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Effects of antibiotics on hydrolase activity and structure of microbial community during aerobic co-composting of food waste with sewage sludge

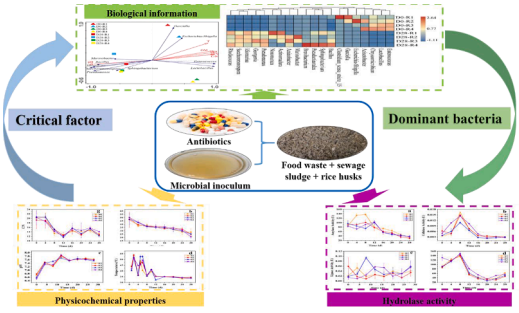
Zhou Chen [a,b], Yanzeng Li [a,b], Yanyan Peng [a], Chengsong Ye [c], Shenghua Zhang [a,*]

[a] Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, People's Republic of China
[b] University of Chinese Academy of Science, Beijing 100049, People's Republic of China
[c] Xiamen University, Xiamen 361102, People's Republic of China

HIGHLIGHTS

- 5 mg/kg of antibiotics decreased cellulase activity and increased lipase activity.
- The contents of Zn, Cu, and Hg increased at 20 mg/kg of antibiotics.
- The microbial community showed the highest diversity at 5 mg/kg of antibiotic.
- 20 mg/kg antibiotics associated with the lowest microbial community richness.
- pH and temperature were the most impactful factors that affecting compost microbes.

GRAPHICAL ABSTRACT



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ABSTRACT

This study aimed to investigate the effects of antibiotics on environmental factors, hydrolase activity, and microbial community during aerobic co-composting of food waste and sewage sludge. The results showed that 5 mg/kg of antibiotics decreased cellulase activity and increased lipase and proteinase activity, while 20 mg/kg of antibiotics also decreased cellulase activity and increased the contents of Zn, Cu, and Hg. The dominant bacterial genera of the four treatment groups were Enterococcus, Pseudomonas, Idiomarina, Lactobacillus, and Bacillus. The addition of antibiotics affected the succession of microbial community structure. Microbial communities treated with 5 mg/kg antibiotics had the highest in diversity, while those treated with 20 mg/kg antibiotics had the lowest in richness. Redundancy analysis (RDA) revealed that the pH and temperature were the most important environmental factors that affected microbial community succession, followed by total nitrogen and moisture content during co-composting of food waste and sewage sludge.

1. Introduction

Food waste (FW) is raw food materials and processed leftovers produced by restaurants, families, and other places during the cooking process (Li et al., 2020). It accounts for as much as 20 ~ 45% of municipal solid waste in Asian and European countries, which has attracted worldwide attention (Awasthi et al., 2018a; 2018b; 2018c). Given that FW is often large in quantity, high in moisture content and

* Corresponding author.
E-mail address: shzhang@iue.ac.cn (S. Zhang).

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organic matter, low in calorific value, and easy contamination, the unsustainable disposal mode of traditional landfill and incineration is not suitable for the terminal disposal (Cerda et al., 2018). In the early 21st century, China had issued relevant policies to encourage the recycling of FW that aimed to reduce the possible environmental impact and public health risks caused by landfill and incineration of FW (Chen, 2016). FW contains abundant nutrients, including protein, sugar, starch, and fat, which varied with time and place, can be converted into fertilizer, methane, and other products through aerobic composting (Liao et al., 2019) or anaerobic digestion (Akindele and Sartaj, 2018) to achieve the goal of harmlessness, reduction, and recycling.

Sewage sludge (SS) is a by-product of the activated sludge process in wastewater treatment plants. In China, approximately 45 million tons of SS (dry weight) are produced annually, and the cost of SS treatment accounts for about 50% of the total operating costs of treatment plants (Jang et al., 2018). SS contains large amounts of heavy metals, organic micro-pollutants, and pathogens. Thus, improper SS disposal may lead to water, air, and soil pollution and even threaten human safety. Nevertheless, SS is rich in organic components and biodegradable, which can be transformed into value-added products by aerobic composting (Awasthi et al., 2018a; 2018b; 2018c). In recent years, aerobic composting through microbial activities has been widely utilized for sludge stabilization and resource recovery to reduce risks to the environment, but low C/N (only around 6–9) limits the independent biological treatment of SS (Fang et al., 2019).

Antibiotics are widely used prophylactically to prevent animal diseases and promote growth. SS is a repository for antibiotics, given that unmetabolized antibiotics in both humans and animals are discharged into municipal sewer networks and being concentrated in SS. SS contains abundant nutrients that can be used for soil amendments after composting, which can lead to unremoved antibiotics entering farmland soil and being absorbed by plants, and eventually enter the human body through the food chain. All in all, FW becomes an important reservoir of antibiotics (Liao et al., 2019) and a potential source of developing antibiotic resistance. Therefore, the effect of antibiotics on the co-composting process of FW with SS is worthy of further exploration.

Aerobic composting can render solid waste harmless, stable, and resource-efficient through biological reactions. It has the advantages of being a simple and stable operating system with high degradative efficiency of organic matter, which produces organic fertilizer. Besides, aerobic composting has broad applicability that enables home composting, which then avoids the need for municipal waste collection, alleviates problems caused by waste sorting and transportation, and reduces land use and central infrastructure materials (Zorpas et al., 2018). Based on these desirable properties, aerobic co-composting of FW and SS may represent a novel strategy to overcome the consequences of increasing organic perishable solid waste to human society and economic development.

Aerobic co-composting of FW and SS has been barely reported, and its physiochemical properties, hydrolase activity, and microbial succession remain largely unclear. In this study, FW and SS were used as substrates for aerobic composting. Rice husks were utilized as bulking agent, because it could be used to adjust the moisture content, C/N ratio and void spaces between particle, which can accelerate composting rate and reach higher temperature (Onwosi et al., 2017; Soobhany, 2019). The recycling effects of FW and SS as well as the impacts of variable doses of microbial inoculum and six antibiotics on the composting process were explored. Using Illumina Hiseq 2500 sequencing of bacterial 16S rRNA gene combined with the physiochemical properties and hydrolase activity of composting, this study aimed to: (1) investigate the effects of antibiotics on physiochemical properties and hydrolase activity in the composting process; (2) study the succession of microbial community during composting under the influence of antibiotics; (3) discover key influencing factors that affect the composting process using RDA analysis.

2. Materials and methods

2.1. Raw material and composting experiment setup

2.1.1. Raw materials

The raw materials contained FW, SS, antibiotics, rice husks, microbial inoculum. FW was collected from the dining hall of the Institute of Urban Environment (Xiamen, China). SS was collected from the Jimei Wastewater Treatment Plant (Xiamen, China). The oxidation ditch process was used in this wastewater treatment plant, which formed an aerobic zone, hypoxic zone and anaerobic zone in space. The excess sludge from the oxidation ditch process passed through to the secondary sedimentation tank and sludge concentration tank, and finally went through the plate-frame pressure filtration and dehydration to obtain the sludge used in our experiment. The properties of raw materials were shown in Table 1. Microbial inoculum (including *Natto bacteria*, *Bacillus*, *Actinomycetes*, *Yeast, Lactobacillus*, and photosynthetic bacteria) was purchased from Shangdong Junde Biotechnology Co., Ltd (China). Antibiotics (including ciprofloxacin, norfloxacin, sulfadiazine, sulfadimethoxil, roxithromycin, and clarithromycin) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd., China.

2.1.2. Setup of the composting experiment

A total of 4 separate reactors were used as carriers for composting. The size of the reactor was 32 cm long × 15 cm wide × 15 cm high. The experiments were conducted in four groups (R1-4), as outlined in Table 2. The experiment period was 28 days (Li et al., 2020). Samples were taken every four days from D0 to D28, which were collected separately from the surface, center and bottom layer of the reactors, with parts of the samples stored at 4 ℃ for the measurement of physiochemical parameters, while others were stored at −80 ℃ for biological information. The composting device was shown in Fig. 1.

2.2. Physicochemical properties and enzymatic analysis

Portable thermometers were used to measure temperature (T). Samples were dried to constant weight at 105 ℃ to determine the moisture content (MC). The oven-dried samples were further heated at 550 ℃ for 5 h in a muffle furnace for the determination of organic matter content (OM). The pH value was measured using a pH probe (pH700, Eutech, China). The total carbon (TC), total nitrogen (TN), and carbon/nitrogen ratio (C/N) analyses were conducted using a CNS elemental analyzer (Vario MAX). Heavy metals were determined by an inductively coupled plasma-mass spectrometer (Agilent 7500cx) after HNO_3-HF-$HClO_4$ digestion process using a microwave digestion instrument (Master). An atmospheric trace mercury analyzer (Tekran2527B/1130/1135) was used to determine the Hg content. A Soxhlet extractor was employed for oil and fat content tests (Wang et al., 2016a; 2016b). The

Table 1
The characteristics of the raw materials.

Parameters	Food waste	Sewage sludge	Blending materials
pH	4.33 ± 0.06	8.00 ± 0.05	6.91 ± 0.03
TC (%)	47.96 ± 0.07	12.48 ± 0.12	32.46 ± 0.46
TN (%)	2.18 ± 0.00	1.60 ± 0.00	1.60 ± 0.00
C/N (%)	22.00 ± 0.47	7.80 ± 0.17	20.29 ± 0.10
Moisture content (%)	72.19 ± 0.00	4.77 ± 0.00	53.13 ± 0.00
Organic matter (%)	96.21 ± 0.00	68.00 ± 0.00	50.27 ± 0.00
Protein (mg/g DW)	73.57 ± 8.58	6.70 ± 0.71	14.89 ± 3.10
Starch (mg/g DW)	146.28 ± 1.06	19.86 ± 2.49	41.72 ± 2.73
Lipid (mg/g DW)	377.19 ± 7.59	0.6 ± 0.24	51.46 ± 6.89
Cellulose (mg/g DW)	247.77 ± 29.51	35.24 ± 1.46	59.66 ± 1.77
Hemicellulose (mg/g DW)	0.03 ± 0.00	1.59 ± 0.11	1.21 ± 0.11

*DW: Dry weight.

Table 2
The compositions of composting.

Compostions	R1	R2	R3	R4
food waste (g)	2000	2000	2000	2000
sewage sludge (g)	750	750	750	750
rice husks (g)	250	250	250	250
microbial inoculum (g)	0	30	30	30
deionized water (g)	80	50	50	50
ciprofloxacin (mg)	0	0	15	60
norfloxacin (mg)	0	0	15	60
sulfadiazine (mg)	0	0	15	60
sulfadimethoxil (mg)	0	0	15	60
roxithromycin (mg)	0	0	15	60
clarithromycin (mg)	0	0	15	60

starch content was measured using a starch determination kit (YX-W-C400). The Coomassie bright blue protein assay kit was used to determine soluble protein content (YX-W-C202). The cellulose content was tested using a cellulose assay kit (YX-C-B634). Lignin content assay kit was used to measure lignin content (YX-W-B636). All the kits were purchased from Sinobestbio Co., LTD. (Shanghai, China). The germination index (GI) was determined according to Awasthi et al., (2014). Enzymatic activities were estimated by testing of the fresh composting crude enzymes. The detailed determination methods of GI and enzymatic activities were presented in MethodsX. Analyses were performed with triplicate samples.

2.3. DNA extraction and 16S rRNA gene amplification and sequencing

DNA was extracted from 0.25 g freeze-dried samples using the FastDNA Spin Kit for Soil (MP Biomedical, France) according to the manufacturer's protocol. To investigate the variation in the bacterial community composition during composting, the V3-V4 region of the bacterial 16S rRNA gene was amplified using the primers 515F and 806R (Liao et al., 2019). PCR reactions were performed in triplicate before sequencing with an Illumina Hiseq 2500 platform. To obtain high-quality clean reads, raw reads were further filtered using FASTP. Noisy sequences of raw tags were filtered by the QIIME pipeline to obtain a high-quality sequence (Caporaso et al., 2010). The sequences were clustered into operational taxonomic units (OTUs) at 97% level similarity using the UPARSE pipeline. A representative sequence of each OTU was assigned to a taxonomy class using an RDP classifier. Rarefaction curves and the alpha diversity including "ACE", "Chao1", and "Simpson" diversity indexes were determined to compare the level of bacterial OTU diversity among different samples.

2.4. Statistical analysis

SPSS (version 19.0) was used for a one-way analysis of variance (ANOVA), and the significance of the results was tested. A *P*-value of <0.05 was considered statistically significant. The redundancy analysis (RDA) was performed to identify the correlation between the environmental factors and the microbial community during composting using the Canoco 5.0 software.

3. Results and discussion

3.1. Changes in physicochemical characteristics during composting

Physicochemical indexes including OM, MC, pH, T, C/N are commonly used to characterize the aerobic composting process. OM is an important indicator of composting maturity because OM is mineralized into carbon and nitrogen sources for microbial use in the process of aerobic composting (Petric et al., 2012). MC affects microbial metabolism, ventilation, and helps to maintain the thermophilic phase, given that water provides the medium for dissolving nutrients and affects the physical structure and degradation of organic matter (Awasthi et al., 2018a; 2018b; 2018c). pH level determines microbial activity, in which a pH of 7–8 is optimal in the composting process (Wang et al., 2013). T is an important indicator of microbial changes during composting, indicating the degradation rate of OM. Carbon is an essential element that fulfills the energy requirement of microorganisms, and nitrogen is an important component of proteins, nucleic acids, and enzymes, therefore, the C/N has an important impact on the growth and reproduction of microorganisms and the quality of organic fertilizers (Cerda et al., 2018). In our study, various indexes at different stages of aerobic composting were examined, and the results were shown in Fig. 2.

On D0, the MC of R1, R2, R3, and R4 were 49.20%, 48.91%, 49.72%, and 51.20%, respectively (Fig. 2a), while the OM were 50.21%, 46.86, 47.75% and 50.21%, respectively (Fig. 2b). Microorganisms took advantage of sufficient water and biodegradable organic matter as substrates and multiplied in a large number within a short period of time (Yu et al., 2018), following which a large amount of heat was released. The T of all the groups reached above 50 ℃ on D2 (Fig. 2d), leading to rapid evaporation of water (Wang et al., 2016a; 2016b) and consequently, the MC and OM rapidly decreased from D0 to D8. The T of R1 and R2 peaked on D2 at 53.9 ℃, indicating that the microbial inoculum used in this experiment has little influence on the T of the composting process, which may be related to the lack of adaptability of the microbial inoculum in the piles. In R4, the peak was also recorded on D2 but at a lower temperature of 51.2 ℃, likely because the increase of antibiotic concentrations affected the microbial activity and delayed the release of heat, leading to the decrease of thermophilic temperature (Ezzariai et al., 2018). As the T rose, nitrogen-containing organic matter such as protein was positively decomposed (Fig. 2b) and converted into ammonium nitrogen. Thus, the pH of all the groups (R1, R2, R3, and R4) increased rapidly from 6.71, 6.78, 6.6, and 6.51 to 7.4, 7.46, 7.41, and 7.33 respectively, from D0 to D4 (Fig. 2c). On D4 and D8, the piles were turned by mixing the materials in the piles, following which the T had risen again because of the supplement of oxygen required by the biological process (Zhang et al., 2019). On D8, the MC had decreased to about 30%, hence deionized water was added to adjust the MC to approximately 55% to create a suitable condition when the temperature noticeably rose again due to increased microbial activity and higher degradation rate of OM. As demonstrated in Fig. 2g, at D12, the C/N decreased rapidly at the initial stage of composting and reached the lowest values at 12.76, 12.50, 11.61, and 12.15 in R1, R2, R3, and R4, respectively.

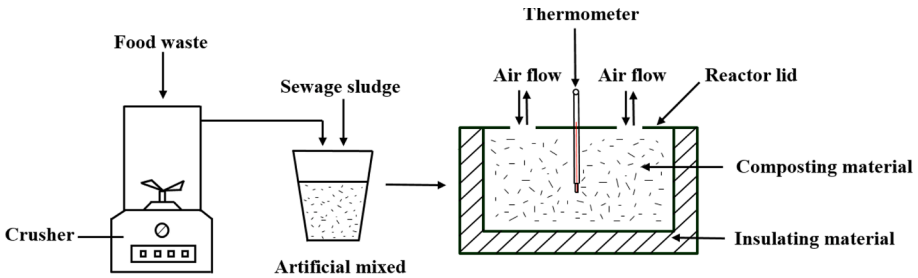

Fig. 1. Schematic diagram of aerobic composting.

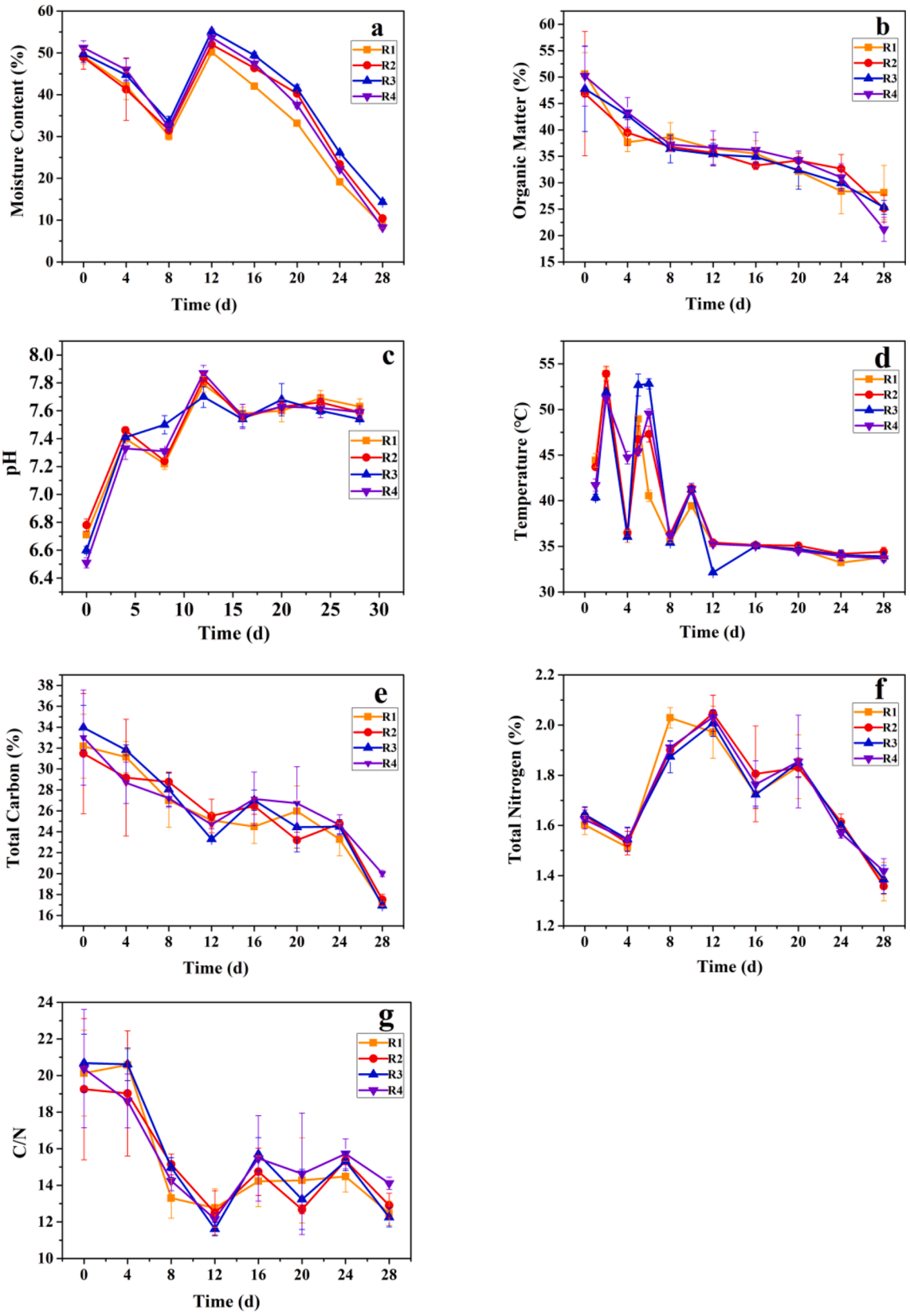

Fig. 2. Changes in physicochemical characteristics of different piles during the composting process. a: moisture content; b: organic matter; c: pH; d: temperature; e: total carbon; f: total nitrogen; g: C/N. Error bars represented standard deviation (n = 3).

From D12 to D28, the MC showed a steady trend of decline. Throughout the composting process, the MC of R1, R2, R3, R4 were reduced by 40.51%, 38.48%, 35.39%, and 42.95%, respectively, as a result of water evaporation by microbial heat generation. In addition, the bulking agent used in this study was rice husk, which contributed to the large porosity of the piles and further accelerated water evaporation. When there were reductions in the biodegradable organic matter and MC, the microbial activity became weakened, followed by slowing and decreasing in OM degradation, recorded at 22.45%, 21.73%, 22.41%, and 29.03% in R1, R2, R3, and R4, respectively. Of all the groups, R4 had the highest OM degradation rate, likely due to a higher relative abundance of *Bacillus* (Fig. 4b) in R4 was higher, which hydrolyzed hemi-cellulose, cellulose, and lignin, and promoted the degradation of refractory organic matter (Awasthi et al., 2018a; 2018b; 2018c). On D28, the TC (Fig. 2e) in R4 was significantly higher than the three other treatment groups ($P < 0.05$). This could be due to a lower temperature in R4, which resulted in a poor degradation effect on organic matters, and therefore less TC consumption. The TN (Fig. 2f) was slightly higher in R4 compared with the other treatment groups, possibly because the introduction of SAs inhibited the basic respiration of composting and reduced the conversion of nutrients such as ammonia and nitrate (Liu et al., 2015). The C/N of R4 (14.11) was the highest among the four groups (12.47, 12.90, and 12.25 in R1, R2, and R3, respectively). This could be explained by the high concentration of antibiotics that might have changed the microbial community through the selection of antibiotic pressure, resulting in the development of antibiotic resistance in the microorganisms. Therefore, to maintain the metabolism of microorganisms with active antibiotic resistance, the metabolic efficiency of other microorganisms would be reduced, thus leading to a higher C/N in R4 (Wepking et al., 2019).

3.2. Changes in heavy metals during composting

Heavy metals in composting enter the food chain through the soil, groundwater, and plants, which may adversely affect the health of animals and humans, such as Zn, Cu, Ni, Cd, Pb, Cr, and Hg. Therefore, it is paramount in the quantification of heavy metals in composting. As outlined in Table 3, the heavy metal concentration of all treatment groups was found to be increased on D28 when compared with the blending materials, which could be due to microbial degradation of some organic compounds and the loss of volatile solids (Deng et al., 2020). The concentrations of Cr in R2, R3, and R4 were significantly lower than that in R1 ($P < 0.05$), indicating that the addition of microbial inoculum potentiated the passivation of these heavy metals. This could be because the microorganisms in the microbial inoculum contributed to the conversion of water-soluble heavy metals into an organic binding state, sulfide binding state, and iron-manganese oxidation state, leading to the decrease of the biological availability of heavy metals (Awasthi et al., 2020). Another possible reason was that the oxidation process and the formation of organo-metallic complexes taken place during composting could reduce the soluble contents of these heavy metals (He et al., 2009). In our study, the highest concentrations of Hg, Zn, and Cu were observed in R4, which might be attributed to the exposure of antibiotics to large quantities of these metals that could impede growth, affect morphology and biochemical activity, leading to poor removal of heavy metals by microorganisms (Deng et al., 2020). In summary, except for As, the amount of heavy metals in this study conformed to the limits of grade B sludge products according to the National Standard of the People's Republic of China (GB 4284-2018).

Table 3
Changes of heavy metals in co-composting of food waste and sewage sludge.

Heavy metals (mg/kg)	Blending materials	R1	R2	R3	R4	Limit of grade B sludge products (mg/kg)
Hg	0.41 ± 0.02	0.76 ± 0.13	0.82 ± 0.07	0.834 ± 0.10	0.930.02	<15
As	4.31 ± 0.34	122.63 ± 10.11	127.57 ± 125.23	44.69 ± 9.14	76.12 ± 53.21	<75
Pb	112.6 ± 1.14	283.1 ± 3.82	265.6 ± 8.56	249.53 ± 6.34	240.8 ± 8.34	<1000
Cd	1.43 ± 0.03	3.33 ± 0.44	3.08 ± 0.81	2.92 ± 0.05	2.74 ± 0.40	<15
Cr	281.69 ± 0.92	576.82 ± 1.06	482.36 ± 9.12	515.49 ± 3.46	481.12 ± 12.80	<1000
Ni	30.95 ± 0.38	69.41 ± 3.36	90.77 ± 3.48	76.77 ± 1.67	87.95 ± 2.69	<200
Zn	274.35 ± 7.99	493.68 ± 1.84	535.92 ± 22.70	550.45 ± 5.30	665.12 ± 22.91	<3000
Cu	48.07 ± 1.06	93.62 ± 3.39	109.77 ± 3.18	104.29 ± 8.03	111.99 ± 0.07	<1500

3.3. Seed germination test

The germination index (GI) is an indicator of maturity and plant toxicity caused by low molecular weight substances produced during the decomposition of organic waste (Awasthi et al., 2014). A GI > 50% indicated that the compost was basically mature. After 28 days of composting, the GI of R1, R2, and R4 were 75.84%, 80.26%, and 74.32%, respectively. However, the GI of R3 was only 37.85%, which could be attributed to the high concentration of low molecular weight substances such as organic acids in the pile (Liu et al., 2011).

3.4. Evolution of hydrolytic enzyme activities during the composting process

Composting is a process that relies on microorganisms to decompose organic substances into humus, which is mainly dependent on various hydrolytic enzymes secreted by microorganisms in the process of growth and metabolism, including cellulase, protease, lipase, and amylase (Li et al., 2020). Characterization and quantification of the hydrolytic enzyme activities can reflect the decomposition and transformation of organic matter in the composting process, which enables delineation of the action process of composting more objectively (Vargas-Garcia et al., 2010). Therefore, in our study, the activities of cellulase, protease, lipase, and amylase were examined to further understand the effect of hydrolytic enzymes on substrate degradation.

Amylase is one of the main hydrolytic enzymes in compost that hydrolyzes starch into simple by-products such as glucose and maltose (Awasthi et al., 2018a; 2018b; 2018c). As demonstrated in Fig. 3a, the amylase activities in R1, R2, R3, and R4 reached their peaks on D8 (136.54 U), D8 (101.29 U), D12 (100.95 U), and D12 (103.02 U), respectively. This observation could be explained by the high starch content (Table 2) in the initial composting process that stimulated the growth of microorganisms and encouraged the synthesis of amylase under the appropriate conditions of external temperature, pH, and moisture content (Echeverria et al., 2012). On D4 and D8 of composting, the amylase activity of R1 was significantly higher than that of the other three groups ($P < 0.05$), which might be associated with the microbial community. As the starch being consumed, its availability in the piles decreased (Raut et al., 2008), resulting in a gradual decline in the amylase activity of R1, R2, R3, and R4 in the later stage of composting.

Cellulase plays a key role in the degradation of cellulose, hemicelluloses, and lignin, which requires the involvement of both fungi and bacteria, such as cracking bacteria and aspergillus (Raut et al., 2008). The changes in cellulase activity during the composting process were illustrated in Fig. 3b. In all the treatment groups (R1, R2, R3, and R4), the cellulase activities (0.017 U, 0.015 U, 0.011 U, and 0.014 U, respectively) peaked on D8, indicating the consumption and catabolism of the biodegradable organic substances such as cellulose and other

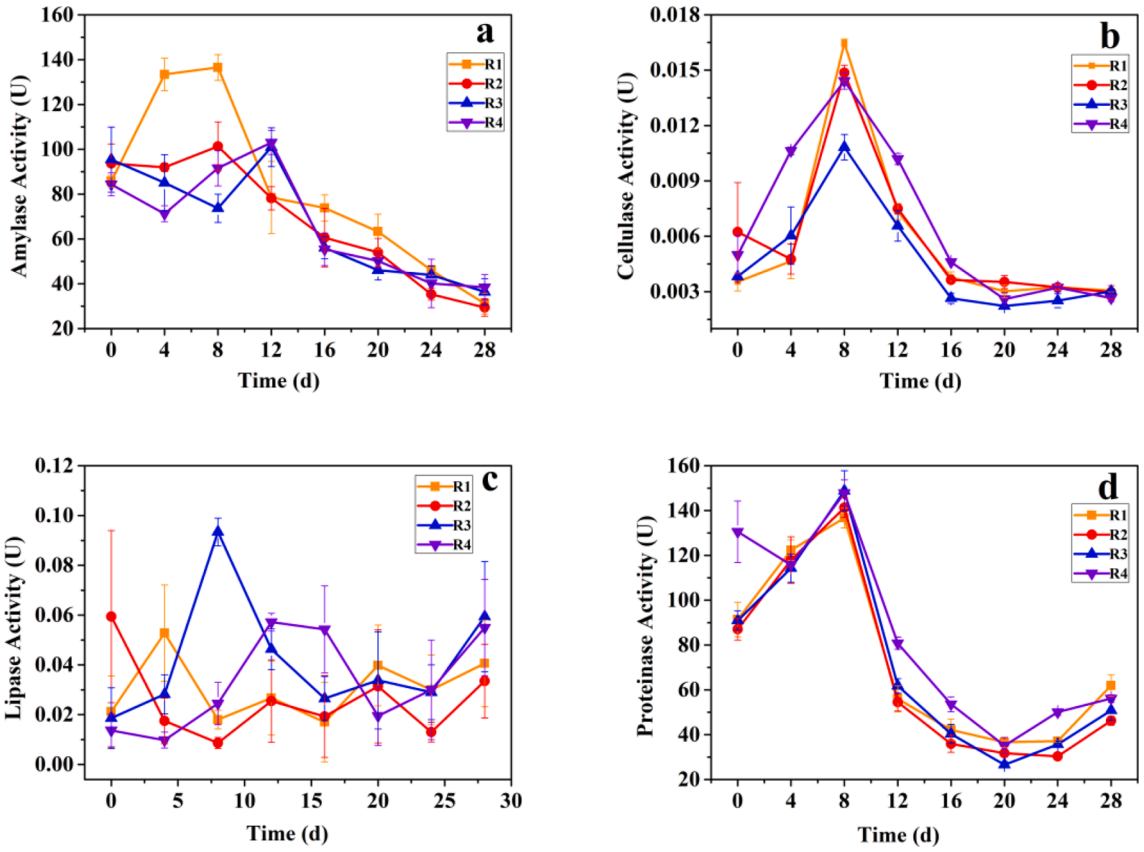

Fig. 3. Evolution of hydrolytic enzyme activities in different files during the composting process. a: amylase; b: cellulase; c: lipase; d: proteinase. Error bars represented standard deviation (n = 3).

refractory organic substances, which stimulated the production of cellulase and led to the increased cellulase activity. On D12, the cellulase activity in R4 was 0.01 U, which was significantly higher than R1 (0.0073 U), R2 (0.0075 U), and R3 (0.0066 U) ($P < 0.05$). This could be associated with a higher abundance of *Bacillus* in R4 (Fig. 4b), which secreted more cellulase and promoted cellulose degradation (Awasthi et al., 2018a; 2018b; 2018c). Additionally, the increase of *Pseudomonas* abundance might contribute to the increase of cellulase activity in R4, given that *Pseudomonas* represented a major decomposer of complex polymers such as lignocellulose (Casacchia et al., 2011). The significant differences ($P < 0.05$) in the cellulase activity of the four treatment groups suggested the influential role of microbial inoculum and antibiotics on cellulase activity.

Lipase hydrolyzes acylglycerol that contains a carbon number of ≥10 in a fatty acid chain to form fatty acids and glycerol and release ester bonds (Hrebeckova et al., 2019). In our study, the lipase activity in R1, R2, R3, and R4 peaked at 0.053 U, 0.059 U, 0.093 U, and 0.057 U, respectively (Fig. 3c), which was maintained at a lower level compared with the amylase (Fig. 3a) and proteinase activity (Fig. 3d). This could be due to fats (Table 2) being harder to be degraded than proteins and starches, whereby its presence in large quantities in the piles could impair the rate of oxygen transfer and microbial activity (Li et al., 2020), leading to the inhibition of lipase activity. Interestingly, on D9, the lipase activity in R3 (0.093 U) was the highest and significantly different from the other treatment groups (R1 [0.018 U], R2 [0.009 U], and R4 [0.025 U]) ($P < 0.05$). This could be due to a higher abundance of *Pseudomonas* (Fig. 4b) in R3 than in R1, R2, and R4, given that *Pseudomonas* is a lipase producer (Abooalfazl et al., 2014). Overall, the levels of lipase activity levels were significantly different among the four groups ($P < 0.05$), and the microbial inoculum had increased the lipase activity.

Protease is an important indicator of the degree of decomposition of organic matter. It is mainly responsible for the degradation of proteins and peptides (Castaldi et al., 2008). The protease activities in R1, R2, R3, and R4 had rapidly increased within 8 days and peaked at 136.74 U, 141.31 U, 148.82 U, and 147.79 U, respectively on D8 (Fig. 3d), likely as a result of piles containing abundant proteins and peptides (Castaldi et al., 2008). On D12, the protease activity in R4 reached 80.77 U, which was significantly higher than all the other treatment groups ($P < 0.05$). The higher abundance of *Pseudomonas* and *Bacillus* (Fig. 4b) in R4 than the other treatment groups could account for this phenomenon, given that both of these were proteolytic enzyme-secreting microorganisms that promote proteolytic hydrolysis (Casacchia et al., 2011; Awasthi et al., 2018a; 2018b; 2018c). In the later stage of composting, the availability of proteins and peptides availability gradually reduced, hence the protease activity gradually decreased and became stable (Castaldi et al., 2008).

All in all, the variation trend of cellulase and amylase activity in the four treatment groups were similar, and the activity of those in R1 was slightly higher than in R2, R3, and R4. Also, different additives had little effects on protease activity in this study. On the other hand, compared with R1, R2, and R4, the lipase activity in R3 peaked earlier and appeared higher level. Except for the lipase activity, the treatment without antibiotics was better than that with antibiotics.

3.5. Changes in microbial communities during composting

To investigate the variation in the diversity and richness of microbial communities in different treatment groups, the alpha diversity indices (Simpson, Chao1, and ACE) are statistically analyzed. Greater Simpson index signifies lower community diversity. On the other hand, larger Chao1 and ACE indexes indicate higher microbial community richness

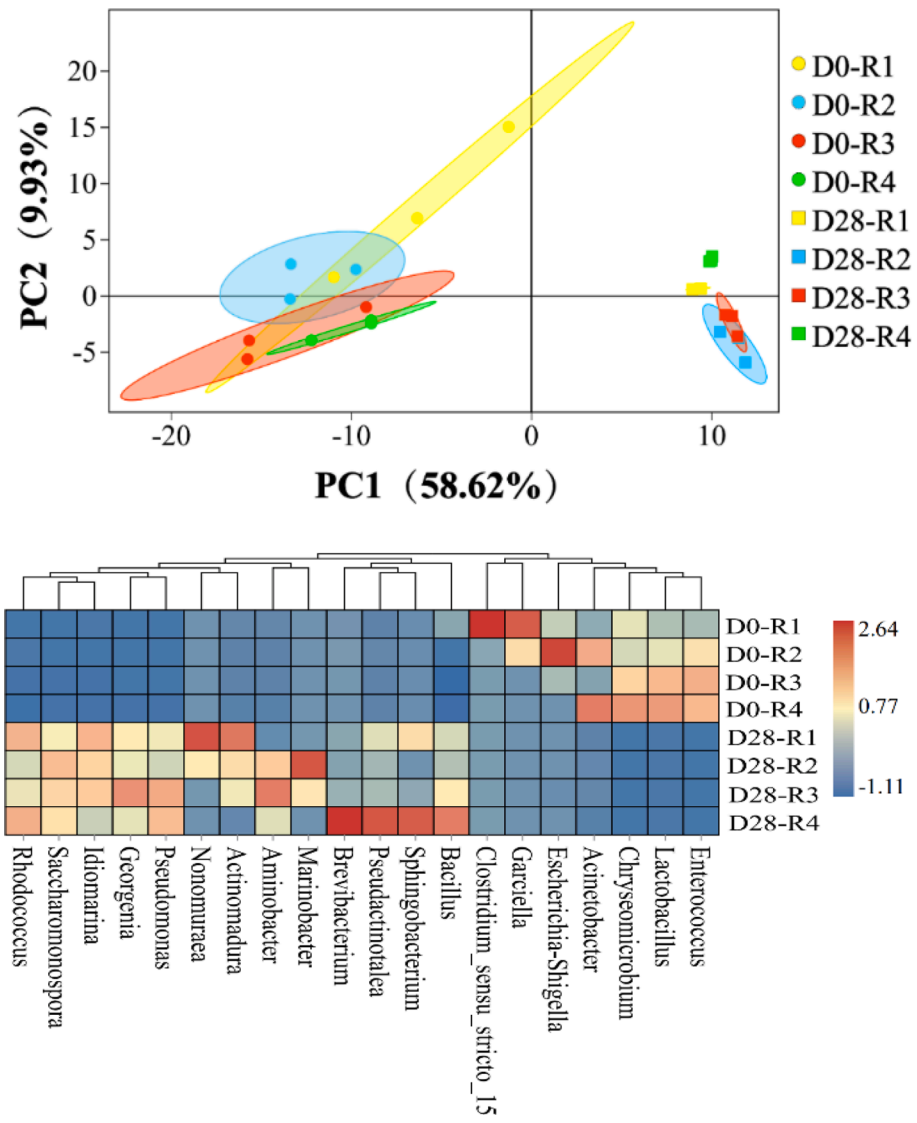

Fig. 4. Principal component analysis (PCA) based on the total operational taxonomic units (OTUs) among composting treatments (a) and microbial community composition at the genus level (b) during the composting process.

(Yu et al., 2018). The T-test revealed that the microbial community richness and diversity on D0 were significantly higher than those on D28 in all treatment groups ($P < 0.05$). As presented in Table 4, the Simpson index showed an increasing trend from D0 to D28, suggesting that the microbial community diversity increased after 28 days of composting, which was consistent with the findings by Zhang et al. (2016). A large increment of the Simpson index between R3 and R4 (0.027 and 0.008, respectively) was observed, which suggested that low concentration of antibiotics promoted microbial community diversity while a high concentration of antibiotics resulted in the opposite effect. The Chao1 and ACE diversity indices decreased from D0 to D28, indicating that the microbial community richness decreased during composting, which could be explained by the high composting temperature resulting in the removal of thermolabile bacteria (Liao et al., 2019).

Principal component analysis (PCA) is commonly used to investigate the similarity between different samples. The higher the similarity between the compositions of samples, the closer the distance reflected in the PCA diagram. The similarity in the microbial community among the four treatment groups in the composting process was demonstrated in Fig. 4a, with PC1 and PC2 demonstrated 58.62% and 9.93% of the total variation, respectively. The structure of the microbial community changed significantly in different stages of composting, which was consistent with the previous study by Zhang et al. (2016). R1 was far away from the other three groups on D0, which could be due to the addition of microbial inoculum and resulting in the variation of the microbial community in R2, R3, and R4 at the initial stage of

Table 4
Alpha-diversity of microbial community structure during composting in different groups.

Sample Name	R1		R2		R3		R4	
Alpha diversity	D0	D28	D0	D28	D0	D28	D0	D28
Simpson	0.967	0.983	0.955	0.972	0.950	0.977	0.967	0.975
Chao1	2868.393	2096.209	2966.863	2281.542	2834.311	2102.489	2880.088	2102.380
ACE	2973.18	2178.335	3061.929	2371.950	2884.413	2171.149	2977.682	2203.334

composting. On D28, the samples of R1, R2, R3, and R4 were close to each other. This might be associated with microbial inoculum and antibiotics which had caused a transient disturbance to the microbial community (Fig. 4b) at the early stage but had a lesser effect at the later stage of composting. Our findings were consistent with the results of a previous study by Ma et al. (2018) that the samples were obviously classified into the heating phase and cooling phase with the progress of composting.

The microbial community also demonstrated distinct variation at the genus level during composting, with those top 20 of the community outlined in Fig. 4b. The dominant microbials observed were *Enterococcus*, *Pseudomonas*, *Idiomarina*, *Lactobacillus*, and *Bacillus*. *Enterococcus* was a food-borne pathogen. The relative abundance of *Enterococcus* appeared highest on D0, with R3 (41.08%) and R4 (40.17%) being significantly higher than R1 (18.08%) and R2 (32.73%). This phenomenon observed might be secondary to the antibiotic-induced proliferation of *Enterococcus*. However, towards the late stage of composting, the presence of *Enterococcus* was barely observed in all 4 treatment groups (R1, R2, R3, and R4, with the relative abundance of 0.37, 0.28, 0.24, and 0.40%, respectively), suggesting that composting could effectively remove pathogenic microorganisms (Liao et al., 2018). *Pseudomonas* is a mesophilic bacterium, with the abundance of it in R1, R2, R3, and R4 were 0.49, 0.51, 0.29, 0.13%, respectively. Evidently, the abundance of *Pseudomonas* increased in the late stage of composting in all groups (R1, R2, R3, and R4, with the abundance of 13.05, 11.09, 18.97, and 17.57%, respectively), corresponding to the stage of the composting mainly comprised of refractory substances of which *Pseudomonas* represented the major decomposer of complex polymers such as lignocellulose. The relative abundance of *Pseudomonas* of R3 and R4 were higher than that of R1 and R2, possibly as a result of antibiotics changing the succession of the microbial community (Casacchia et al., 2011). In our study, from D0 to D28, *Idiomarina* in R1, R2, R3, and R4 increased from 0.46, 0.34, 0.12, and 0.040% to 11.79, 10.31, 10.63, and 6.71%, respectively. In the late stage of composting, as the piles had shrunk and the moisture content decreased while the salinity had increased, these conditions provided a conducive environment for the growth of *Idiomarina* and contributing to its abundance on D28 and higher than that on D0. On the contrary, *Lactobacillus* was largely abundant on D0 especially in R2, R3, and R4, likely from the microbial inoculum containing *Lactobacillus* used in this experiment. On D28, the relative abundance of *Lactobacillus* decreased to<0.08%. Given that *Lactobacillus* is acidogenic bacteria and more suited in acidic conditions, the high pH of the piles to above 7 (Fig. 2a) in the late stage of composting produced an environment not conducive to the growth of *Lactobacillus*, resulted in the reduction of *Lactobacillus* abundance greatly (Wang et al., 2019). Besides, the temperature during the initial composting was low, which was not conducive to the growth of *Bacillus*. When the temperature in the piles raised to higher than 50℃, *Bacillus* as a heat-resistant bacteria rapidly proliferated and dominated in the thermophilic period of composting and secreted large quantity of amylase (Fig. 3a) and protease (Fig. 3d) for the biodegradation of organic matter (Awasthi et al., 2018a; 2018b; 2018c). In addition, *Bacillus* also hydrolyzed hemi-cellulose, cellulase, and lignin (Fig. 3b), which accelerated the degradation of macromolecular substances in the piles and promoted stability (Awasthi et al., 2018a; 2018b; 2018c). The relative abundance of *Bacillus* in R4 was higher than the other groups, this could be attributed to temperature variation, given that *Bacillus* was positively correlated with temperature and the high temperature had lasted longer in R4 (Wang et al., 2018).

Redundancy analysis (RDA) is a multivariate regression method assuming linear response of species to the underlying environmental gradient, which can be used to assess the potential relationship between the species and the environmental variables (Lee et al., 2012). In our study, redundancy analysis (RDA) was conducted to evaluate the potential effects of physiochemical properties on the microbial community structure during composting. As demonstrated in Fig. 5, RDA1 and RDA2 accounted for 91.55% of the total explanatory variable. The most important variable among the selected environmental parameters was the pH, followed by the T and TN. Also, pH and T were significantly associated with the microbial community ($P < 0.05$), which was consistent with previous studies (Awasthi et al., 2018a; 2018b; 2018c). A positive correlation between pH and microbial community in R1, R3, and R4 on D28 was observed but especially in R1, indicating that the microbial community in R1 was more sensitive to pH variation than in R3 and R4. Composting samples were obviously distributed to either the rising phase or the cooling phase. In R1 and R2, a different microbial community was observed in both D0 and D28, indicating that the microbial inoculum used in this study resulted in the succession of the microbial community. On the other hand, while R3 and R4 also had similar microbial communities in both D0 and D28, they were dissimilar from those in R1 and R2, suggesting that the addition of antibiotics affected the microbial community structure. All in all, our findings established that composting conditions should be optimized to improve composting efficiency.

4. Conclusion

In the process of aerobic co-composting of FW with SS, the activity of hydrolase was significantly influenced by antibiotics. The dominant bacterial genera of the R1, R2, R3, and R4 were *Enterococcus*, *Pseudomonas*, *Idiomarina*, *Lactobacillus*, and *Bacillus*. Furthermore, the diversity and richness of microbial communities were affected by antibiotics. The RDA analysis revealed that the pH and temperature were the most important environmental factors that affected microbial community succession. These findings provide further knowledge in the treatment of perishable organic solid waste and will shed light on strategies to improve the co-composting efficiency in the future.

CRediT authorship contribution statement

Zhou Chen: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Yanzeng Li:** Data curation, Formal analysis, Investigation, Methodology, Writing - review & editing. **Yanyan Peng:** Formal analysis, Investigation, Methodology. **Chengsong Ye:** . **:** Formal analysis, Data curation. **Shenghua Zhang:** Funding acquisition, Writing - review & editing, Supervision.

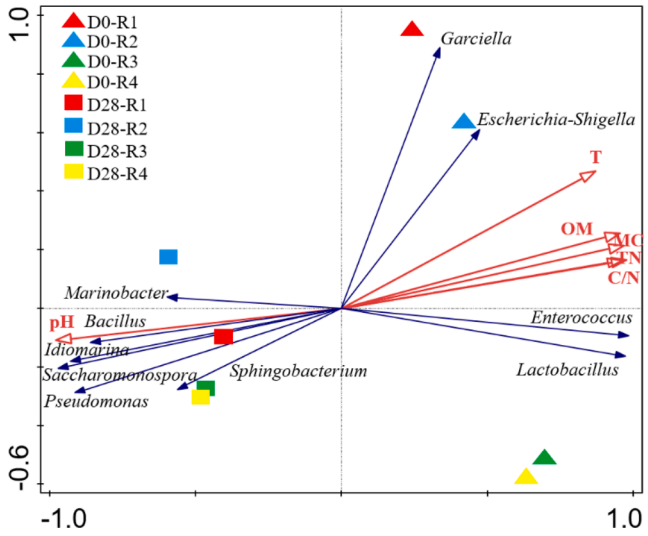

Fig. 5. Redundancy analysis (RDA) of selected environmental parameters and top 10 genera of composting samples.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2020.124506.

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