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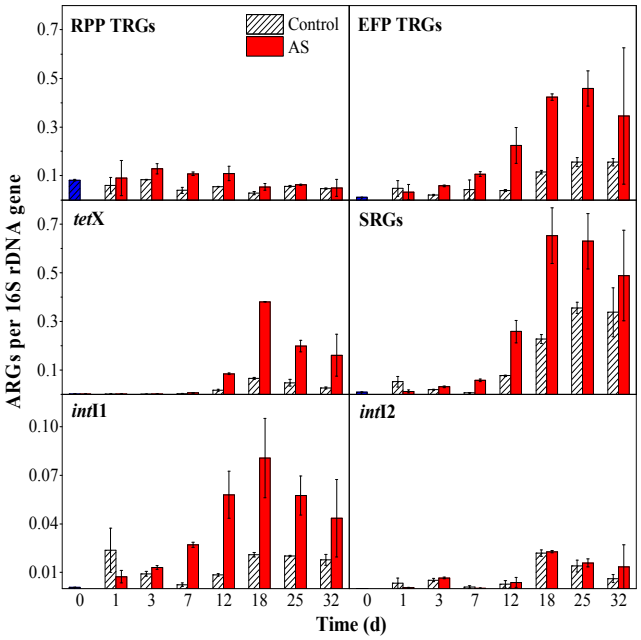
Graphical Abstract

This study investigated the removal of oxytetracycline and sulfamethazine as well as the behavior of antibiotic resistance genes during thermophilic composting of swine manure.



Environmental Impact

The contamination of antibiotics and proliferation of antibiotic resistance genes (ARGs) in the environment have been concerned worldwide. Concentrated animal feeding operations represent an important pollution source of antibiotics and ARGs. This study investigated the removal of two commonly-used veterinary antibiotics, namely oxytetracycline and sulfamethazine, and their corresponding ARGs during thermophilic composting of swine manure. It was found that thermophilic composting could effectively remove the target antibiotics, but failed to prevent the proliferation of ARGs. This study helps to understand the fate and behavior of antibiotics and ARGs during composting of animal manure.

Effects of thermophilic composting on oxytetracycline, sulfamethazine, and corresponding resistance genes in swine manure

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ABSTRACT

Environmental contamination caused by residual antibiotics and antibiotic resistance genes (ARGs) in concentrated animal feeding operations has drawn increasing attention. This study investigated the removal of oxytetracycline (OTC) and sulfamethazine (SMN) as well as the behavior of their corresponding ARGs through a series of simulated composting tests with swine manure. Results indicate that the composting piles became fully mature after 32 d when the thermophilic stage was maintained 55 °C for 3.5 d. At an initial spiked concentration of 50 (SMN) and 32 mg kg^{-1} (OTC), their removal efficiency could reach 89.8% and 100%, respectively. Abiotic process was mainly responsible for the degradation of SMN, whereas both abiotic and biotic processes were responsible for the degradation of OTC. Among all the studied ARGs, only the tetracycline resistance genes encoding ribosomal protection proteins (RPP) remained relatively stable throughout the composting process, while those encoding efflux pump (EFP) and enzymatic inactivation (EI) proteins and sulfonamide resistance genes obviously increased when the composting was complete. The addition of antibiotics inhibited the microbial activity in the early of composting but promoted the proliferation of ARGs particularly in the mesophilic stage. Integron-mediated horizontal gene transfers played an important role in the proliferation of most ARG types studied (i.e., EFP TRGs, EI TRG and SRGs). In summary, thermophilic composting of swine manure could remove the studied antibiotics effectively, but failed to prevent the proliferation of corresponding ARGs.

Keywords: Antibiotics; tetracyclines; sulfonamides; antibiotic resistance genes; swine manure; thermophilic composting

1. Introduction

The contamination of antibiotics and proliferation of antibiotic resistance genes (ARGs) in the environment have drawn increasing attention worldwide. Antibiotics are widely used in concentrated animal feeding operations to control diseases and promote growth. However, approximately 30% to 90% of the applied antibiotics cannot be digested but are excreted in their original forms via manure and urine.[1] Tetracyclines and sulfonamides are two commonly-used classes of veterinary antibiotics in China. According to our previous work, the highest detected concentrations of oxytetracycline (OTC) and sulfamethazine (SMN) in swine manure from different regions in China ranged from 59.06 to 172.9 mg kg^{-1} and from 1.73 to 28.7 mg kg^{-1}, respectively.[2,3]

The spread of antibiotics and their corresponding ARGs caused by discharge of swine wastes have been frequently reported.[4–7] Genes encoding ribosomal protection (RPP), efflux pump (EFP), and enzymatic inactivation (EI) proteins represent three main mechanisms of bacteria resistant to tetracyclines.[8,9] The mutation of dihydropteroate synthase genes (*sul*1, *sul*2, and *sul*3) is the primary mechanism of bacterial resistant to sulfonamides.[10] These ARGs are enriched in swine wastes and can be easily disseminated by means of bacterial reproduction and horizontal gene transfers (HGTs) among bacteria in the environment.[11,12] Heuer et al.[13] found that manure application to soils increased the sulfonamide resistance level of soil bacterial communities. Peng et al.[14] also reported that long-term fertilization with untreated and composted swine manure increased the abundance of tetracycline resistance genes (TRGs) in soils.

As the largest country for pork production in the world, China contributed to 46.3% of the global output in 2013.[15] Accordingly, large amounts of swine manure are produced every year, which requires appropriate treatment before being applied to farmlands. Because thermophilic composting can kill pathogens, worm eggs, and weed seeds in manure, it has been long and widely adopted as a practical technology in China to turn manure into organic fertilizer.

Previous studies have shown that composting could effectively remove antibiotics from manure.[16,17] However, only a few studies have examined the removal of ARGs during composting. Selvam et al.[18] found that after 42 d of aerobic composting, the removal of ARGs resistant to tetracyclines, sulfonamides and fluoroquinolones was greater than 98%. Wang et al.[19] reported that composting achieved a higher removal of both bacteria and ARGs resistant to tetracyclines and macrolide-lincosamide-streptogramin B superfamily than lagoon treatment. However, to date, the behavior and fate of ARGs during manure composting still remain largely unknown.

In this work, a series of simulated thermophilic composting tests with swine manure was carried out to investigate the removal of two representative antibiotics (i.e., SMN and OTC). Meanwhile, the variations in the abundances of sulfonamide resistance genes (SRGs) and TRGs were examined simultaneously and their correlations with related parameters were assessed by Pearson's bivariate correlation analysis. This study helps to understand the fate and behavior of antibiotics and corresponding ARGs during composting of animal manure.

2. Materials and methods

2.1. Composting operation

Fresh sow manure, because of its low background antibiotic concentrations, was collected from a concentrated swine feedlot located in Tongzhou District, Beijing. The total detected concentrations of three tetracycline antibiotics (i.e., tetracycline, OTC, and chlorotetracycline) and six sulfonamide antibiotics (i.e., sulfadiazine, sulfathiazole, sulfamethizole, sulfamethoxazole, SMN, and sulfadimethoxine) were less than 1 and 0.01 mg kg^{-1} fresh weight (fw), respectively. The manure was transported immediately to laboratory and stored at 4 °C in a refrigerator. Their physico-chemical parameters were analyzed as follows: pH = 6.97 ± 0.02; moisture content = (74.3 ± 1.4)%; total organic carbon (TOC) = (35.5 ± 0.3)% (dry weight, dw); total nitrogen (TN) = (2.8 ± 0.1)% (dw); and carbon/nitrogen (C/N) ratio = 12.9 ± 0.6.

Three simulated composting groups were prepared including the control,

antibiotics-spiked (AS), and autoclaved and antibiotics-spiked (AAS). For each composting group, 2.35 kg of fresh manure was mixed with 0.65 kg of saw dust (moisture content = 7.0%; TOC = 50.3% (dw); and C/N ratio = 400–500) by using a blender to adjust the final C/N ratio to 30 and the final moisture content to 60%. Afterward, 3 kg of the above mixture was placed in a stainless steel barrel (inner diameter (i.d.) 24 cm and height 24 cm) with a total volume of 8 L. The control was prepared as described above. The AS was spiked with OTC and SMN to reach an initial concentration of 50 and 32 mg kg^{-1} (fw), respectively. The AAS was sterilized at 121 °C for 20 min in a high-pressure autoclave and then spiked with the same amount of antibiotics as the AS. The stainless steel barrels containing the composting piles were placed in a water bath whose temperature program was set as follows: 45 °C for 0.5 d, 55 °C for 3.5 d, 50 °C for 2 d, 45 °C for 2 d, 42 °C for 2 d, 40 °C for 2 d, 38 °C for 2 d, 34 °C for 3 d, 30 °C for 4 d, 28 °C for 4 d, and 25 °C until the completion of the composting (i.e., Day 32). Each composting pile was turned over twice per day in the first week and once per day thereafter to supply oxygen. The barrels were weighed every day and water was added to the composting piles to maintain a constant water content (60%). Composite samples were prepared by mixing three subsamples respectively taken from the bottom, middle and top layers of each pile on Days 0, 1, 3, 7, 12, 18, 25, and 32. A part of each composite sample was used for measurement of germination index (GI) immediately after preparation, and the rest part was stored at –80 °C for later chemical and biological analyses.

2.2. Composting maturity index determination

The temperature of composting piles was recorded twice per day. TOC and TN concentrations were determined using an elemental analyzer (Vario EL III, Elementar, Germany) in duplicate. The GI was determined in triplicate according to former studies.[20,21] In brief, 4 g of each freshly prepared composite sample was added with 40 mL of sterilized water and mixed thoroughly by a vortex. The mixture was shaken at 200 revolutions per minute (rpm) and 28 °C for 24 h and then centrifuged at 2000× *g* for 10 min. Afterward, 5 mL of the supernatant was transferred into a sterilized Petri

dish (i.d. 90 mm) with two stacked pieces of filter paper as the bottom liner. Sterilized water was used as blank control. After the filter paper was soaked by the supernatant, ten Chinese cabbage (*Brassica chinensis*) seeds were distributed evenly on it and cultivated at 28 °C for 48 h. The GI was calculated using the following equation:

$$\mathrm{GI}\ (\%) = \frac{\text{summation of root lengths in the test sample}}{\text{summation of root lengths in the blank control}} \times 100\% \quad (1)$$

2.3. Antibiotic analysis

SMN and OTC were extracted from freeze-dried composite samples,[2] and thereafter determined in triplicate by ultra-high performance liquid chromatography and tandem mass spectrometry (UPLC-MS/MS, ACQUITY UPLC/Quattro Premier XE, Waters, USA) coupled with a Waters Acquity Symmetry C18 column (50 × 2.1 mm, 1.7 μm). Milli-Q water containing 0.2% formic acid (v/v) (A) and acetonitrile (B) were used as mobile phases at a total flow rate of 0.2 mL min^{-1}. The gradient elution program (time in min, % mobile phase B) was set as follows: (0, 30), (3, 50), (6, 100), and (8, 30). The MS parameters were set according to our previous study.[22] The limit of quantification (LOQ) of SMN and OTC was 6.44 and 4.66 μg kg^{-1}, respectively.

2.4. DNA extraction, q-PCR, and bacterial community analysis

Total DNA was extracted from 0.2 g of each composite sample by using FastDNA SPIN kit for soil (MP-bio, USA). The concentration and quality of the extracted DNA were determined by spectrophotometric analysis (Genequant 1300, GE Healthcare, USA) and agarose gel electrophoresis, respectively. The extraction and analysis of DNA were performed in triplicate for each sample.

Nine TRGs, including four RPP (*tet*M, *tet*O, *tet*Q, and *tet*W), four EFP (*tet*A, *tet*C, *tet*G, and *tet*L) and one EI (*tet*X) TRGs, two SRGs (*sul*1 and *sul*2), and two integrase genes (*int*I1 and *int*I2) were quantified by q-PCR. pMD18-T plasmids carrying the gene fragments of target ARGs were extracted by MiniBEST Plasmid Purification Kit (Takara, Japan) and then quantified by spectrophotometric analysis. The molecular weight of the vector carrying each inserted gene can be calculated by the known DNA sequence; as such, the gene copy number can be calculated from the

determined plasmid concentration. q-PCR was run on an ABI Prism 7300 real-time system (Applied Biosystems, USA). The reaction mixture (25 μL) contained 12.5 μL of 2× SYBR Premix Ex *Taq* GC (Takara, Japan), 0.2 μM each primer, 0.1 mg mL^{-1} BSA, 1× ROX ref. dye, and 5 μL of 30-fold diluted DNA template. Ten-fold serial dilutions (10^8 to 1 gene copy number) of the plasmid DNA were performed to establish the standard curve. A standard curve covering at least five orders of magnitude can be used when $R^2 > 0.99$ and amplification efficiency = (90–110)%. The primers, reaction programs, amplification efficiencies, and limit of detection for gene copy number are listed in Tables S1 and S2.

The primer pair of F984-GC (containing GC-clamp) and R1378 was used to amplify 16S rDNA from the total extracted DNA of composting samples.[23] The reaction mixture (50 μL) consisted of 5 μL of 10× PCR buffer (Mg^{2+} Plus), 0.2 μM each primer, 0.1 mg mL^{-1} BSA, 2.5 U of *Taq* DNA polymerase (Takara, Japan), and 5 μL of 30-fold diluted DNA template. GC-PCR was manipulated as follows: initial denaturation at 95 °C for 5 min, followed by 10 cycles of denaturation for 60 s at 95 °C, annealing for 60 s at 60 °C, and extension for 2 min at 72 °C. In the 10-cycle period, the annealing temperature was decreased by 0.7 °C every second cycle. Afterward, the reaction was continued with 25 cycles of 60 s at 95 °C, 60 s at 53 °C, and 2 min at 72 °C, and then a final extension at 72 °C for 6 min.

After amplification, denaturing gradient gel electrophoresis (DGGE) was performed to separate different 16S rDNA bands. Approximately 15 μL of each GC-PCR product (a mixture of triplicates) was loaded into the denaturing gradient gel. Polyacrylamide gel (6% to 9% gradient) corresponding to a denaturant gradient of 26% to 58% (100% denaturant containing 7 M urea and 40% formamide) was selected with a running time of 5 h at 220 V. Electrophoresis was run on a Dcode system (Bio-rad, USA) filled with 1× TAE buffer at 60 °C. Typical bands were excised under an ultraviolet lamp and immersed in sterilized water at 4 °C overnight. Water with leached DNA was used as a template to re-amplify the bands with non-GC-clamp primers. Re-amplicons were sequenced on a sequencing apparatus (ABI3300, Applied Biosystems, USA).

2.5. Statistical analysis

Correlations between the target ARGs and related parameters were evaluated by Pearson's bivariate correlation analysis (SPSS 19.0, IBM, USA). Paired samples *t*-test based on *p*-value was performed to evaluate the significance of difference among different samples.

3. Results and discussion

3.1. Evaluation of composting efficacy

An entire composting process can be divided into three stages according to the temperature change of the composting piles, namely, thermophilic (Days 1 to 7), mesophilic (Days 8 to 25), and maturation (Days 26 to 32) stages (Fig. 1A). The temperature of all the three groups was controlled according to the US EPA standard for composting, which is "maintained at 55 °C or higher for 3 d".[24] Organic matter is generally humified or mineralized by microbes during composting; as such, the variation of C/N ratio is indicative of microbial activity. As shown in Fig.1B, the C/N ratio in the AAS had an insignificant change over 32 d, indicating little microbial activity. By contrast, the C/N ratio in the control and AS remained relatively stable in the thermophilic stage and decreased considerably in the mesophilic stage. Particularly from Days 12 to 18, it decreased fast from 29.0 to 23.0 and 28.7 to 23.5 in the control and AS, respectively; afterward, it decreased slightly until a final value of 22.3 (control) and 22.7 (AS) was reached on Day 32 when the temperature was decreased to about 26 °C.

Fig. 1

GI is a practical index that indicates the maturity of composting. In addition to the samples from Day 32, samples from Day 7 were also collected for the GI analysis to evaluate the efficacy of thermophilic stage. Results indicate that the GIs of the control and AS were 36.8% and 42.2% on Day 7, respectively (Fig. 2), implying that the thermophilic stage could not remove all harmful substances in the composting piles and thus the seed germination was inhibited. On Day 32, the GIs of both the control and AS were higher than 80%, indicating the maturity of composting piles.[21]

This result also indicates that the addition of antibiotics did not retard the composting process.

Fig. 2

3.2. Degradation of antibiotics during composting

The degradation of antibiotics in the AS and AAS during composting is shown in Fig. 3. Results indicate that in the thermophilic stage (i.e., Days 1–7), the removal efficiencies of SMN and OTC increased quickly, reaching 82.1% and 65.1% in the AS and 74.8% and 38.1% in the AAS, respectively. Afterward, the degradation of SMN became much slower, with a final removal of 100% (AS) and 81.6% (AAS) on Day 32. Meanwhile, the OTC concentration continued to decrease notably in the AS but remained nearly unchanged in the AAS, with a final removal of 89.9% and 40.9% on Day 32, respectively. This result indicates that SMN was degraded mainly by abiotic process, while OTC was degraded by both abiotic and biotic processes. It was reported that OTC could be degraded during manure composting processes with a total removal exceeding 90%,[25,26] which is consistent with our result. There exist conflicting results regarding the degradation of SMN during animal manure composting. Dolliver et al.[27] found that SMN was non-degradable during turkey manure composting; however, Mitchell et al.[28] found that more than 95% of SMN was degraded during dairy manure composting due to the combined effects of feedstock solids and temperature. Furthermore, Ho et al.[29] reported that another sulfonamide, sulfadiazine, could be degraded by more than 99% after broiler manure composting as a result of temperature dependent abiotic processes. Our result is in good agreement with the latter two studies.[28,29] In addition, the sawdust added to the composting piles in this study may also adsorb and then transform SMN to non-extractable forms.[30]

Fig. 3

3.3. Change in bacterial communities during composting

GC-PCR and DGGE were performed to investigate the change in bacterial communities during composting. The following sequencing results of typical bands were obtained: CP-1, CP-3, and CP-7, *Pseudomonas* sp.; CP-2, *Cellvibrio* sp.; CP-4,

Enterobacter sp.; CP-5, *Pseudoxanthomonas* sp.; and CP-6, *Luteimonas* sp. (Fig. 4). The addition of antibiotics only induced a slight and transitory perturbation of the bacterial communities during composting. Compared with the control, *Pseudomonas* sp. (CP-3) and *Enterobacter* sp. (CP-4) were inhibited to some extent during the thermophilic stage in the AS. For both the control and AS, some bands (CP-3 and CP-5) disappeared and other bands (CP-6, CP-2 in the control) appeared on Day 12, indicating that the bacterial communities significantly changed with a decrease of temperature when the composting stage was switched from thermophilic to mesophilic. As the temperature and antibiotic concentrations further declined in the mesophilic stage (after Day 12), the selective pressure alleviated substantially, which led to only minor change in the bacterial communities in the control and AS.

Fig. 4

3.4. Behavior of target ARGs during composting and correlation analysis

The variations of the relative abundances of target ARGs and integrase genes with composting time in the control and AS are shown in Fig. 5. The detailed relative and absolute abundance data are presented in Tables S3 and S4, respectively. Results indicate that their relative abundances had higher levels in the AS, but exhibited similar variation patterns between the two groups in the mesophilic stage ($p < 0.05$). Although the antibiotic concentrations became much lower (i.e., OTC = 5.06 mg kg^{-1}, SMN < LOQ) when the composting was complete, it has been reported that antibiotics at sub-therapeutic levels can promote the accumulation of ARGs,[31] and their degradation byproducts may also favor the maintenance of ARGs.[32] Thus, it is possible that the residual antibiotics in the AS could still stimulate the proliferation of ARGs.

Fig. 5

The RPP TRGs (summation of *tet*M, *tet*O, *tet*Q, and *tet*W) initially dominated in the thermophilic stage, decreased slightly in the mesophilic stage, and then remained nearly constant in the maturation stage. By contrast, the EFP TRGs (summation of *tet*A, *tet*C, *tet*G, and *tet*L), EI TRG (*tet*X), and SRGs (summation of *sul*1 and *sul*2)

were initially present at low levels in the thermophilic stage, increased obviously in the mesophilic stage with peak values appearing on Days 18–25 ($p < 0.05$), and then decreased to some extent in the maturation stage. The integrase genes (i.e., *int*I1 and *int*I2) exhibited a similar variation pattern, probably reflecting active integron-mediated HGTs during the mesophilic stage. It is noted that the behavior of ARGs during composting observed in this study was different from other former studies.[18,19] In this study, the composting piles were maintained at 55 °C for 3.5 d, but a higher tempreature or a longer time of composting was adopted by other studies that may faciliate the removal of ARGs.[18,19] Moreover, the water content was maintained constant (i.e., 60%) in this study by periodically supplementing water to the composting piles, which was likely to favor the survival of antibiotic resistance bacteria.[19]

From the C/N ratio variations (Fig. 1B) and the DGGE profiles (Fig. 3), it can be seen that the microbial activity was inhibited and the bacterial communities only changed slightly in the thermophilic stage, which led to minor change of target ARGs. As the temperature decreased quickly in the early half of the mesophilic stage, the bacterial communities changed significantly and the relative abundances of target ARGs changed accordingly. Moreover, the correlation analysis (Table 1) between the Log-transformed ARGs per 16S rDNA gene and related parameters shows that most of the RPP TRGs (*tet*M, *tet*Q, and *tet*W) exhibited significant positive correlations with the temperature and antibiotic concentrations ($p < 0.01$ or $p < 0.05$), while other TRGs (*tet*A, *tet*C, *tet*G, and *tet*X) and SRGs (*sul*1 and *sul*2) were not significantly or even negatively correlated with the two parameters. It suggests that bacteria carrying RPP TRGs were less adaptive than those carrying other TRGs and SRGs in the mesophilic stage as the temperature and antibiotic concentrations decreased, probably because the former mainly consisted of enteric bacteria that tended to diminish during the composting.[8] Thus, in the mesophilic stage, the RPP TRGs decreased to some extent while other TRGs and SRGs increased obviously. In addition, the negative correlation between Log *tet*X and OTC indicates that the biodegradation of OTC was not mainly attributed to *tet*X. *tet*X was first found in anaerobic *Bacteroides*, but its

expressed protein could only degrade tetracyclines enzymatically in the presence of oxygen and nicotinamide adenine dinucleotide phosphate.[33] To date, little information is available on the function of *tet*X in the environment. Likewise, its significant increase in the composting process needs further study.

Table 1

Integrons are important gene elements involved in HGTs.[34,35] Class 1 and Class 2 integrons have been commonly found to be closely related to tetracycline and sulfonamide resistances.[36–39] In this study, *tet*G, *tet*X, *sul*1, and *sul*2 were positively correlated with *int*I1 and *int*I2 ($p < 0.01$ or $p < 0.05$); *tet*L was positively correlated with *int*I1 ($p < 0.05$); but three RPP TRGs (*tet*M, *tet*Q, and *tet*W) were negatively correlated with *int*I1 or *int*I2 ($p < 0.01$ or $p < 0.05$). RPP TRGs are commonly found in conjugative transposons;[4] EFP TRGs and SRGs are likely associated with plasmids that can harbor integrons;[8,10] and *sul*1 and *tet*G are directly associated with integrons.[34,40] The different gene locations of ARGs may account for the contrasting correlations between different ARGs and integrase genes observed in this study. In addition, the positive correlations between most of the studied ARG types (i.e., EFP TRGs, EI TRG, and SRGs) and the integrase genes suggest that integron-mediated HGTs played an important role in the proliferation of ARGs during manure composting.

4. Conclusions

In this study, a series of simulated thermophilic composting tests with swine manure was carried out to investigate the removal of OTC and SMN as well as the behavior of their corresponding ARGs. Based on the experimental results, the following conclusions can be drawn:

- The addition of antibiotics did not retard the maturation of composting piles but induced an obvious proliferation of corresponding ARGs. The removal of OTC and SMN reached 89.9% and 100% after 32 d of composting, respectively.
- RPP TRGs remained quite stable throughout the composting process, while EFP TRGs, EI TRG and SRGs that had initial low levels increased obviously in the

mesophilic stage.

- Most of the RPP TRGs exhibited significant positive correlations with the temperature and antibiotic concentrations, while other TRGs and SRGs showed insignificant or even negative correlations with the two parameters.
- The positive correlations between most of the studied ARG types (i.e., EFP TRGs, EI TRG, and SRGs) and the integrase genes suggest that integron-mediated HGTs were important for the proliferation of ARGs during manure composting.
- Further study is required to investigate the simultaneous removal of antibiotics and their corresponding ARGs by optimizing the composting conditions of animal manure.

Acknowledgments

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Electronic supplementary information (ESI) Available

Additional information is available on PCR primers for target ARGs, integrase genes and 16S rDNA gene; q-PCR conditions; relative and absolute abundances of target ARGs and integrase genes during composting (Tables S1–S4).

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Figure captions

Fig. 1 Variations of temperature (A) and C/N ratio (B) with composting time. AS: antibiotics-spiked; AAS: autoclaved and antibiotics-spiked; error bars represent the relative percent difference (n = 2).

Fig. 2 Variation of germination index (GI) during composting. Error bars represent the standard deviations (n = 3).

Fig. 3 Removal of target antibiotics with composting time. OTC: oxytetracycline; SMN: sulfamethazine; $C_{OTC,0}$ = 50 mg kg^{-1} (fw); $C_{SMN,0}$ = 32 mg kg^{-1} (fw); error bars represent the standard deviations (n = 3).

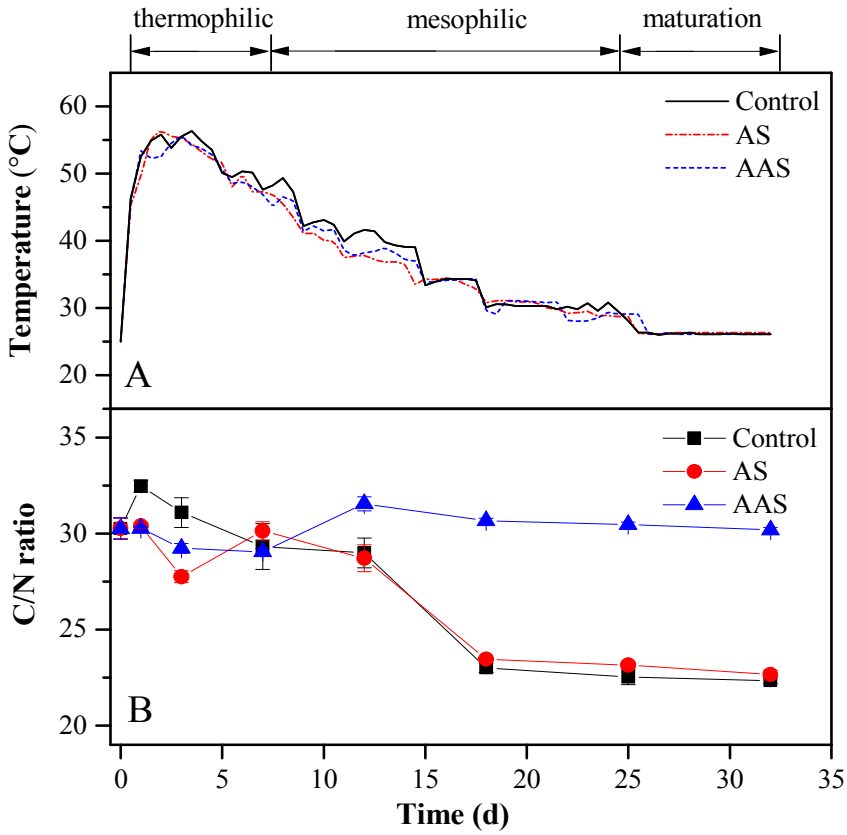
Fig. 4 DGGE profiles of composting samples (with typical bands numbered).

Fig. 5 Variations of the relative abundances of target ARGs and integrase genes with composting time. The data on Day 0 represent the initial relative abundances of target ARGs and integrase genes for both the control and AS; error bars represent the standard deviations (n = 3).

Table 1 Pearson's correlation analysis between Log-transformed ARGs per 16S rDNA gene and related parameters in the composting process

Category	ARG	SMN[a]	OTC[a]	Log *int*I1[b]	Log *int*I2[b]	Temp.[b]
RPP TRGs	Log *tet*M	0.884[c]	0.896[c]	-0.502	-0.631[c]	0.953[c]
	Log *tet*O	0.600	0.365	0.450	-0.078	0.248
	Log *tet*Q	0.837[d]	0.985[c]	-0.692[c]	-0.568[d]	0.818[c]
	Log *tet*W	0.867[d]	0.916[c]	-0.295	-0.618[d]	0.931[c]
EFP TRGs	Log *tet*A	-0.783[d]	-0.600	0.410	-0.133	0.255
	Log *tet*C	-0.859[d]	-0.950[c]	0.325	0.210	-0.106
	Log *tet*G	-0.685	-0.914[c]	0.690[c]	0.623[d]	-0.803[c]
	Log *tet*L	0.172	-0.202	0.579[d]	-0.095	0.152
EI TRG	Log *tet*X	-0.937[c]	-0.942[c]	0.715[c]	0.596[d]	-0.902[c]
SRGs	Log *sul*1	-0.841[d]	-0.963[c]	0.888[c]	0.655[d]	-0.827[c]
	Log *sul*2	-0.897[c]	-0.867[d]	0.770[c]	0.786[c]	-0.891[c]

[a] Data from the antibiotics-spiked (AS) group (n = 7). [b] Data from the control and AS groups (n = 15). [c] Significance level = 0.01. [d] Significance level = 0.05.



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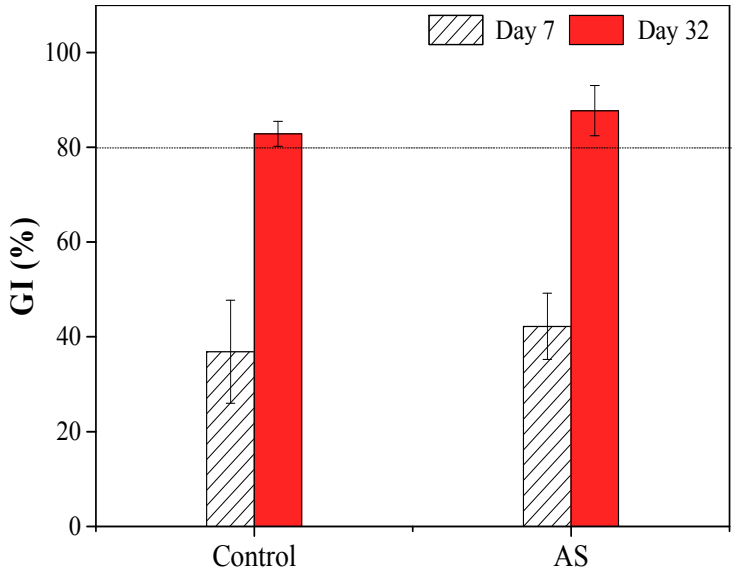

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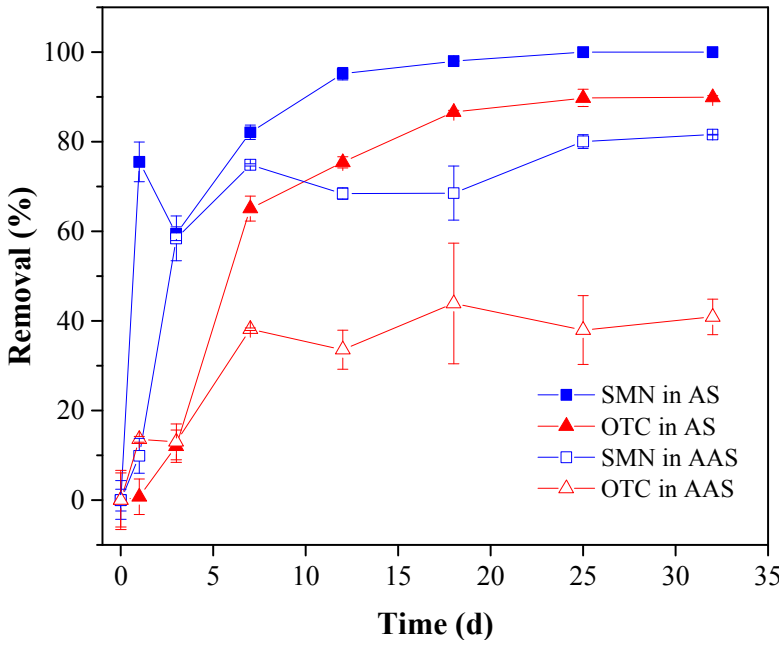

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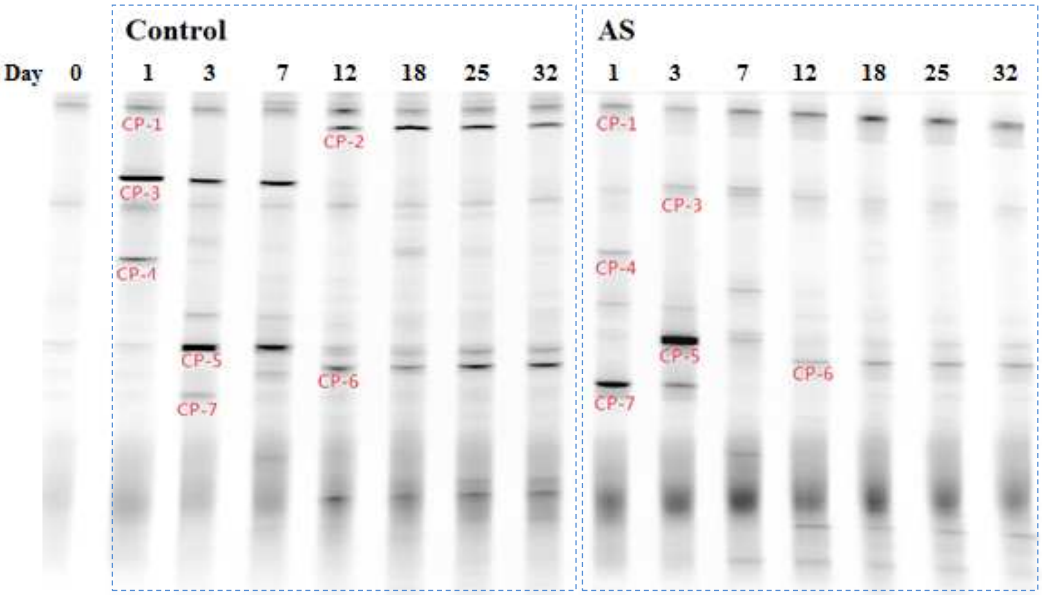

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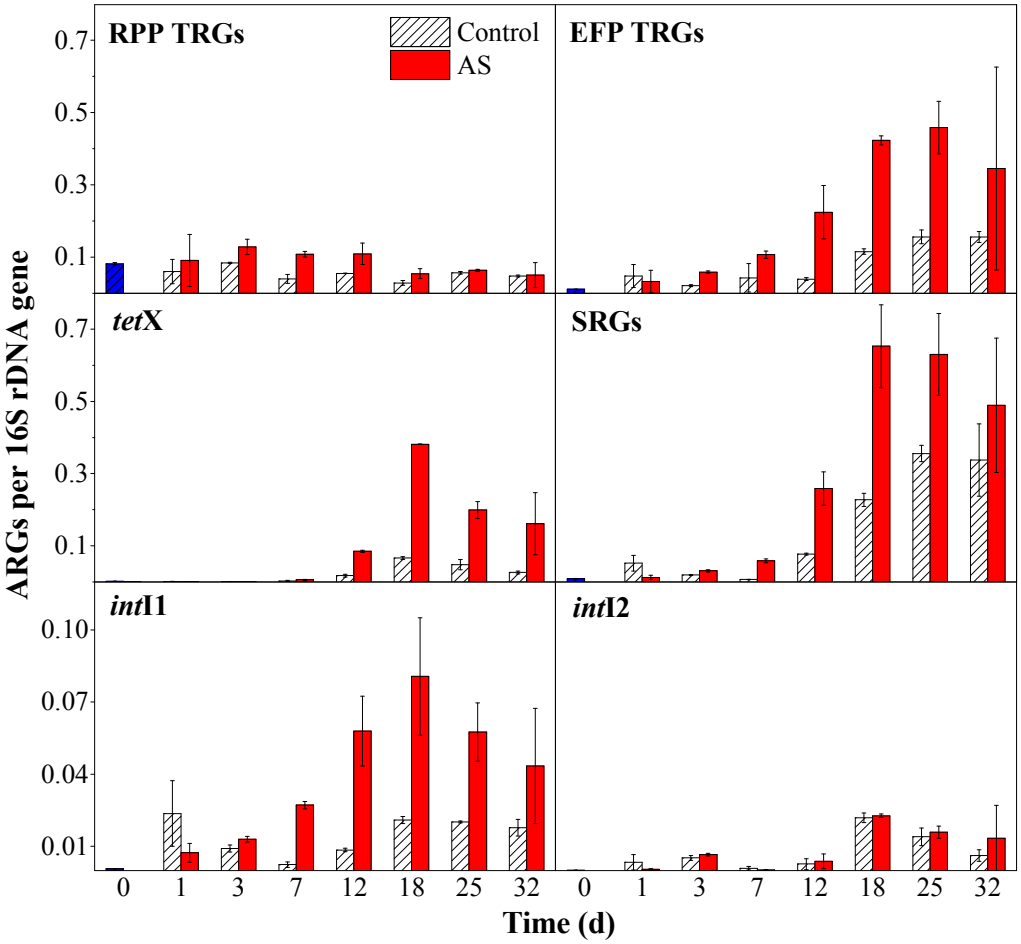

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