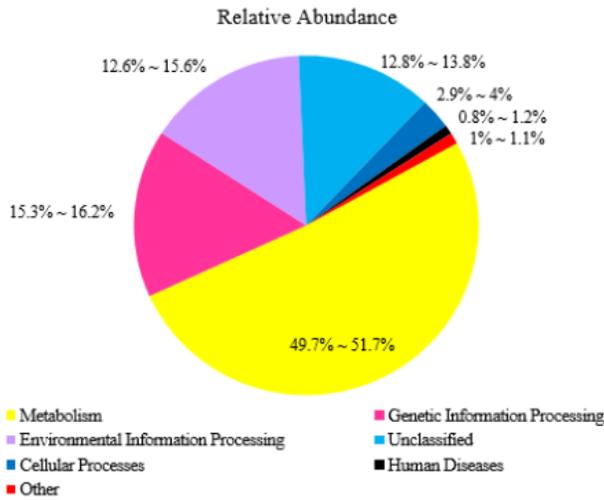
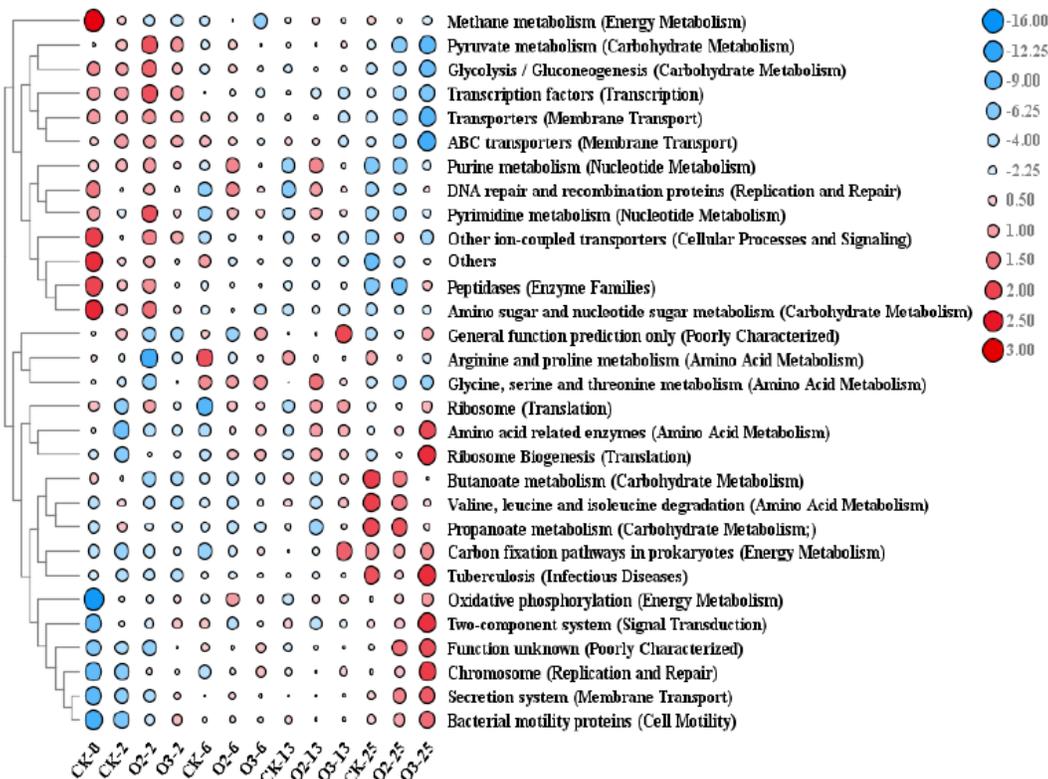


Figure 3

Changes in relative abundances of bacteria during composting: (a) relative abundances of bacterial phyla and (b) relative abundances of bacterial genera.



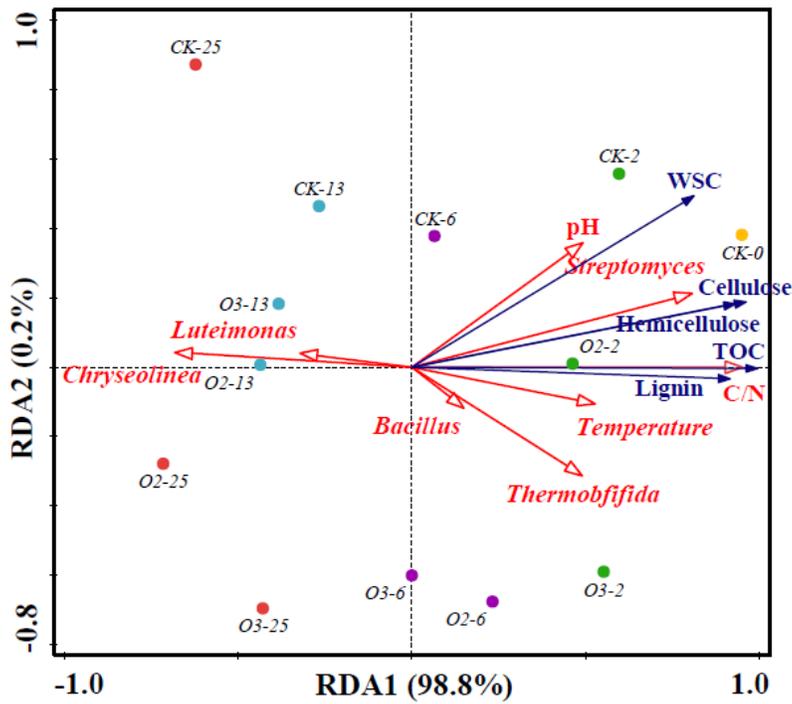
(a)



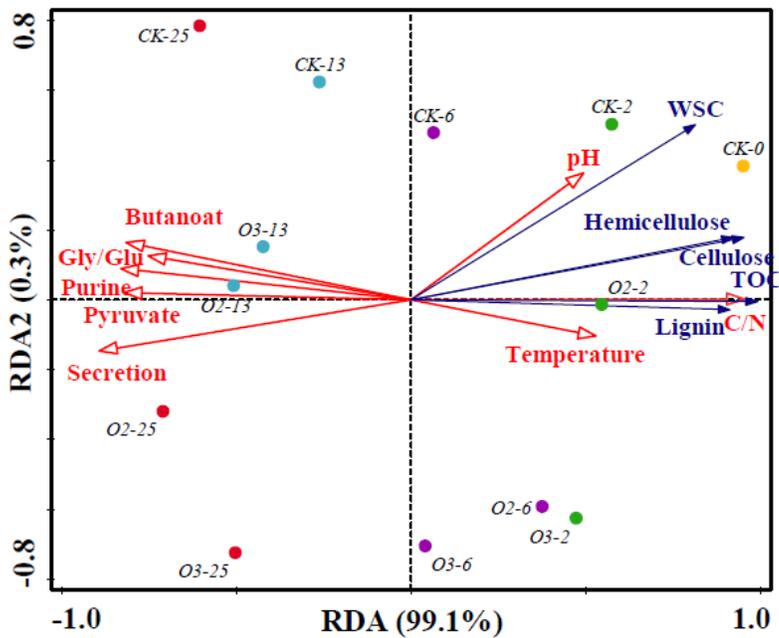
(b)

Figure 4

Changes in relative abundances of microbial functional genes during composting: (a) biochemical functional gene pathways, and (b) level 3 KEGG function predictions for the 30 main functional genes.



(a)



(b)

Figure 5

Redundancy analysis based on the compost properties, bacterial abundances, functional genes, and lignocellulose and carbon contents, where blue arrows represent lignocellulose and carbon, red arrows represent the compost properties, bacterial abundances, and functional genes, and dots represent the samples.