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Title:

Dynamics of bacterial composition and the fate of antibiotic resistance genes and mobile genetic elements during the co-composting with gentamicin fermentation residue and lovastatin fermentation residue

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Abstract Dynamics in bacterial community composition, along with 13 antibiotic resistance genes (ARGs) and eight mobile genetic elements (MGEs), were assessed during co-composting with gentamicin and lovastatin fermentation residue (GFR and LFR, respectively). Using next generation sequencing, the key bacterial taxa associated with the different stages of composting were identified. Most importantly, *Bacillus*, belonging to Phylum Firmicutes, was associated with enhanced degradation of gentamicin, decomposition of organic matter (OM) and dissolved organic carbon (DOC), and also extension of the thermophilic phase of the composting cycle. During the course of composting, the patterns of different ARGs/MGEs varied. However, the total and the normalized (to bacterial numbers) copies both remained high. The abundance of various ARGs was related to bacterial abundance and community composition, and the changing pattern of individual ARGs was influenced by the selectivity of MGEs and bacteria.

Key words gentamicin; antibiotic resistance genes; composting; bacterial diversity; mobile genetic elements

1. Introduction

The emergence of antibiotic resistance genes (ARGs) and their distribution through microbial ecosystems has resulted in the emergence of antibiotic resistant bacteria (ARB). This has important implications for human health, as many traditional therapies for disease control (i.e. bacterial infections) have been lost, resulting in a significant challenge to effective clinical practice globally (Levy et al., 2004; Zhu et al., 2013).

It has been widely reported that evolution of a range of ARGs are induced by the presence ubiquitous antibiotics present in animal manure and antibiotic fermentation residues (Luo et al. 2010; Huijbers et al. 2015). As these residues are nutrient rich, they are often applied to land as fertilizers. Given this, it is imperative that the antibiotic residues in these residues are effectively removed. Furthermore, the abundance of ARGs in these residues needs to be qualitatively and quantitatively determined.

Gentamicin is a broad spectrum aminoglycoside antibiotic widely used in China and elsewhere. Large volumes of gentamicin fermentation residue (GFR) are generated as part of the antibiotic production process; and GFR contains high concentrations of residual gentamicin (Liu et al. 2017a). In order to recycle this waste, which contains high concentrations of valuable plant nutrients, it is important to remove gentamicin residue and ARGs in GFR (Marti et al., 2013). Previously, we have described the co-composting of GFR with lovastatin fermentation residue (LFR) as a process to remove antibiotic residues (Liu et al. 2017a). In this process, the fungus *Aspergillus terreus* (an isolate able to efficiently degrade gentamicin), was inoculated into the composting process (Liu et al., 2016), and most gentamicin was metabolized. However, the fate of ARGs in this compost has not been evaluated. Given the selection and evolutionary process involved (i.e. previous high antibiotic-levels), there may remain significant levels of ARGs within the microbial population, posing a significant environmental risk.

Although composting has been regarded as an effective bioremediation technology for the removal of ARGs, (Gou et al., 2018; Selvam et al. 2012), this view has been challenged by recent findings. Su et al. (2015) found that the abundance and diversity of

ARGs were enriched during composting of sewage sludge. Similarly, Wang et al (2015) evaluated changes in tetracycline resistance genes during composting and found that while some remained static, others significantly increased. The different fates of ARGs (reduction, stasis, or increase), may be associated with the composition of the composting materials, environmental parameters, or the bacterial community structure during the composting process (Tien et al., 2017). Of these, changes in bacterial community composition have been found to be most closely coupled with the dynamics of ARGs (Su et al., 2015; Qian et al., 2016a). Furthermore, mobile genetic elements (MGEs), such as plasmids, integrons, and transposons, represent further factors that may influence the spread of ARGs via horizontal gene transfer (HGT) (Ma et al., 2017; Sentchilo et al., 2013; Yin et al., 2017). However, until now, few studies have assessed the fates of ARGs during composting of GFR, nor the factors influencing their abundance.

This study aimed to: (1) investigate dynamics of bacterial community composition throughout the co-composting process under different proportions of GFR and LFR; (2) detect changes in aminoglycoside ARGs and MGEs during composting; and (3), analyze the primary factors influencing the fate of different ARGs, and underlying mechanisms, over the composting process.

2. Materials and Methods

2.1 Composting process and sampling

Details of the composting process have been provided in full previously (Liu et al., 2017a). Briefly, raw materials of GFR and LFR were obtained from Zhichu

Pharmaceutical Factory (Shandong Province, China). Urea, used to adjust the carbon to nitrogen ratio (C: N), was sourced from Xinlong Fertilizer Factory (Yantai, Shandong, China). *Aspergillus terreus* FZC3, originally isolated from the solid waste product of gentamicin production, was sourced from the China General Microbiological Cultural Collection Center (CGMCC; accession CGMCC 12072).

The study investigated four composting treatments: (1) a high proportion of GFR to LFR (15:1, w/w; HCK); (2) a high proportion of GFR to LFR (15:1, w/w) + 2.5% *A. terreus* FZC3 (v/w; HFZC3); (3) a medium proportion of GFR to LFR (10:1, w/w) + 2.5% *A. terreus* FZC3 (v/w; MFZC3); (4) a low proportion of GFR to LFR (5:1, w/w) + 2.5% *A. terreus* FZC3 (v/w; LFZC3).

For each treatment, GFR and LFR were mixed thoroughly to achieve desired proportions. Urea was added to adjust the C:N ratio to approximately 20:1. *Aspergillus terreus* FZC3 (8.2 g L^{-1} dry biomass), pre-cultivated *in vitro* for five days, was added to compost mixes and the final moisture content adjusted to 60 %. Treatment batches of compost were remixed and 100 kg batches were transferred to 240 l plastic vessels.

Composting occurred over 21 days. During this time, aeration was achieved by turning the compost heap every day after temperature rose to 50°C, and every two days after the pile temperature dropped below 50°C. There were triplicates batches of compost for each of the treatments.

Samples were collected on days 1, 7, and 21; these represented mesophilic, thermophilic, and mature phases of composting, respectively. At each collection time, subsamples of the upper, middle, and lower layers were collected at three random sites.

Sub-samples were stored at -80°C for DNA extraction.

2.2 DNA extraction and high-throughput sequencing of bacterial 16S rRNA genes

Total DNA was extracted from 0.5 g of compost samples using the PowerSoil DNA isolation kit according to the manufacturer's instructions (Omega Bio-tek, Norcross, GA, U.S.). DNA purity was measured by NanoDrop spectroscopy (Thermo Fisher Scientific, Waltham, MA, USA), and DNA concentration and integrity by Qubit fluorescence and agarose gel electrophoresis, respectively. Samples of purified DNA were stored at -20°C until use.

To characterize the bacterial community present in the samples of compost, DNA was sent to the Total Genomics Solution (TGS) Institute (Shenzhen, China) for high-throughput sequencing of the V3 and V4 regions of the 16S rRNA gene using the Illumina HiSeq 2500 platform. Barcoded fusion primers were added to the 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') primers, allowing the assignment of DNA reads into specific treatment groups. PCR and thermal cycling parameters were the same as those given previously (Liu et al., 2017b). PCR products were purified using a DNA Extraction Kit (Tiangen DNA extraction Kit, China).

2.3 Real-time qPCR

The abundances of 13 ARGs were determined using quantitative (real time) qPCR. These were: *aac(3)-I*, *aac(3)-II*, *aac(3)-Vb*, *aac(6')-I*, *aac(6')-II*, *aac(6')-IIb*, *aac(6')/aph(2'')*, *aph(3')-III*, *ant-(2')-I*, *ant-(3'')-I*, *rmtA*, *tlyA*, *strB*. In addition, eight MGEs, including four

plasmids genes (*IncP-1*, *IncQ*, *IncW*, *IncN*), two integrase genes (*int1* and *int2*), and two transposons genes (*IS613* and *tnpA*), were quantified along with the total bacterial community size (16S rRNA gene abundance) using qPCR.

A standard plasmid containing a cloned and sequenced 16S rRNA gene fragment was used to create a six-point calibration curves from 10-fold dilutions. The stock was determined to be 1.79×10^{10} copies L^{-1}. Real time PCR reactions were run in triplicate, and included both negative and positive controls. A threshold cycle (Ct) < 31 and amplifying efficiency range of 80-120% in technical triplicates for each sample were regarded as positive amplifications. The average Ct values from the technical triplicates were used for analysis. The absolute abundance (copies g^{-1} dry samples) of each ARG in each sample was calculated by multiplying the absolute copy number of 16S rRNA genes by the relative copy number of this ARG: Relative copy number = $\frac{10^{(31-Ct_{(ARG)})/(10/3)}}{10^{(31-Ct_{(16S)})/(10/3)}}$, where Ct(ARG) is the threshold cycle of ARG, and Ct(16S) is the threshold cycle of 16S rRNA genes. The absolute copy number of each MGE was similarly calculated. Bacterial cell numbers, and the normalized numbers of ARGs and MGEs per bacterial cell, were calculated according to the method described by Su et al. (2015).

The qPCR reaction system (10 µl) comprised 5 µl of Roche FastStart Universal SYBR Green Master (ROX), 0.75 µl of both forward and reverse primers (Wcgene Biotech, Shanghai, China), 0.5 µl of template DNA (or control), and 3 µl of double distilled water. The qPCR conditions were as follows: Initial hold for 10 min at 95°C, followed by 40 cycles of 95°C for 30 sec, 40 cycles of 60°C for 30 sec, and then 72°C for 10 min (Pu et al., 2018).

Quantitative PCRs were conducted on ABI VIIA ™ 7 Real-Time PCR system (Wcgene Biotechnology, Shang-hai).

2.4 Bioinformatics and statistical analyses

The raw pair-end reads were assembled following filtering for correct adaptors and the removal of low-quality reads and sequences with ambiguous "N" using QIIME (V1.9.0, http://qiime.org/index.html). Overlapping criteria was determined as overlapping lengths >10 bp and mismatch ratio lower than 0.2. Chimera tags were filtered out using the Gold database by UCHIME (version 4.2.40). Resultant high-quality reads were clustered into operational taxonomic units (OTUs) based on 97% similarity in nucleotide composition using Uparse (http://drive5.com/uparse/) (Edgar, 2013). Each OTU was taxonomically assigned to the silva database using the RDP classifier. OTUs with relative abundance values >0.001% (i.e. greater than three tags in at least one sample were retained.

Alpha diversity (Shannon's index) of the bacterial community for each sample was calculated by alpha_diversity.py (http:qiime.org/scripts/alpha_diversity.html) included in the QIIME and was diagramed using R 3.1.0 (Bates et al., 2013; R Core Team, 2014). To assess similarity of community composition among samples, beta diversity (based on Bray curtis, Weighted Unifrac and Unweighted Unifrac distances) were calculated by jackknifed_beta_diversity.py (http://qiime.org/scripts/jackknifed_beta_diversity.html) included in the QIIME. Principal component analysis (PCA) was performed to assess beta diversity. Permutational multivariate analysis of variance (PERMANOVA) was performed

using R 3.1.0 to formally test for differences among treatment groups (R Core Team, 2014). Correlations between bacterial community composition and the physicochemical properties of the compost over time were calculated using Spearmans rank method.

Redundancy analysis (RDA), along with partial RDA, was performed to establish underlying relationships between ARGs/MGEs, bacterial community composition, and the physicochemical properties of the compost environment (R Core Team, 2014). Correlation between ARGs and MGEs, and other statistical significance tests, were conducted using SPSS V.19 (IBM, USA). Graphs were generated in OriginPro 8.5 (OriginLab, USA) and Excel 2007 (Microsoft, USA).

3 Results and Discussion

3.1 Bacterial diversity

According to the changes of Shannons index, differences in alpha diversity between the three composting phases were highly significant ($p=0<0.001$), with the thermophilic phase having highest diversity of bacterial OTUs. In contrast, the richness and evenness of the fungal community was similar between GFR and LFR treatments ($p=0.072$), and this supported results from our previous study (Liu et al., 2017a). This discrepancy in response between the bacterial and fungal communities highlights the fact that, to some extent, bacteria play a greater (or at least functionally different), role in the thermophilic phase than fungi. Furthermore, significantly higher Shannons index values were observed in samples on day 21 than day 1, suggesting that co-composting with GFR and LFR was associated with more diverse bacterial communities (Li et al., 2018).

According to the ordination (PCA graph), axis 1 (PC1) explained 76.7% of the community variation, and PC2 explained 13.5%. The composition of the bacterial community clearly separated into three groups associated with the phase of composting: mesophilic, thermophilic, and mature. Similar results were found by Qian et al. (2016a). Importantly, the results also showed that addition of *A. terreus* FZC3 and the ratio of GFR to LFR had little effect on bacterial composition during composting.

3.2 Bacterial composition at phyla level

Phylum-level changes in bacterial community composition during composting are shown in **Fig. 1A**. Four major phyla, Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria, accounted for 49.33%, 39.30%, 10.79% and 0.38% of the total sequence reads, respectively.

These findings supported those of Zhang et al. (2016), whereby Proteobacteria also exhibited the highest relative abundance (approximately 37%) during composting with wheat straw. While the population size of Proteobacteria stayed stable in the study of Zhang et al. (2016), we found that the relative abundance of Proteobacteria could reach 88.71%-92.91% during the initial stage of co-composting of GFR with LFR, a , decreased sharply to 11.82%-22.54% during the thermophilic phase, and recovered again to 32.10%-54.61% at compost maturation. This variation demonstrated that most of the Proteobacteria appeared in the present composting process are mesophilic (Chandna et al., 2013).

The second most abundant phylum detected throughout the composting was

Firmicutes. We found that the relative abundance of this bacteria was much higher than results reported in other studies, such as during co-composting of cow manure with rice straw (Ren et al., 2016), manure with hardwood/hay (Neher et al., 2013) or swine manure with red mud (Wang et al., 2016). This further shows the important effects that different substrates had on the bacterial communities during the composting process. The dramatically higher abundance of Firmicutes during the thermophilic phase (i.e. compared with the mesophilic and mature phase) supports the thermophilic tolerance characteristic of most Firmicutes taxa. When comparing the abundance of Firmicutes in different treatments at thermophilic phase, treatment MFZC3 showed much higher abundance (**Fig. 1A**), and this was accompanied with a longer thermophilic phase and more rapid decomposition of organic matter (OM) and dissolved organic carbon (DOC) (Liu et al., 2017a). This suggested coupling between Firmicutes abundance and heat release. Furthermore, the rapid degradation of gentamicin during the thermophilic phase provides an associative role for Firmicutes in gentamicin removal (Liu et al., 2017a).

Bacteroidetes were found to increase during the thermophilic phase of co-composting with cow manure and rice straw by Ren et al. (2016) and co-composting with dairy manure and rice chaff by Tian et al. (2013). These findings are inconsistent with our study in which Bacteroidetes were in higher abundance (31.25%) at mature phase than that in mesophilic (0.88%) and thermophilic phase (0.24%). In consideration that, different from manure, rice straw and chaff, GFR and LFR were fermented substrates and contained little refractory matters. Therefore, the variations of Bacteroidetes contents between the previous studies (Ren et al., 2016; Tian et al., 2013) and our present one may

result from that Bacteroidetes are related to the decomposition of some refractory matters (Zhao et al., 2016).

3.3 Bacterial composition at genera level

Variations in bacterial genera among the four treatments and over the composting cycle are displayed in **Fig. 1B**. By comparing the abundance of bacteria in different composting phases, it was found that more major genera (i.e. >1.0%) appeared at the thermophilic (11 genera) and mature phase (12 genera), compared with than mesophilic phase (5 genera).

During mesophilic phase, *Psychrobacter* were dominant, however these largely disappeared from the community during the later two phases of composting. Furthermore, lower ratios of GFR: LFR were more suitable for survival of *Psychrobacter*.

Bacillus, belonging to the phylum Firmicutes, genereally maintained high population sizes even through to the thermophilic phase. This is likely a result of the thermostability of endospores typically produced by these bacteria (Qian et al., 2016a). The abundance of *Bacillus* in treatment MFZC3 was 1.38-2.96 times greater than that in other treatments. According to changes in physicochemical properties reported in our previous study (Liu et al., 2017a), this suggests that *Bacillus* may enhance decomposition of OM and DOC, promote the degradation of gentamicin, and prolong the thermophilic phase.

Caldicoprobacter, another genus of Firmicutes, is a thermophilic anaerobic bacterium with xylanas activity (Bouanane-Darenfed et al., 2011). The abundance of *Caldicoprobacter* reached a maximum during the thermophilic phase of composting. In

addition to thermotolerance, this may also be related to the presence of hypoxic conditions. The abundance of *Caldicoprobacter* was greatest in treatment LFZC3. This was associated with a higher proportion of LFR in LFZC3 and high viscosity-gravity of LFR, along with extended anaerobic conditions.

3.4 Associations between bacteria community and physicochemical properties

Associations between the main bacteria present during composting and environmental conditions were tested (PERMANOVA analysis; **Fig. 2**). Different bacterial profiles were present in the three phases of composting, which was supported by the PCA ordination. Taxa that were dominant during the mesophilic phase (cluster A) were significantly correlated ($p \leq 0.05$) with total nitrogen (TN), OM, DOC, and moisture content (Liu et al., 2017a). Bacteria, including the genera *Bacillus*, *Caldicoprobacter, Tepidimicrobium,* and *Sinibacillus* and others (cluster B) were dominant during the thermophilic phase. This group was significantly related to increased temperature and electrical conductivity (EC). Bacteria in the C-cluster had significant positive correlations ($p \leq 0.05$) with ammonium nitrogen (NH_4^+-N) and pH (Liu et al., 2017a).

In other studies, where stock material such as swine manure (Wang et al., 2016), cow manure (Qian et al., 2016a), wheat straw (Zhang et al., 2016) and sewage sludge were composted, the main bacterial taxa associated with composting were different to those reported here. This may result from the unique chemical nature of the stock material itself, differences in nutrient ratios, and variation in environmental conditions (redox, pH, etc) incurred by the different substrates. For example, the presence of GFR in the compost

imposes an antibiotic-based selection on the community and results in a substrate with high pH). Although many bacteria differ between composting systems, *Bacillus*, is largely ubiquities. This reflects the key role *Bacillus* has in most composting processes, and particularly during the thermophilic phase and for decomposition of OM and DOC (Liu et al., 2017a).

3.5 Quantification of ARGs/MGEs

Thirteen ARGs, eight MGEs, and 16S rRNA genes were detected in samples of compost. The abundances of these, calculated as copy numbers per gram of dry material, are shown in **Fig. 3**. Variation in abundances of ARGs/MGEs could be categorized into four groups: (I) ARGs that increased throughout the composting; (II) ARGs that decreased throughout the composting; (III) ARGs that increased at thermophilic phase and then decreased; (IV) ARGs that decreased at thermophilic phase and then increased. Among the detected ARGs/MGEs in the four treatments, *ant-2'-I* belongs to group (I); *aac(3)-II*, *aac6'-II*, *aac6'-IIb*, *IncN* and *IS613* belong to group (II); *ant-(3'')-I* and *tlyA* belong to group (III); *aac(3)-I*, *aac(3)-Vb*, *aac6'-I*, *aac6'/aph(2'')*, *strB* and *Int1*, belong to group (IV). The rest, including *aph3'-III*, *rmtA*, *IncW*, *IncQ*, *IncP-1*, *Int2,* and *tnpA,* followed different trends in variation across the four different treatments, suggesting that the addition of *A. terreus*, and the proportions of GFR and LFR, both influenced the dynamics of the abundances of these genes.

It has been widely demonstrated that ARGs could be efficiently removed from waste streams based on dairy manure (Qian et al., 2016b), chicken manure (Guan et al., 2007),

or swine manure (Wang et al., 2016) following composting. In this study, the abundance of ARGs and MGEs initially declined and then recovered to levels greater than that present initially. However, the abundances of ARGs at the later phase of composting were not significantly different from those present during the mesophilic phase, except for treatment LFZC3 (significantly elevated). Similarly, Su et al. (2015) also showed that the abundance of ARGs remained high in material after composting. This may be due to poor-ventilation during the composting undertaken in these two studies. In addition, by comparing the dynamics of ARGs and cell number g^{-1} dry samples in different studies (Qian et al., 2016b; Su et al., 2015), it can be concluded that the abundance of ARGs are positively related to the total bacterial population. That is, the greater the number of bacteria in the sample, the higher the frequency of occurrence of antibiotic resistance genes in the community.

Based on changes of normalized copy numbers of ARGs (i.e. copies per bacterial cell), ARGs decreased in the initial phase of composting when LFR was added in the substrate material. For treatment LFZC3, the thermophilic phase displayed the largest normalized copy number (about 2.3).

For normalized abundance of MGEs, a sharp increase was observed after composting for every treatment. Furthermore, the treatment LFZC3 obtained the highest copy number (3.0), and this was more than four times that of treatment MFZC3.

3.6 Correlations among ARGs/MGEs, bacterial community, and physicochemical properties

A Venn diagram, based on partial RDA, was conducted to assess the separate and

joint effects among MGEs, bacterial community composition, and the physicochemical properties of compost on changes in ARGs (**Fig 4**). Three factors were found to account for 96.99% of the variation, and separately explained 11.45%, 9.33%, and 6.97%, respectively. The interaction between MGEs and the physicochemical properties of the compost explained the proportion of variation (ca. 29.41%), followed by MGEs and bacterial community composition (ca. 21.08%). Associations between bacterial community composition and physicochemical properties explained 18.43% of the variation, while only 0.32% was due to the interactions between the three variables. These results indicated that the changes of MGEs played a more crucial role in the alternations of relevant aminoglycoside ARGs than bacterial community structure, which is consistent with results reported by Su et al. (2015).

Variation in MGEs explained a relatively low proportion (ca. 2.6%) of total variation in ARGs (including the ARGs of 9 categories of antibiotics). In previous research (Su et al., 2015), the abundance of aminoglycoside ARGs was significantly ($p \leq 0.01$) related to MGEs, which supported the present study. This could, at least partially, demonstrate that the mechanisms involved in the transformation of diverse ARGs differ, and the fates of aminoglycoside ARGs are strongly associated with shifts of MGEs.

Recently, it has been reported that MGEs and ARGs are genetically connected and some subtypes of *acc* family-genes were easily captured by integrons (Gou et al., 2018; Zhu et al., 2017). Ma et al. (2017) also found that, when comparing with other kinds of ARGs, aminoglycoside ARGs were more frequently carried by class 1 integrons. According to the Pearson correlation analysis (**Table 1**), *aac(3)-I*, *aac(3)-Vb*, *aac(6')-I*,

aac(6')/aph(2''), and *ant-(2')-I* were significantly ($p \leq 0.01$) related the abundance of integrase genes (*int1* and *int2*) and the plasmids genes (*IncQ* and *IncW*). This indicated that the distribution of the five aminoglycoside ARGs was linked to the accumulation of transposable elements (integrons), providing a mechanism for mobilization ARGs onto plasmids and potential for HGT (Sentchilo et al., 2013; Riccio et al. 2003; Gou et al., 2018). The plasmid gene (*IncP-1*) and transposon gene (*IS613*) were both positively correlated with *aac(6')-IIb* and *strB* ($p \leq 0.01$), suggesting *IS613* may have inserted into *IncP-1*. This could lead to the spread of these two aminoglycoside ARGs via the transfer of *IncP-1*. Li et al. (2016) also found that this mechanism of transfer played a vital role in the distribution of ARGs. Negative correlations between *IS613* with *aph(3')-III* and *ant-(2')-I*, as well as between *tnpA* with *aac(6')-I* and *ant-(2')-I*, may be due to the opposite variation measured among their hosts (Qian et al., 2016a).

According to the RDA analysis shown in **Fig. 5**, variation in the dominant bacterial genera could explain 80.66% of variance in occurrence of ARGs and MGEs. These groups, representing three composting stages (mesophilic, thermophilic, and mature phase), were separated into the third, first and fourth quartiles, respectively, based on the variances of ARGs/MGEs. The positive or negative correlations between different ARGs and MGEs (**Table 1)** were also supported by the acute angles or obtuse angles between particular ARGs and MGEs in **Fig. 5** and the network analysis. Among the ARGs showing insignificant correlations with MGEs (**Table 1**), *aac(3)-II* and *aac(6')-II* were associated with the abundance of *Psychrobacter* (*Psy.*) and *Oceanisphaera* (*Oce.*), both of which occurred mainly during the mesophilic phase of composting. Variation in the abundance of

ant-(3'')-I and *tlyA* genes were correlated with that of thermophilic bacteria, including *Bacillus* (*Bac.*), *Sinibacillus* (*Sin.*), *Caldicoprobacter* (*Cal.*), and *Tepidimicrobium* (*Tep.*). The increasing abundance of *RmtA* during the mature phase of composting was associated with enrichment of *Paenalcaligenes* (*Pae.*), *Pusillimonas* (*Pus.*) and *Moheibacter* (*Moh.*). In addition, Actinobacteria, which are applied to produce antibiotics and often harbor a large number of ARGs, maintained a low relative abundance (less than 0.5%) during the course of composting. This latter finding may be related to the lethal effects that pretreatment of GFR with gypsum has on Actinobacteria. Overall, variation in physicochemical properties of the compost, over time, was strongly coupled with the fates of ARGs and changes in the bacterial communities.

In the present study it has been proved that the transfer of aminoglycoside ARGs is related to specific MGEs and bacteria, and the relevant mechanisms associated with this are hypothesized. This provides a solid base for the future research to test opportunities to reducing ARGs, especially aminoglycoside ARGs, in the environment. For example, this may be achieved through alteration of the bacterial community composition, through modification of the physicochemical habitat, to alleviate the spread of ARGs and MGEs. Furthermore, use of ozone treatment and biochar addition has also been shown to affect the fates of ARGs by influencing bacterial community composition (Cui et al., 2017; Czekalski et al., 2016). Together, these methods should be considered to enhance the reduction of aminoglycoside ARGs.

4. Conclusion

During co-composting with GFR and LFR, the key bacterial taxa and ARGs were classified to three distinct subgroups according to different composting stages. *Bacillus*, relatively abundant during the thermophilic stage, was associated with enhanced degradation of gentamicin, decomposition of OM and DOC, and a prolonged thermophilic phase of composting. Different ARGs/MGEs had varied in their occurrence in the composting systems. However, total and the normalized abundances both remained high. It was also found that the abundance of ARGs was affected by bacterial propagation and composition, and the structure of ARGs and its individual changing patterns were strongly influenced by MGEs.

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Appendix A. Supplementary data

E-supplementary data for this work can be found in e-version of this paper online.

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Figure captions

Fig. 1 Bacterial composition at phylum (A) and genus (B) levels associated with different treatments at day 1, day 7, and day 21 of composting. Bars display the standard error.

Fig. 2 Heat-map, displaying correlations between dominant bacteria in different treatments and physicochemical properties during composting. Physicochemical properties grouped on the X-axis, and dominant bacteria on the Y-axis. Blue and red represent negative and positive correlations, respectively, with darker colors represents higher correlations. "+" and "*" represent that correlation is significant at the 0.05 and 0.01 level, respectively. Bacterial taxa, in different colors, represent the corresponding genera enriched in different treatments during composting. (A) Genera dominated at mesophilic stage; (B) genera enriched at thermophilic stage, (C) genera dominated at mature stage. EC, electrical conductivity; OM, organic matter; DOC, dissolved organic matter; TN, total nitrogen; NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen.

Fig. 3 Changes of the abundances (copies of gene g^{-1} per dry samples) of individual ARGs/MGEs during composting. Results are the mean of three replicates; bars display the standard error. Treatments with the same letter on the column are not significantly different from one another ($P \leq 0.05$).

Fig. 4 Partial redundancy analysis (RDA) assessing the separate and joint effects of MGEs,

bacterial community and physicochemical properties on the variances of ARGs during composting

Fig. 5 RDA triplots showing the relationship between the key bacterial genera and the related ARGs/MGEs on different time-points after commencement of composting (1, 7, and 21 days). Blue arrows represent the main ARGs/MGEs, while red arrows represent the dominant bacteria. The angle between the blue arrow and the axis represents the correlation between a genus and effects associated with the axis. The acute angle between the genus and ARGs/MGEs indicates a positive correlation; the obtuse angle indicates a negative correlation. Square symbols represent the treatment HCK. Circle symbols represent the treatment HFZC3. Downward triangle symbols represent treatment MFZC3. Diamond symbols represent treatment LFZC3. The purple symbols are treatments at mesophilic stage. The green symbols are treatments at thermophilic stage. The red symbols are treatments at mature stage.

Table 1 Pearson correlation between ARGs and MGEs

	Int1	*Int2*	*IncP-1*	*IncQ*	*IncN*	*IncW*	*IS613*	*tnpA*
aac(3)-I	**0.689**[a]	**0.620**[a]	0.152	**0.649**[a]	-0.135	**0.587**[a]	-0.11	-0.257
aac(3)-II	-0.108	-0.109	0.191	-0.139	-0.042	-0.099	0.151	0.084
aac(3)-Vb	**0.647**[a]	**0.451**[a]	-0.152	**0.521**[a]	-0.126	**0.467**[a]	-0.291	-0.316
aac(6')-I	**0.995**[a]	**0.819**[a]	0.026	**0.795**[a]	-0.17	**0.730**[a]	-0.252	**-0.338**[b]
aac(6')-II	-0.123	-0.116	0.267	-0.155	-0.41	-0.112	0.271	0.106
aac(6')-IIb	-194	-0.165	**0.440**[a]	-0.209	0.012	-0.159	**0.490**[a]	0.035
aac(6')/aph(2'')	**0.752**[a]	**0.559**[a]	0.066	**0.692**[a]	-0.054	**0.563**[a]	-0.15	-0.307
aph(3')-III	0.098	-0.006	-0.253	0.087	-0.218	0.056	**-0.550**[a]	-0.04
ant-(2')-I	**0.803**[a]	**0.665**[a]	-0.16	**0.758**[a]	-0.153	**0.506**[a]	**-0.417**[b]	**-0.353**[b]
ant-(3'')-I	-0.184	-0.179	0.047	-0.219	0.001	-0.102	-0.274	0.209
rmtA	0.187	0.008	-0.153	0.198	-0.049	0.087	-0.164	-0.24
tlyA	-0.246	-0.135	0.124	-0.156	-0.21	-0.142	-0.28	0.016
strB	-0.173	-0.168	**0.473**[a]	-0.214	0.031	-0.165	**0.510**[a]	0.106

[a] Correlation significant at $p \leq 0.01$.

[b] Correlation significant at $p \leq 0.05$.

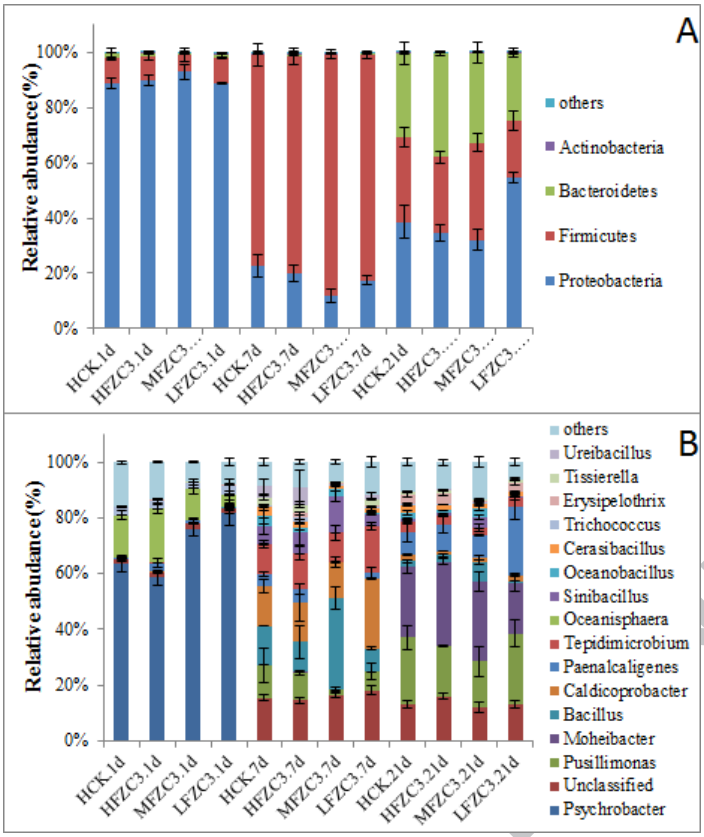

Fig. 1

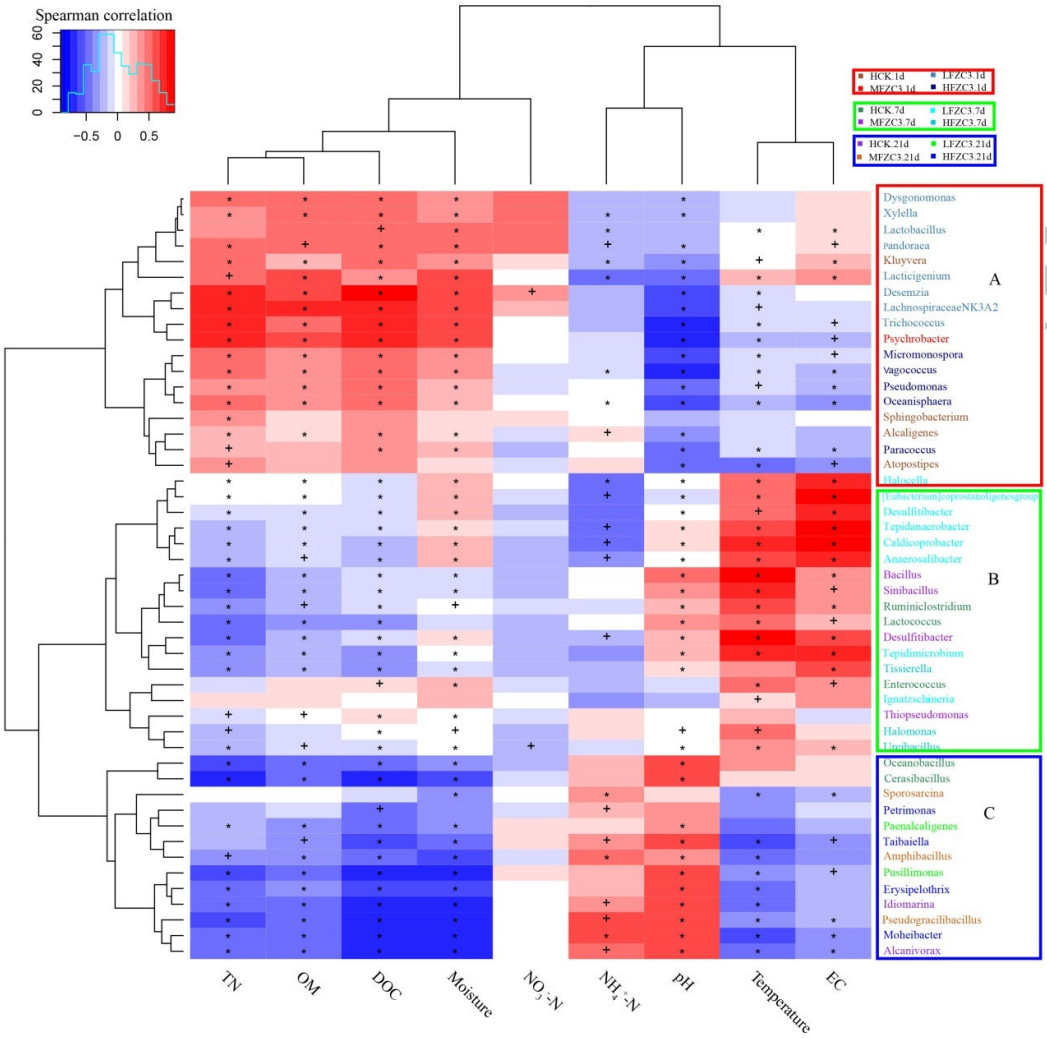

Fig. 2

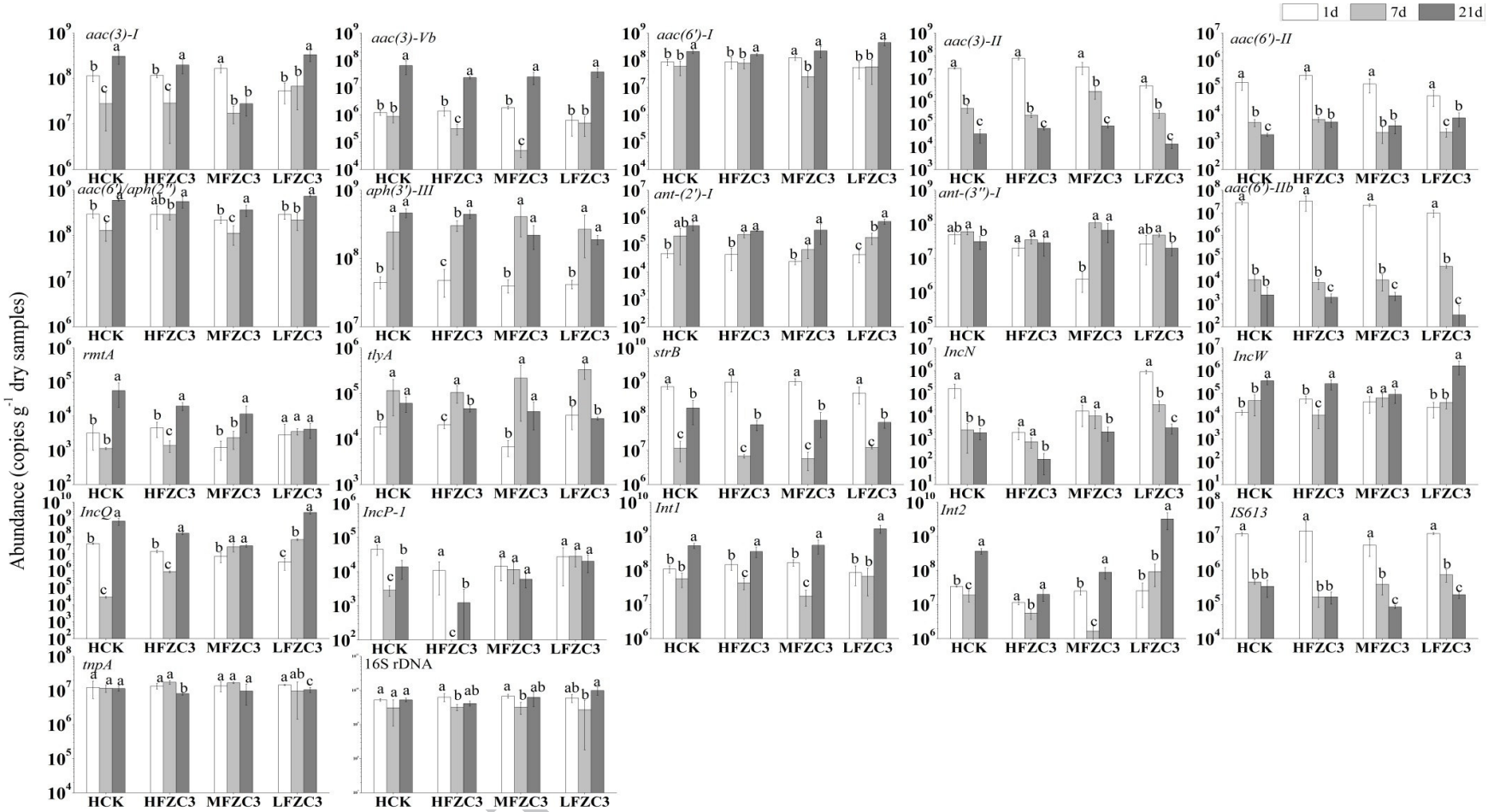

Fig. 3

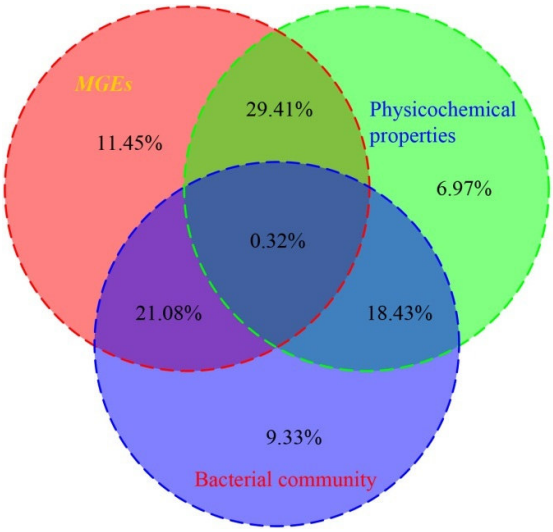

Fig. 4

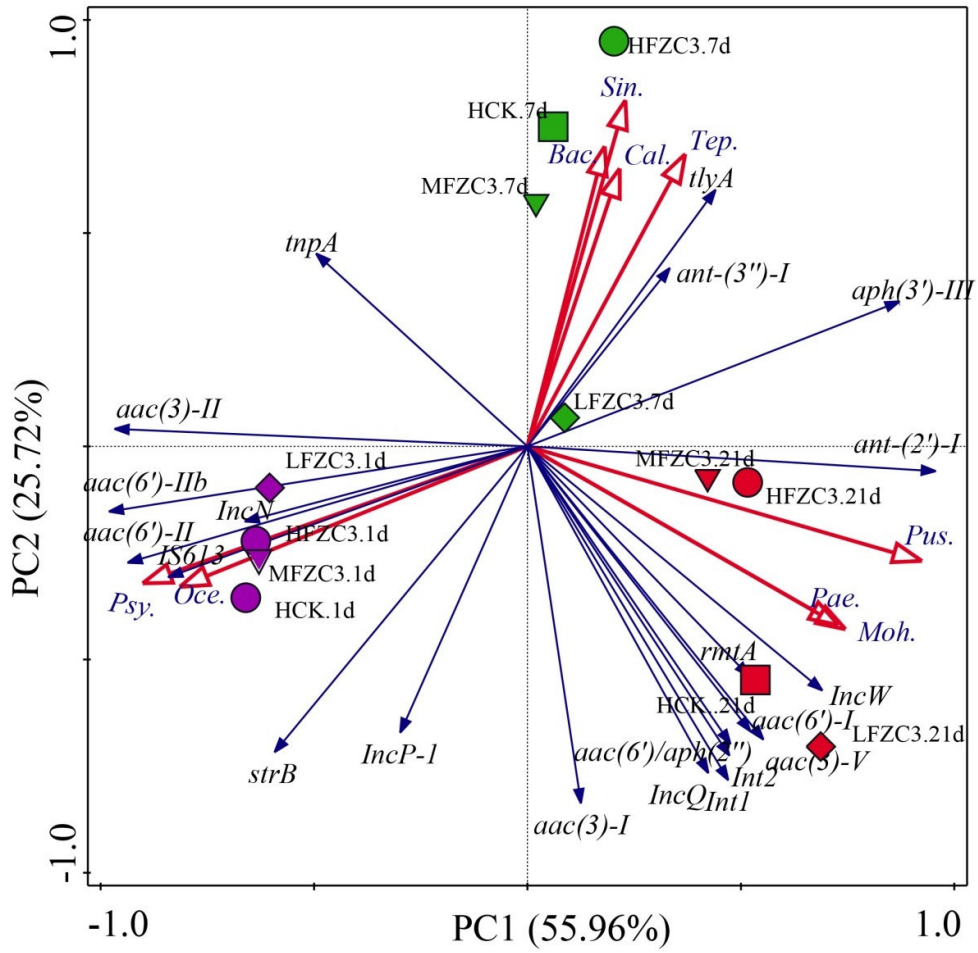

Fig. 5

- *Bacillus* was associated with enhanced degradation of gentamicin and decomposition of OM and DOC
- *Bacillus* was associated with extended thermophilic phase of composting
- The total abundance of ARGs/MGEs remained high values during composting
- The abundance of aminoglycoside ARGs was related to MGEs