Effects of Pilot-Scale Co-Composting of Gentamicin Mycelial Residue with Rice Chaff on Gentamicin Degradation, Compost Maturity and Microbial Community Dynamics

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Research Article

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Effects of pilot-scale co-composting of gentamicin mycelial residue with rice chaff on gentamicin degradation, compost maturity and microbial community dynamics

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Abstract

Purpose It is challenging to treat and manage gentamicin mycelial residue (GMR) due to the high residual antibiotic content in GMR. The objective of this study is aimed to investigate the viability of recycling GMR by co-composting with rice chaff, describe the dynamics of the physicochemical and biology parameters and microbial community, and evaluate the maturity of the compost products.

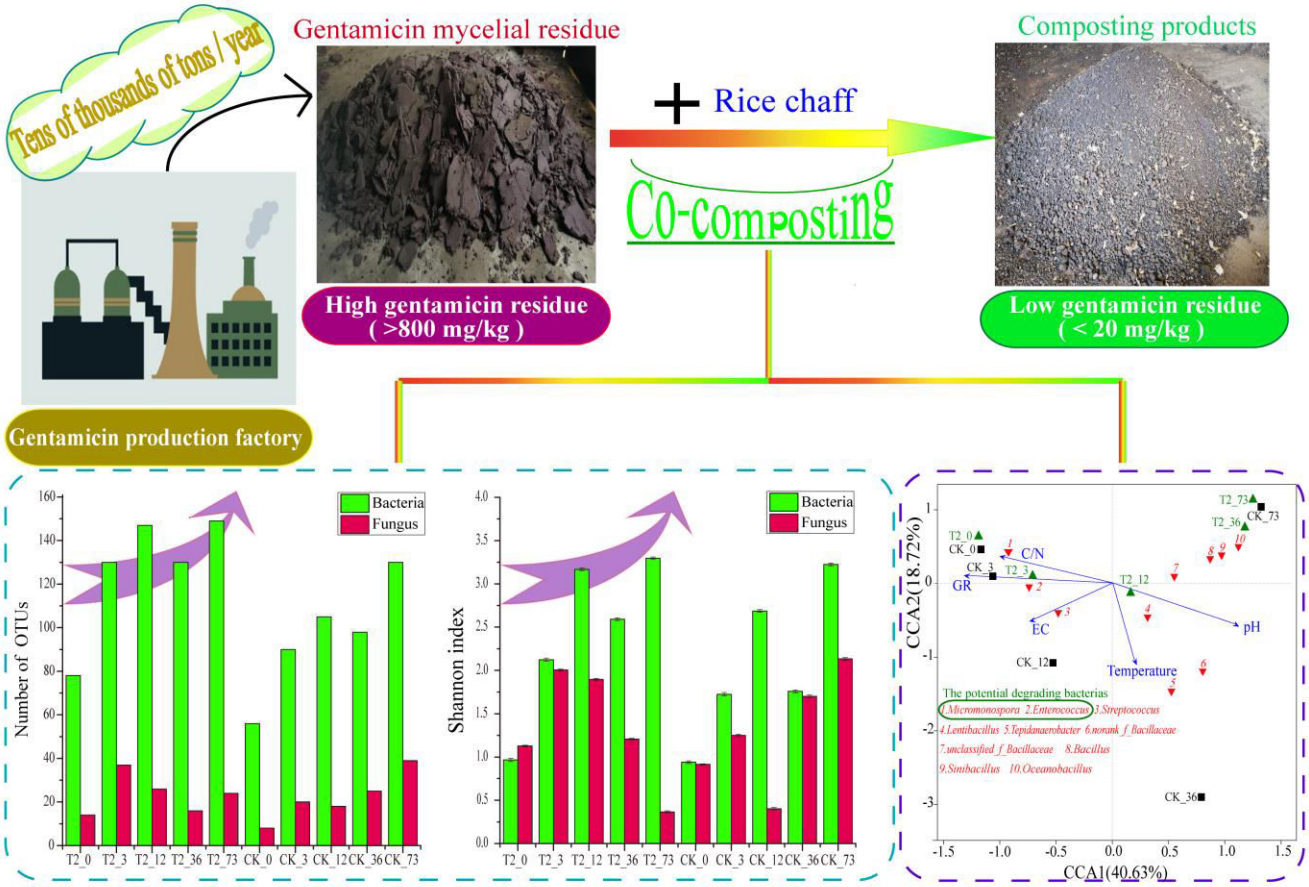
Methods The co-composting process applied was a pilot-scale composting process under the conditions found outside the laboratory. Three 1-tonne piles of fresh GMR were composted in each treatment; test treatment contained rich chaff and the control did not. Dried GMR was made by drying fresh GMR. Three compost treatments were as follows: (1) no rice chaff, 8:1 weight/weight fresh GMR-to-dried GMR (CK); (2) 8:1 weight/weight fresh GMR-to-rice chaff (T1); and (3) 4:1 weight/weight fresh GMR-to-rice chaff (T2). The raw materials were made into three compost cones of 2 m diameter and 1.5 m height.

Results The optimal fresh GMR:rice chaff ratio (w/w) was 4:1. Over 99% of gentamicin was degraded after 73 days of co-composting. The key parameters of the final products, such as the pH, C/N ratio, germination index and crop growth indexes, all met the national standards for compost maturity indicators. Compared with those of fungi, the abundance and diversity of bacteria obviously increased during co-composting. Canonical correlation analysis (CCA) revealed that the bacterial community dynamics were closely correlated with the amount of residual gentamicin. *Micromonospora* and *Enterococcus* may have been the key microorganisms degrading the gentamicin.

Conclusion The addition of rice chaff improved the decomposition of gentamicin residue in the GMR and made the GMR usable in fertilizer; this result could help antibiotic production factories recycle

more of their waste products. The results provide new insight into the potential for co-composting with rice chaff to achieve sustainable GMR management.

Graphic Abstract



Statement of Novelty

The antibiotic production factories biowastes containing the high residual antibiotic will be a potential threat to ecological environmental systems and eventually human health. However, as antibiotics mycelial residue contains high levels of organic nutrients such as protein and crude fat, it may have potential to become a valuable organic fertilizer. The aims of our study is to explore a feasible disposal of antibiotics mycelial residue to reduce the environmental impact, and concurrently recycle nutrients.

Keywords Gentamicin mycelial residue · Co-composting · Gentamicin degradation · Rice chaff · Microbial diversity

Introduction

Gentamicin is a crucial aminoglycoside antibiotic reliably used to cure serious and life-threatening infections mainly caused by gram-negative bacteria in humans and animals [1]. Gentamicin mycelial residue (GMR), containing gentamicin and its precursors, is a major byproduct, with tens of thousands of tons produced per year in China. High concentrations of antibiotic residue have made GMR treatment an urgent environmental challenge. Inappropriate treatment of GMR may make gentamicin spread into the natural environment, which will increase drug-resistant bacteria and be a potential threat to ecological environmental systems and eventually human health. In 2008, GMR was classified as hazardous waste, which is treated with approved methods, including incineration, biomass pyrolysis, removal to landfills and other treatment technologies [2]. However, the costs of incineration and biomass pyrolysis are high. In landfills, a large amount of GMR will not only affect a large land area but also potentially leach toxic chemicals to soils, surface water and even groundwater. Furthermore, some studies have shown that antibiotics in soil can induce environmental microorganisms to produce antibiotic resistance genes (ARGs) and enhance their adaptability to antibiotics [3]. Pathogens have transmitted ARGs, becoming antibiotic resistant in clinics [4] and subsequently harming human health and public safety. Antibiotics can also affect plant growth, potentially leading to crop losses or even food shortages. Zhang et al. [5] showed that sulfadimidine could reduce folic acid synthesis and ultimately inhibit Arabidopsis growth by inhibiting DNA synthesis. Boxall et al. [6] found that enoxacin significantly inhibited the growth of carrots and lettuce. As GMR contains high levels of organic nutrients such as protein and crude fat, it may have potential to become a valuable organic

77 fertilizer. However, as yet there has been few research on pilot-scale composting of GMR. Therefore, it
78 is urgent to find a sustainable method to degrade residual gentamicin in GMR and concurrently recycle
79 nutrients.

80 Composting is an effective and suitable technology for transforming organic residues such as sludge
81 and agricultural waste into fertilizer or conditioner [7]. At present, increasing attention has been paid to
82 removing antibiotics in livestock manure or antibiotic mycelial residue by composting to stabilize the
83 remaining organic matter and inhibit pathogens. And then the final compost product may be used to
84 fertilize farmland, thus ensuring that crops absorb most of the essential nutrients needed for growth and
85 development. Ezzariai et al. [8] investigated whether composting sludge or manure could be effective
86 in reducing the levels of antibiotics and ARGs. The results showed that the antibiotic removal ranged
87 from 17-100%. Zhang et al. [9] suggested that under the condition of laboratory aerobic co-composting
88 was a practicable method to treat penicillin fermentation fungal residue (PFFR). Similarily, Zhang et al.
89 [10] also demonstrated that aerobic composting could be a practicable programme to remove 64.7% of
90 the detected antibiotic in swine manure. However, sewage sludge can not be composted alone because
91 of its high moisture content (about 80%) and poor air permeability [11], and therefore must be mixed
92 with bulking agents to reduce the moisture content and improve the porosity of composting raw
93 materials [12].

94 Mature compost is used to fertilize and improve soil organically and safely, so firstly, it must be
95 non-toxic and then supply nutrients and structure to soil and plants [13]. Hence, the parameter of
96 maturity is often used to evaluate the stability and safety of compost. Researchers generally believe that
97 it is hard to confirm the chemical and biological stability of compost with a single parameter, and
98 several or more parameters should be used to estimate the stability [13, 14]. Using physicochemical

methods, biological activity and plant toxicity analysis, it is more credible to versatilely observe the compost maturation and stability. In these studies, the moisture content, temperature, pH, electrical conductivity (EC), C/N ratio and germination index (GI) were measured to assess maturity [9, 10]. So, applicable monitoring methods would measure both the physicochemical and the plant growth indicators, as well as the relationships between the physicochemical and the biological indicators, during the whole composting process. Furthermore, the time period of composting and overall content of the compost were highly correlated with microbial composition and diversity [15]; composting was fundamentally a dynamic process in which microbial flora play a key role, and composting quality can be measured by analyzing the activity and diversity of microorganisms [14]. Therefore, the analysis of microbial communities may contribute to uncovering the mechanisms of antibiotic degradation during composting. However, few reports have been done on the treatment and management of GMR as a bioresource, e.g., a fertilizer, from pilot-scale co-composting experiments. Hence, a pilot-scale experiment on the co-composting of GMR and rice chaff was performed in this study. Moreover, the gentamicin degradation process, physicochemical characteristics, biologic parameters and microbial community evolution were analyzed to provide more information about full-scale composting treatment of GMR. All experiments involved pilot-scale composting under the conditions found outside the laboratory.

Materials and Methods

Feedstock and Sample Collection

Fresh GMR and dried GMR were provided by Henan RenHua Biotechnology Co., Ltd. Dried GMR was made by drying fresh GMR. Rice chaff used as bulking agents to increase the porosity and adjust the C/N ration, were obtained from a local farm. The main physicochemical properties of the

feedstocks are listed in Table 1.

Table 1 Physicochemical properties of the feedstocks. Values are means (±SD) : n = 3.

Parameter	Fresh GMR	Dried GMR	Rice chaff
pH	6.47±0.14	6.02±0.09	6.05±0.11
Moisture (%)	66.04±0.69	16.12±0.07	10.55±0.05
OM[a] (%)	80.77±0.21	44.78±0.22	78.28±0.19
TN[b] (%)	2.38±0.15	2.05±0.12	0.34±0.02
C/N	19.65±0.82	12.66±0.04	116.18±7.66

[a] means organic matter; [b] means total nitrogen.

Experimental Procedure and Sampling

The experiment was carried out in a plant of Henan RenHua Biotechnology Co., Ltd, from July to September, during a period of 73 days. The feedstocks were mixed for composting in three treatments, all including a fixed 1000 kg fresh GMR. The compost treatments were as follows: (1) no rice chaff, 8:1 weight/weight fresh GMR-to-dried GMR (CK); (2) 8:1 weight/weight fresh GMR-to-rice chaff (T1); and (3) 4:1 weight/weight fresh GMR-to-rice chaff (T2). After being manually mixed, the raw materials were made into conical piles with approximately 1.57 m^3 each (diameter × height: 2 m × 1.5 m) as illustrated in Table 2. These treatments included artificial turning every three days to increase the oxygen intake of the materials. Meanwhile, the moisture content was tested and adjusted to 50-60% with tap water.

Table 2 Design of the experiment

Treatment	Fresh GMR (kg)	Dried GMR (kg)	Rice chaff (kg)
CK	1000	125	-
T1	1000	-	125
T2	1000	-	250

Approximately 500 g of composting material was randomly obtained from each pile at a depth of 50 cm and mixed to obtain a cross-sectional and homogeneous sample. One part of each sample was preserved at -20°C for molecular assessment of microbial diversity and determination of gentamicin concentrations. Another part of the three samples was used to measure other physicochemical properties and the GI.

Physicochemical Parameters

During composting, the temperatures at five locations (the midline of north-south-east-west and the top, 25-30 cm) in the compost pile and ambient temperature were measured twice per day. The moisture contents of the composting samples were measured by drying the samples to reach a constant weight at 105°C in an oven. 10 g fresh samples were extracted with deionized water 1:10 (w/v) for 5 h. The pH value and EC of the suspensions were measured by a pH meter (Mettler Toledo Co., Shanghai, China) and an EC meter (Leici Chuangyi Instrument Co., Shanghai, China), respectively. The sample stored at 4°C was subjected to total nitrogen (TN) determination by the Kjeldahl digestion method [16]. The quantitative organic fertilizer samples were mixed with concentrated sulfuric acid, added H_2O_2 into the mixture, and then put it into a graphite digester, which was digested at 380℃, and the organic nitrogen was converted into ammonium nitrogen. After digestion, it was distilled with automatic Kjeldahl apparatus and titrated with HCl. According to the China national organic fertilizer standard (NY 525-2012), organic matter (OM) in the sample was determined by the potassium dichromate method.

According to the consumption of potassium dichromate and sulfuric acid before and after oxidation, the organic carbon content was calculated, and the organic carbon content multiplied by coefficient 1.724 was the organic matter (OM) content of mycelial residue compost. So the C/N ratio was calculated with the following formula: $C/N = \frac{OM}{1.724 \times TN}$ [17, 18].

Biological Parameters

The phytotoxicity analysis includes the germination test and the plant growth, which can give an intuitive indication of compost maturation. The germination test was carried out by using cabbage seeds. The GI was measured as described in [19]. Ten milliliters of compost filtrate of each sample was placed in a sterilized dish containing two pieces of filter paper. Ten cabbage seeds were cultured in each dish for 48 h at 25±1°C. Each treatment was repeated three times with distilled water as blank control. The seed germination percentage and root lengthening were determined, and the GI (%) was calculated based on the following formula: GI (%) = (seed germination percentage (%) × mean of root lengthening in treatment) × 100%/(seed germination percentage (%) × mean of root lengthening in control).

Chinese cabbage has a short growth period and is easy to cultivate and sensitive to pollutants, so it is often used in experiments to evaluate pollutant absorption and toxicity effects. The Chinese cabbage seeds were purchased from a seed store in Zhengzhou. This study was designed to include four treatment groups: cow manure/soil (2 g/kg, CM treatment), gentamicin compost product/soil (2 g/kg, GL treatment), gentamicin compost product/soil (4 g/kg, GH treatment) and soil (CK treatment). The soil was obtained from bare soil near the south gate of Zhengzhou University and was moisture soil. Plastic pots were each filled with 3 kg of soil, and the soil moisture content was kept at the maximum soil water capacity. Ten seeds were planted in each pot. The cabbages were harvested after 75 days of

175 growth. Ten Chinese cabbages with uniform growth from different pots were selected and cleaned with
176 deionized water. Their emergence rate (MR), height from roots to top (Height), fresh weight (Weight)
177 and gentamicin residue (GR) were measured. Each processing group consisted of four independent
178 replicates.

179 **Determination of Gentamicin Concentrations**

180 Gentamicin is easily dissolved in water and adsorbed by resin in acidic environments and desorbed in
181 alkaline environments. By changing the pH value of the extract, the GR in the compost samples was
182 extracted. Five grams of freeze-dried samples were diluted 1:40 in distilled water. The solution pH was
183 first adjusted to 4.5 with oxalic acid and then 1.5 with dilute sulfuric acid. The suspensions were
184 incubated for 1 h at 60°C at 120 rpm. After the incubation period, the liquids were left, and the solids
185 were centrifuged. The solids were cleaned once with 10% methanol solution (water: methanol = 90:10),
186 and the resulting suspensions were collected. Two grams of resin was added to the supernatant
187 (adjusted to pH 4.0 using 40% sodium hydroxide) and incubated for 1 h at 45°C and 120 rpm. Then,
188 the shaker was run at 20°C for 1 h at 120 rpm. The suspensions were discarded, and the resin was
189 collected in a conical flask. One hundred milliliters of 5% ammonia water was poured into the conical
190 flask, and it was placed in the shaker at 30°C and 120 rpm for 3 h. Finally, the liquid was evaporated
191 and collected at 60°C at 80 rpm. The resulting extracts were fully dissolved with ultrapure water to 10
192 ml and filtered through a 0.22 μm pore size membrane. Gentamicin concentrations were measured
193 using HPLC according to the Chinese Pharmacopoeia (2015).

194 The gentamicin degradation percentage (GDP) was calculated by the following formula:

195 $$GDP(\%) = \frac{C_0 - C_t}{C_0} \times 100\%;$$

196 where

C_0: the initial gentamicin concentration in compost, mg/kg;

C_t: gentamicin concentration in compost on day t, mg/kg.

Dynamic Model of Gentamicin Biodegradation during Composting

A pseudo-first-order kinetics reaction equation was used to reflect the dynamics of antibiotic biodegradation [20, 21]. Thus, in this study, the biodegradation dynamics of gentamicin were fitted with the following formula: $C_t = C_0 e^{-kt}$, where C_0 and C_t were the same as in the above formula, and k was the rate constant of antibiotic degradation (day^{-1}). Using this equation, the half-life of gentamicin was calculated as follows: $t_{1/2} = -\ln 2/k$.

Microbial community diversity and structure

To determine the bacterial and fungal diversity during the composting process, microbial community genomic DNA was extracted from each pile in the initial phase (day 0), mesophilic phase (day 3), thermophilic phase (day 12 and 36), and cooling phase (day 73) using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the changes of pile temperature. The DNA extract was checked on 1% agarose gel, and DNA concentration and purity were determined with NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). High-throughput sequencing of the 16S and 18S rRNA genes was performed using the Illumina MiSeq PE300 platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The V3-V4 hypervariable region of the bacterial 16S rDNA was amplified with the primers 338F (ACTCCTACGGGAGGCAGCAG') and 806R (GGACTACHVGGGTWTCTAAT'), purified, quantified, and sequenced. Primers SSU0817F (TTAGCATGGAATAATRRAATAGGA) and 1196R (TCTGGACCTGGTGAGTTTCC), targeting the V5-V7 hypervariable region of the fungal 18S rDNA, were chosen for amplification and subsequent sequencing of the polymerase chain reaction

(PCR) products.

Coupled reads in the raw sequences were merged into a sequence, and quality control and filtration were performed to adjust for read quality and splicing effects. Sequences with a quality score < 20 in the tail were removed, and the sequences longer than 50 bp were retained. Operational taxonomic units (OTUs) with 97% similarity cutoff were clustered using UPARSE (version 7.1, http://drive5.com/uparse), and chimeric sequences were identified and removed.

Statistical Analysis

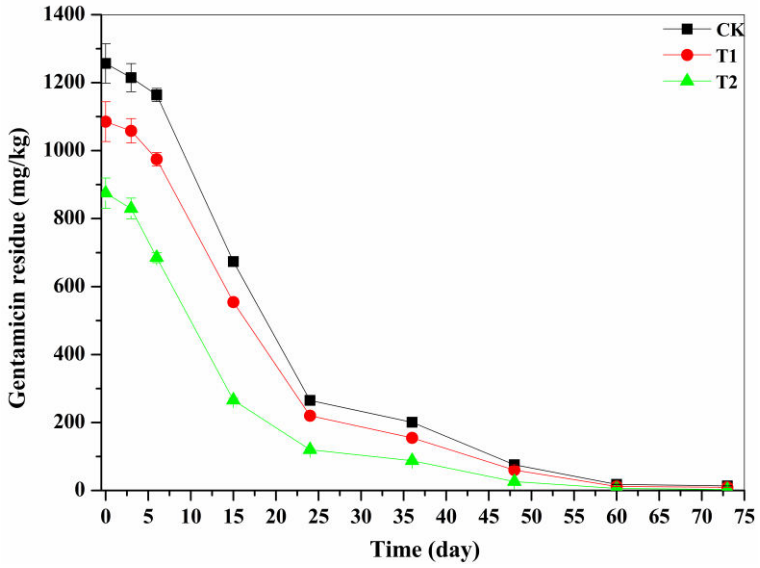
The abundance and diversity of the microbial community was evaluated with the alpha diversity. Based on the relative abundances of OTUs, the Chao1 index for community richness, Shannon index for community diversity, and community coverage were calculated. Correlations between bacterial communities and physicochemical properties were assessed using canonical correlation analysis (CCA). The correlations between the ten most abundant bacteria and environmental factors were revealed by Spearman's correlation coefficients. SPSS 19.0 software was used to analyze gentamicin degradation and physicochemical parameters. Origin 9.0 and CANOCO 5.0 were used to generate graphs in the study.

Results and Discussion

Changes in Gentamicin Residue

A number of previous studies have shown that composting has been generally studied as an econmical and environment-friendly method to treat antibiotic residues [9, 10, 22]. Figure. 1 shows the evolution of gentamicin residues during 73 days of co-composting. In the initial compost samples, CK, T1 and T2 exhibited gentamicin concentrations of 1257 mg/kg, 1085 mg/kg and 875 mg/kg, respectively. In the first 15 days of composting, the gentamicin concentrations decreased sharply in all three treatments,

241 and the degradation percentages were all above 63%. At the end of composting, the residual
242 concentrations of gentamicin in CK, T1 and T2 were 13.71 mg/kg, 9.31 mg/kg and 3.0 mg/kg,
243 respectively. This established the effectiveness and operability of co-composting of GMR and rice
244 chaff for removal of residual gentamicin. Among them, the GDP in T2 was the highest, 99.66%. In T2,
245 the content of rice chaff was the highest (250 kg), and the initial C/N ratio was also the highest. As such,
246 the proportion of GMR and rich chaff likely affects the composting process and gentmicin degradation.
247 Many studies suggested that the antibiotics degradation was closely related to microbial catabolism.
248 Ezzariai et al. [8] reported that the antibiotics degradation could be contributed by microorganism. In
249 this case, sufficient nutrition ensured the growth and metabolism of microorganisms, which had a
250 positive impact on the composting process and gentamicin degradation [23].



251

252 **Fig. 1** Gentamicin degradation during co-composting of GMR with/without rice chaff for
253 treatments*. *CK: fresh GMR and dried GMR (8:1, w:w); T1: fresh GMR and rice chaff (8:1, w:w); T2:
254 fresh GMR and rice chaff (4:1, w:w)

255 The gentamicin degradation rates of CK, T1 and T2 during the composting are shown in Table 3.
256 The first-order equation closely fitted the degradation kinetics of gentamicin, and the correlation

coefficients (R^2) were between 0.91 and 0.92. In all treatments, the reaction constant (k = 0.0260 day^{-1}) of T2 was the highest, and the half-life ($t_{1/2}$ = 22.0 days) was the shortest, demonstrating that gentamicin was degraded most quickly in the T2 compost. In contrast, the reaction constant (k = 0.0233 day^{-1}) of CK was the lowest, and the half-life ($t_{1/2}$ = 26.1 days) was the longest, demonstrating that gentamicin degradation was slowest in CK. Considering the degradation percentages and rates, a GMR-to-rice chaff ratio of 4:1 was optimal for gentamicin degradation; at this ratio, the content of rice chaff and the C/N ratio both were their highest values.

Many studies have reported that the degradation half-lives of different antibiotics in composting vary greatly, which may be related to the extensive differences in substrates and conditions [21, 24, 25]. Wu et al. [26] reported that the half-life of tetracycline was 11.75 days during composting of pig manure with mushroom residues (1:2 v/v). However, after 42 days composting of pig manure with sawdust (1:1 w/w, dw) the tetracycline degradation was 91.6%, with the half-life of 10.02 [27]. Obviously, compared with these antibiotics, gentamicin was more stable in structure and more difficult to be decomposed by microorganisms. Therefore, it is possible to modulate the microbial communities by changing the types of raw materials and physicochemical conditions of the composting, which has the potential to shorten the composting cycle and improve the composting quality [28].

Table 3 Fitted kinetic equations of gentamicin degradation during composting.

Treatments*	Fitted equation	R^2	k (day^{-1})	$t_{1/2}$ (day)
CK	$C=C_0 e^{-0.0233t-0.0808}$	0.911	0.0233	26.1
T1	$C=C_0 e^{-0.0253t-0.0518}$	0.918	0.0253	24.6
T2	$C=C_0 e^{-0.0260t-0.1170}$	0.920	0.0260	22.0

*CK: fresh GMR and dried GMR (8:1, w:w)

T1: fresh GMR and rice chaff (8:1, w:w)

T2: fresh GMR and rice chaff (4:1, w:w)

Changes in Temperature

Temperature is considered one of the key factors for successful composting operations since temperature changes are closely tied to the biotransformation of OM and dynamic changes in the microbial community [29]. It is usually used as a parameter to indicatie the good initiation and the end fo the composting progression. During the composting process, the evolution of the temperature of the material gives an indication of the efficiency of the composting process. The changes in temperature in the three treatments were recorded during composting, and the developmental trends of different phases were similar (Fig. 2). The three kinds of compost experienced the mesophilic phase (1-8 days), followed by the thermophilic phase (9-57 days) and, finally, by the cooling/mature phase (58-73 days). The ambient temperature ranged from 18°C to 39°C.

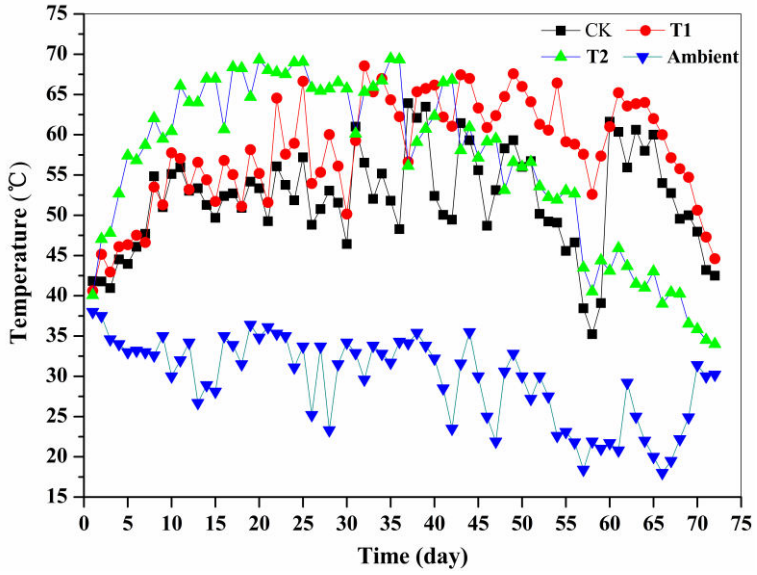


Fig. 2 Variation in temperature during co-composting for treatments*. CK: fresh GMR and dried GMR (8:1, w:w); T1: fresh GMR and rice chaff (8:1, w:w); T2: fresh GMR and rice chaff (4:1, w:w)

The first phase was characterized by heat raise from 40 to 50 °C observed in 4 days, showing a rapid

291 colonization of mesophilic microbial populations. After the 4th day of composting, the temperature of
292 the T2 treatment rose rapidly above 50°C, and the high-temperature period lasted 53 days. Compared
293 with that of the T2 treatment, the temperatures of the T1 and CK treatments slowly increased, and the
294 high-temperature period was postponed for 4 days. This rapid progress from mesophilic to thermophilic
295 phase can be attributed to the high microbial activity generated by the presence of easily degradable
296 organic compounds. In the first 42 days of composting, the average temperatures of the three
297 treatments decreased in the order T2>T1>CK. After 42 days, the temperatures of T2 and CK dropped
298 sharply, and the samples entered a cooling period; meanwhile, the average temperature of T1 was still
299 over 50°C until 70 days, when it entered the cooling period. Indeed, the conversion and biodegradation
300 of the organic matter during the two previous phases (mesophilic and thermophilic) enriched the
301 windrow in stable and hygienic mineral matter which greatly reduces the microbiological activity and
302 consequently the decrease of the temperature of the pile. However, on the 61st day, the CK temperature
303 rose rapidly from 37°C to 60°C, where it stayed for 8 days before the sample entered a cooling period.
304 These differences in behavior probably occurred because the content of gentamicin in the CK, T1 and
305 T2 treatments decreased at different times. Initially, high residual gentamicin strongly inhibited
306 microbial growth. However, with the composting process, gentamicin was gradually degraded, and its
307 inhibitory effect also gradually weakened, making more microorganisms grow rapidly. Another reason
308 might be that the porosity in the CK, T1 and T2 treatments increased at different times, which was
309 beneficial to the growth and metabolism of aerobic microbes [30]. Therefore, T2 entered the
310 thermophilic stage and completed the aging stage fastest. Microorganisms in T1 were active and
311 continued to decompose OM. In CK, most OM was decomposed in the thermophilic period. The
312 material became loose, and the oxygen content increased, which caused thermophilic microorganisms

to become active and the sample to re-enter a high-temperature period to decompose OM. In this study, the numbers of days above 50°C in the CK, T1 and T2 treatments were 46, 63 and 53, respectively. According to the health standard for harmless composting (GB7989-87), the temperature of the composting pile must be maintained at more than 50°C for 7 days to prevent the free breeding of weed seeds and pathogens. All three treatments met this standard.

Evolution of Physicochemical Parameters and Biological Parameters

Changes in pH, EC and C/N during Composting

The changes in physicochemical parameters in different piles during composting are shown in Fig. 3a-c. The pH of compost has an important influence on the metabolic activity and composition of the microbial community [31]. Attaining and holding the correct pH is important for effective composting and a pH range of 6.5-9.0 supports good microbial activity during the composting process. Thus, different metabolites are produced after the material is degraded by microorganisms [32]. In CK, T1 and T2, the pH value changed gradually (Fig. 3a). In the medium-temperature phase, the pH values of CK, T1 and T2 rose rapidly until the 30^{th} day and reached maximum values of 8.89, 9.07 and 8.94, respectively. Then, from the high-temperature period to the cooling period, they remained at approximately 8.0. In the medium-temperature phase, the mineralization of OM generally releases volatile ammonia and ammonium, leading to an increase in pH [33]. As the composting continues, thermophilic microbes work diligently to decompose organic matter and produce small molecule acids, causing the pH to fluctuate around approximately 8.0 [34]. During the cooling/maturation phase, small molecule organic content was low and NH_4^+-N gradully oxidized to NO_3^--N, thus further reducing the pH of compost. According to the China national organic fertilizer standard (NY 525-2012), the optimal pH value of compost is 5.5-8.5. During the mature/cooling periods, the pH values of CK, T1 and T2

were 8.1, 8.14 and 8.12, respectively, which was in line with the national standard.

EC reflects the mineralization of organic matter and the soluble salt content in compost. The content of soluble salt is one of the important indexes to evaluate whether it has toxicity to plants. During the composting process, EC reflects the content of soluble salt in compost extract, which affects the plant toxicity of compost. Thus, to a certain extent, EC demonstrates the maturity degree of compost products and high EC in compost is undesirable. In the medium-temperature phase, the EC values of CK, T1 and T2 increased rapidly (Fig. 3b). The EC value of T2 peaked at 4.62 mS/cm on the 9^{th} day, and the EC values of CK and T1 reached their highest values on the 12th day, at 7.19 mS/cm and 6.64 mS/cm, respectively. The increase in EC may be related to the accelerated release of a large number of small molecules and ions, which was caused by composting microorganisms rapidly decomposing organic matter and nitrogen source [35]. After 24 d of composting, EC have appeared the second increase in CK, T1 and T2 treatment, which may have due to both evaporation of water and decomposition of OM caused a net of dry matter. From 30^{th} to the end of compostin, the EC values gradually decreased and remained stable, which were 2.49 mS/cm, 2.48 mS/cm and 2.43 mS/cm, respectively, indicating that they did not inhibit seed germination [36]. EC decreased slightly, possible due to either volatilization of ammonia or precipitation of mineral salts [13].

In composting, carbon and nitrogen sources provide the energy and nutrients required by microorganisms, and the C/N ratio is considered an important indicator of the maturity of compost [37]. In CK, T1 and T2, the changes in the C/N ratio were similar (Fig. 3c). As composting progressed, the C/N ratio gradually decreased. Their initial C/N ratios were 15.66, 21.57 and 23.43, except for CK, the C/N ratio of T1 and T2 ranged between the better C/N ratio of 20-25:1, which could provide the balance nutrition for microorganismal growth. During composting, the gradual C/N ratio decline has

occurred mainly due to the decomposition of organism matter. Meanwhile some N in the raw material decomposes and was used by microorganisms, while some was discharged as NH_3, N_2, and NO_X. However, most of the N was mineralized by microorganisms as NO_3^--N in the compost. In the high-temperature phase, the compost lost more C than N as OM decomposition accelerated, so the C/N ratio gradually decreased. However, entering the cooling stage, the metabolic activity of microorganisms receded, which resulted in little OM and TN changes and a steady C/N ratio. At the end of composting, the ratio C/N finally decreased to 7.13, 7.48 and 8.6, respectively. Studies have shown that compost is mature and safe when its solid-phase C/N ratio decreases from an initial (20-30):1 ratio to under (15-20):1 [9]. T1 and T2 met these conditions, suggesting that they were mature compost. The initial C/N ratio for the CK sample was less than 20, which was not suitable for the composting treatment. Another criterion for evaluating maturity is T = (endpoint C/N ratio)/(initial C/N ratio); when T<0.7, compost is deemed to be mature [38]. The T value of the CK treatment was 0.46, indicating that the CK compost reached the criterion of maturity.

Changes in Germination Index during Composting

If the compost final product was unstable and immature, it would be harmful for seed germination, plant growth, and the soil environment due to the phytotoxic compounds was created by the lack to oxygen exchange and bioavailable N. The GI is relevant to the plant toxicity of compost products and is a quick and efficient indicator of compost maturity. The GI values of CK, T1, and T2 were 0% at the beginning of composting (Fig. 3d), indicating that these samples were highly toxic to plants. The GI values gradually increased during composting until the end, which were 78.4%, 80.2% and 96.06%, respectively. According to Luo et al. [39], if a GI value is higher than 80%, the compost is usually not toxic to plants. Therefore, the T1 and T2 composts were believed to be nonphytotoxic and mature. In a

word, the results demonstrated a better physicochemical and biological performance was shown in 4:1 weight/weight fresh GMR-to-rice chaff (T2).

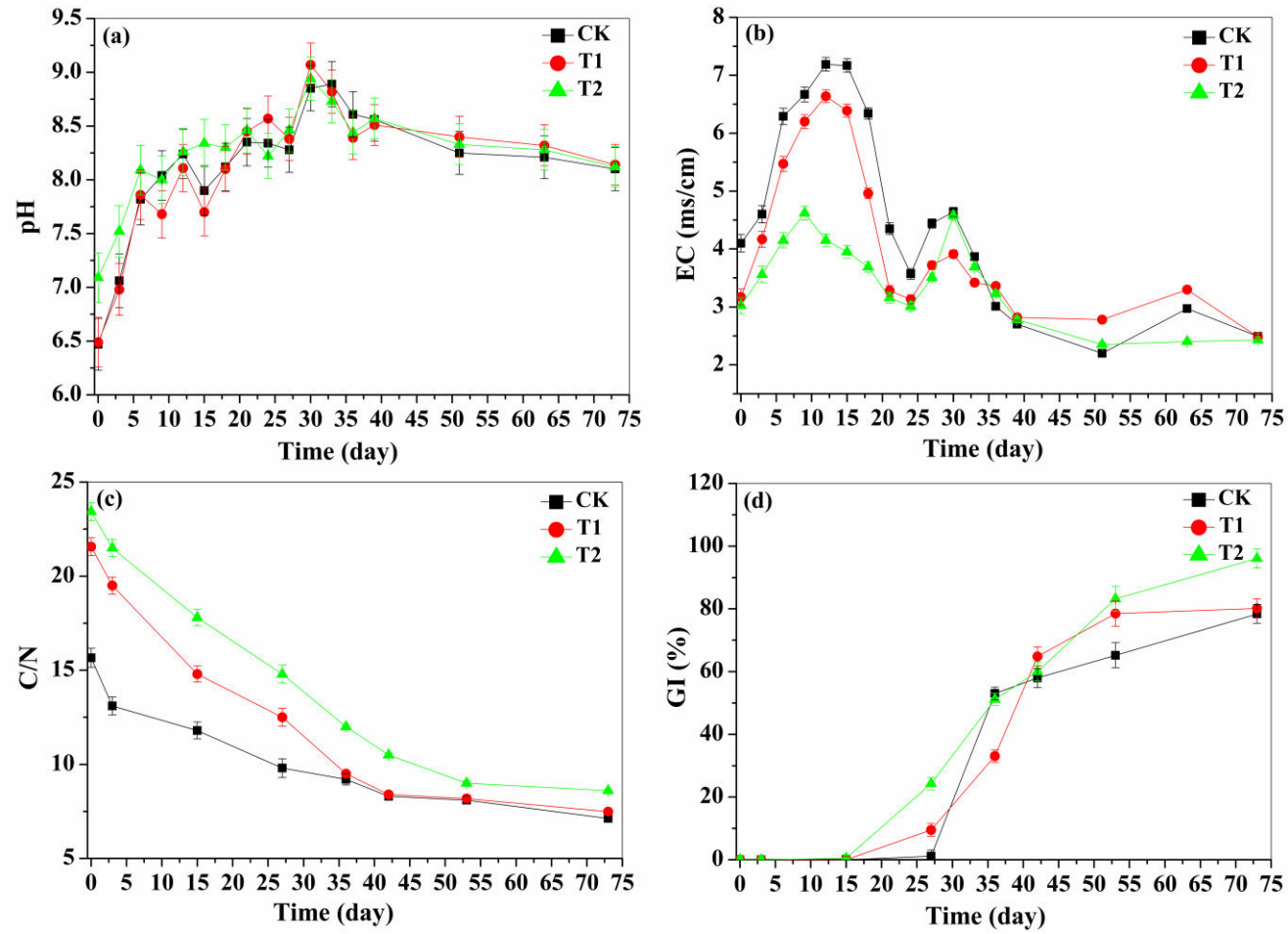


Fig. 3 Variations in pH (**a**), EC (**b**), C/N (**c**) and GI (**d**) during co-composting for treatments*. CK: fresh GMR and dried GMR (8:1, w:w); T1: fresh GMR and rice chaff (8:1, w:w); T2: fresh GMR and rice chaff (4:1, w:w)

Correlations between Parameters

Composting is actually an aerobic biodegradation of organic matter by microorganisms, with the microorganisms playing a key role [37]. However, the growth and metabolic activities of microorganisms are also affected by environmental factors. The high residual gentamicin in GMR may influence microbial activity and even inhibit composting. Correlations among the parameters were analyzed, and they are shown in Table 4. In the CK, T1 and T2 treatments, the GR was significantly

correlated with the pH, C/N ratio and GI, indicating that the degradation of gentamicin was related to the properties and proportions of the compost raw materials [40]. The GDP might be improved by further changing the raw materials, their proportions or the compost conditions.

Table 4 Correlations between parameters for T1, T2 and CK composts.

Parameter	Temperature	pH	EC	C/N	GI	GR
T1 treatment*						
Temperature	1	0.784*	0.050	-0.615	0.352	-0.662
pH		1	-0.255	-0.938**	0.657	**-0.959****
EC			1	0.413	-0.680	0.397
C/N				1	-0.854**	**0.986****
GI					1	**-0.804***
GR						1
T2 treatment*						
Temperature	1	0.579	0.596	0.005	-0.331	-0.235
pH		1	-0.061	-0.779*	0.510	**-0.918****
EC			1	0.642	-0.847**	0.397
C/N				1	-0.936**	**0.942****
GI					1	**-0.779***
GR						1
CK treatment*						
Temperature	1	0.751*	-0.100	-0.415	0.237	-0.496
pH		1	-0.344	-0.892**	0.622	**-0.918****

EC	1	0.582	-0.815*	0.565
C/N		1	-0.836**	**0.967****
GI			1	**-0.802***
GR				1

*CK: fresh GMR and dried GMR (8:1, w:w)

T1: fresh GMR and rice chaff (8:1, w:w)

T2: fresh GMR and rice chaff (4:1, w:w)

*: $0.01 \leq p < 0.05$; **: $p < 0.01$.

Crop Growth Indexes

In order to further determine the plant toxicity and maturity of the composting products, pot experiments were conducted with Chinese cabbage. The gentamicin compost product was prepared by co-composting GMR with rice chaff (4:1) for 73 days. The compost final product had the following basic physical and chemical properties: pH, 8.12; moisture content, 24%; organic matter content, 58.41%; total C, 33.88%; total N, 3.94%; C/N, 8.60; residual concentration of gentamicin, 3.0 mg/kg. The emergence rate and quality of Chinese cabbage in different treatments are shown in Table 5. The emergence rate, plant height and fresh weight of Chinese cabbage growing for 75 days in the different treatments decreased in the order GH>GL>CM>CK, indicating that the gentamicin composting product promoted crop growth better than ordinary cow manure and that high fertilizer application was beneficial to crop growth. The soluble sugars in vegetables mainly include glucose, fructose, sucrose, and others. The soluble sugar content reflects the taste of vegetables to a certain extent, so it can be used as an important indicator of vegetable quality. The soluble sugar level in the different treatments was GL>CM>GH>CK. No GR was detected by HPLC in the stems and leaves of Chinese cabbage on

413 the 75th day of growth. The comprehensive analysis showed that gentamicin composting not only
414 promoted the growth of the potted crops but also improved their quality; these results further indicate
415 that the composting product was mature.

416 **Table 5** Emergence rate and quality of Chinese cabbage in different treatments.

Parameter	CK	CM	GL	GH
MR[a] (%)	65	77.5	80	82.5
Height[b] (cm)	10.0 ± 0.12	12.5 ± 0.32	13.6 ± 0.24	17.8 ± 0.22
Weight[c] (g)	5.3	6.3	7.2	10.1
SS[d] (%)	0.52 ± 0.02	0.60 ± 0.03	0.63 ± 0.01	0.58 ± 0.02
GR[e] (mg/kg)	0	0	0	0

417 [a] means emergence rate; [b] means average height from root to top;
418 [c] means average fresh weight; [d] means soluble sugar content; [e] means gentamicin residue.
419 Mean values ± standard errors.

420 **Microbial Community Diversity**

421 The types of compost substrates have a crucial influence on microbial community composition during
422 composting [41]. To determine the relationships between different compost substrates, GR and
423 microbial community diversity, CK (without rice chaff) and T2 (250 kg of rice chaff) samples were
424 analyzed.

425 16S rRNA HiSeq sequencing identified 56-149 OTUs for each compost sample, for a total of 1113
426 OTUs. As shown in Fig. 4a, the coverage indexes of all samples were above 0.999, indicating that the
427 sequencing had good species coverage and reflected the actual communities. As seen from the observed
428 richness (S_{obs}) of OTUs (Fig. 4b) and the Chao1 index (Fig. 4d), the taxonomic richness in T2 was

generally higher than that in CK. The lowest richness value appeared in CK_0, while the highest richness value appeared in T2_73. Alpha diversity indexes can reflect community diversity. The Shannon index of CK was smaller than that of T2 (Fig. 4c). As composting progressed, GR gradually decreased, and the Shannon indexes of T2 and CK both showed an upward trend until the 73rd day. Their Shannon indexes peaked at 3.295 and 3.224, respectively. These results showed that GR had a strong inhibitory effect on bacterial abundance and diversity and that co-composting had a significant effect on increasing bacterial richness and diversity. The Chao1 and Shannon indexes showed that the abundance and diversity of the bacterial community in T2 was higher than that in CK. The high microbial diversity in T2 might be attributed to the compost materials, which was beneficial to the growth of microorganisms. [42, 43].

18S rRNA HiSeq sequencing identified only 227 OTUs for fungi, much fewer than for bacteria. The coverage indexes (Fig. 4a) of the fungi were also above 0.999, indicating that the obtained sequences reflected the actual communities. According to the S_{obs} of OTUs (Fig. 4b) and the Chao1 index (Fig. 4d), the change trend of fungi was similar to that of bacteria, but the richness and Chao1 index of fungi were much lower than those of bacteria. Meanwhile, the Shannon index (Fig. 4c) indicated that the fungal diversity was lower than the bacterial diversity, with values of 1.127, 2.004, 1.894, 1.206, and 0.365 in T2 and 0.914, 1.250, 0.402, 1.700, and 2.134 in CK at different stages. In this study, the OTU number (Fig. 4b) and the Shannon index (Fig. 4c) of fungi were significantly lower than those of bacteria, suggesting that fungi grew poorly during high-temperature aerobic composting.

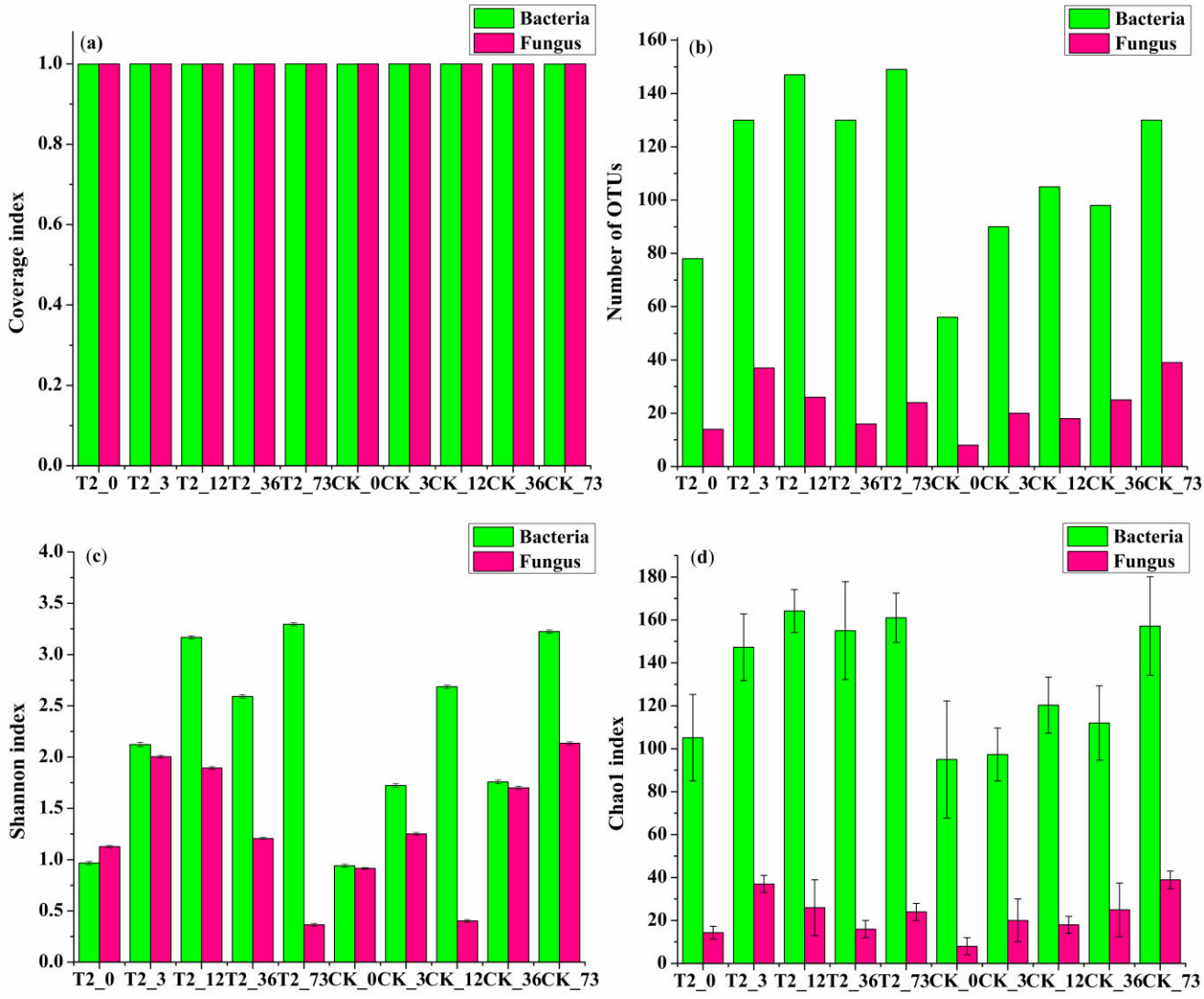


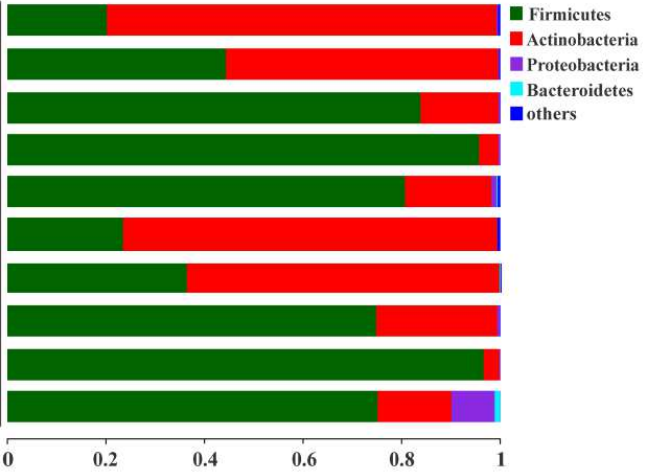
Fig. 4 Alpha diversity of the bacterial and fungal communities in compost T2 and CK: coverage index (**a**), number of OTUs (**b**), Shannon index (**c**) and Chao1 index (**d**). CK: fresh GMR and dried GMR (8:1, w:w); T2: fresh GMR and rice chaff (4:1, w:w)

Microbial Composition

The compositions of the bacterial communities of T2 and CK composts as composting progressed are shown in Fig. 5a. During the entire composting process, three phylum-level groups were predominant (accounting for > 99% of the total bacterial sequences) in T2 treatment: Firmicutes (20.19-80.70%), Actinobacteria (3.90-79.09%) and Proteobacteria (0.19-1.04%). Three phylum-level groups were predominant (accounting for > 98% of the total bacterial sequences) in CK treatment: Firmicutes

458 (23.46-96.66%), Actinobacteria (3.18-75.96%) and Proteobacteria (0-8.79%). The two most abundant
459 phylum were same in T2 and CK composting treatments (Firmicutes and Actinobacteria). The dynamics
460 of the compost microflora indicated that these phyla were key players in decomposing OM during the
461 whole composting process [37]. During the thermophilic phase, Firmicutes were the most predominant
462 component, contributing 95.72% and 96.66% of the total bacteria in T2 and CK, respectively. The
463 possible reason was that GMR was rich in cellulose. Firmicutes were the dominant microflora of cellulose
464 degradation [44]. As composting continued, Actinobacteria first decreased and then increased, accounting
465 for 17.52% and 14.97% of the total in T2 and CK, respectively, in the cooling period. Zhong et al. [28]
466 also demonstrated that *Actinobacteria* had higher relative abundance in cooling period during dairy
467 manure composting. Furthermore, Huerta et al. [45] found that ARGs were possibly carried and
468 disseminated by Firmicutes and Actinobacteria. Therefore, more studies are needed to focus on
469 environmental safety assessment risks of GMR composting.
470 However, the compositions of the fungal communities were not similar to those of the bacteria.
471 Ascomycota and Basidiomycota were the main phyla, accounting for more than 98% of the fungi in the
472 two piles (Fig. 5b). Neher et al. [46] found that in composting, the main fungal phyla were Ascomycota
473 and Basidiomycota under heat stress and different compost recipes. The high percentages of Ascomycota
474 and Basidiomycota may be due to the persistent continuous heat and the addition of rice chaff.

Others	Bacteroidetes	Proteobacteria	Actinobacteria	Firmicutes	(a)
0.44	0.00	0.28	79.09	20.19	T2_0
0.24	0.00	0.19	55.24	44.33	T2_3
0.12	0.00	0.21	15.85	83.82	T2_12
0.01	0.00	0.36	3.90	95.72	T2_36
0.53	0.21	1.04	17.52	80.70	T2_73
0.58	0.00	0.00	75.96	23.46	CK_0
0.15	0.00	0.02	63.43	36.39	CK_3
0.09	0.00	0.55	24.53	74.83	CK_12
0.00	0.01	0.16	3.18	96.66	CK_36
0.00	1.11	8.79	14.97	75.13	CK_73



Others	Basidiomycota	Ascomycota	(b)
0.00	62.46	37.54	T2_0
0.71	17.70	81.59	T2_3
1.36	12.86	85.78	T2_12
0.00	3.66	96.34	T2_36
0.00	1.33	98.67	T2_73
0.00	59.36	40.64	CK_0
1.31	5.00	93.68	CK_3
0.15	1.12	98.73	CK_12
0.25	8.46	91.29	CK_36
1.01	10.00	88.99	CK_73

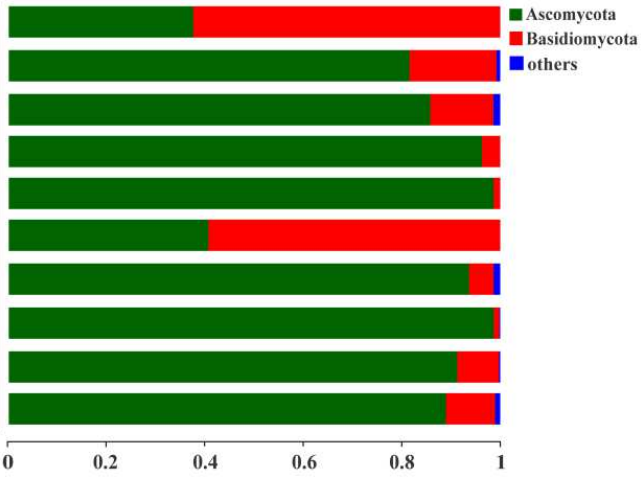
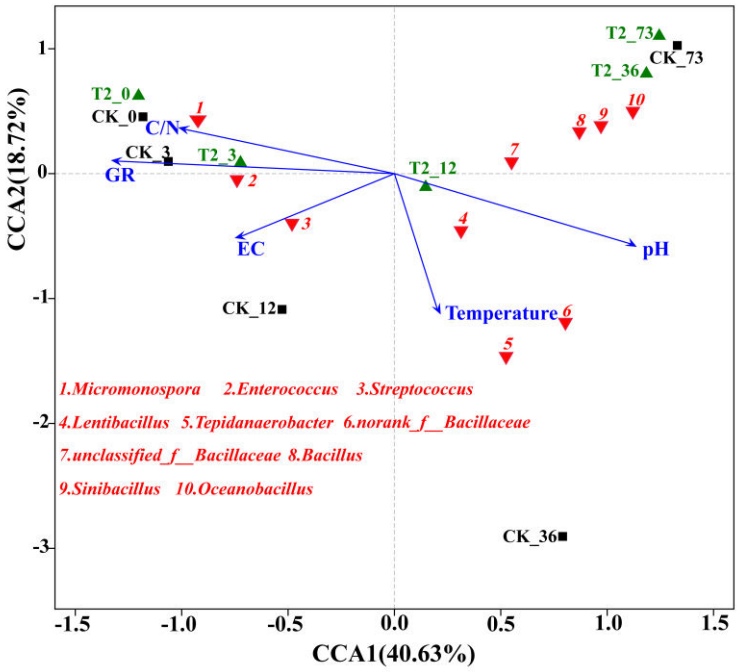


Fig. 5 Relative abundances of (**a**) bacteria and (**b**) fungi in their communities in T2 and CK at the phylum level during the composting process. CK: fresh GMR and dried GMR (8:1, w:w); T2: fresh GMR and rice chaff (4:1, w:w)

Relationship between Physicochemical Parameters and the Bacterial Community Composition

Due to the higher diversity and abundance of the bacterial community and its dominant position over the fungal community in composting [47], the relationships between environmental factors and the bacterial microbial community were further determined to explore the degradation mechanism of gentamicin. During composting, environmental factors can affect microbial community structure by affecting the

decomposition of OM [48]. To better reveal the dynamic correlations between bacterial communities and environmental parameters during composting, CCA (Fig. 6) was used to analyze the bacterial community and physicochemical indicators (GR, temperature, pH, EC and C/N). As shown in Fig. 6, CCA1 and CCA2 respectively explained 40.63% and 18.72% of the variation in bacterial communities. Among the environmental factors, GR played a key role in differentiating the bacteria between T2 and CK. The CCA values of the two groups in the mesophilic period were not particularly far apart (Fig. 6); however, the values at the thermophilic periods were far apart (Fig. 6), indicating that the bacterial community differences were positively influenced by the physicochemical factors during composting. With gentamicin degradation, the bacterial community compositions between T2 and CK composts gradually stabilized, showing little difference in the mature period. To further determine the correlations between bacteria and physicochemical parameters, Spearman's correlation coefficient analysis was used to analyze the environmental factors and the ten most abundant bacteria. As shown in Fig. 7, *Micromonospora* and *Enterococcus* had conspicuous positive correlations ($p<0.05$) with GR, which indicated that gentamicin was probably degraded by these bacteria. *Norank_f_Bacillaceae*, *Bacillus*, *Oceanobacillus* and *unclassified_f_Bacillaceae,* which belong to Bacillaceae and Firmicutes, were notably positively correlated with pH at the thermophilic and cooling periods ($p<0.05$). They could form very thick spore walls to resist high temperatures and high pH values. This could explain the increase in Firmicutes in the community composition of bacteria during the thermophilic and cooling periods (Fig. 5a).



503

504 **Fig. 6** CCA of the relationships between physicochemical parameters and the bacteria of the top 10

505 genera (explanatory variables of the top 10). The blue arrows indicate the physicochemical parameters.

506 The red triangles represent genera. The green triangles and black squares represent samples of the CK and

507 T2 treatments, respectively, at different composting phases. CK: fresh GMR and dried GMR (8:1, w:w);

508 T2: fresh GMR and rice chaff (4:1, w:w)

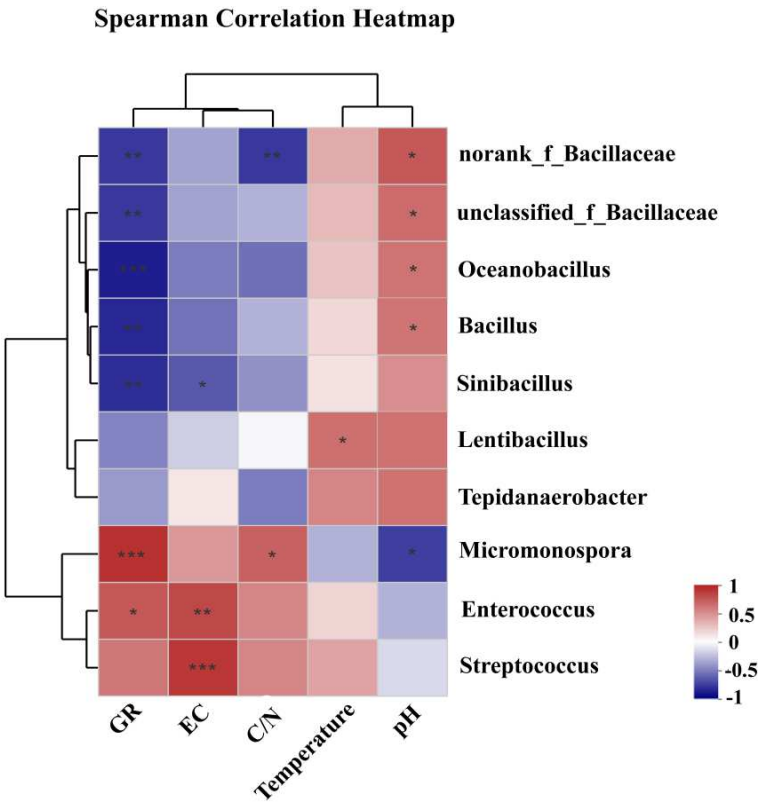


Fig. 7 Heatmap of correlations between the top ten genera in composting samples and physicochemical factors. *P < 0.05, **P < 0.01, ***P < 0.001

Conclusions

In this study, parameters such as GR, temperature, pH, C/N ratio, EC, GI, and crop growth indexes as well as assessments of microbial communities were used to estimate the effectiveness of co-composting GMR with rice chaff. The results demonstrated that co-composting is a practical technological measure for treating and managing GMR. CCA showed that bacterial communities were greatly influenced by residual gentamicin. *Micromonospora* and *Enterococcus* were the bacterial genera that might degrade gentamicin. However, gentamicin-resistant bacteria and genes should be further investigated during the composting process to predict the effects of application of GMR to soil.

Acknowledgments

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Declarations

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Conflicts of interest/Competing interests

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

Availability of data and material

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

Authors' contributions

Investigation, Data curation, Writing-Original: **Wenjing Bu**; Writing - Review & Editing: **Junfeng Wan**; Resources: **Huimin Zhang**; Data curation, Validation: **Nan Liu**; Methodology, Software: **Ke Wang**; Conceptualization, Supervision: **Yan Wang**.

Ethics approval

This is our original work and we certify that we have participated sufficiently in the work to take public responsibility for the appropriateness of the experimental design and method, as well as the collection, analysis, and interpretation of the data. This manuscript has not been published previously.

Consent to participate

All the authors have reviewed the final version of the manuscript and mutually agreed that it should be submitted to "***Waste and Biomass Valorization***".

Consent for publication

If ***"Waste and Biomass Valorization "*** accepts our manuscript, all the authors will agree to public in it.