



# Occurrence of heavy metals, antibiotics, and antibiotic resistance genes in different kinds of land-applied manure in China

Jiajia Xue<sup>1,2</sup> · Jianqiang Wu<sup>3</sup> · Yaru Hu<sup>1</sup> · Chenyan Sha<sup>3</sup> · Shijie Yao<sup>1</sup> · Peng Li<sup>4</sup> · Kuangfei Lin<sup>1</sup> · Changzheng Cui<sup>1,2</sup>

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## Abstract

Various pollutants remaining in the livestock and poultry manures pose potential threat to the soil ecosystem during land application, whose impact should be appreciated. The occurrence of heavy metals, antibiotics, and antibiotic resistance genes (ARGs) in swine manure (SM), chicken manure (CM), and the SM organic fertilizer (OF) were investigated. The order of total concentrations of antibiotics detected in manures was as follows: SM > CM > OF. The amount of ciprofloxacin (CIP) in SM reached up to 6.61 mg/kg, which only occupied 1% of the antibiotic concentration reported in the past years. The total concentration of thirteen ARGs in CM ranked first, reaching  $7.35 \times 10^{11}$  copies/g, among which the *strB* gene was detected with the highest concentration. It was worth noting that the *qnr* ARGs were persistent in OF with the absence of corresponding antibiotics, indicating ARGs were harder to remove than antibiotics during manure composting. Zn and Cu (46.5–843 mg/kg) were obviously higher than other seven heavy metals, and significantly correlated with most ARGs ( $p < 0.01$ ). This study provided the basic data of the pollution in animal manures that will be land-applied, illuminating the original source of potential risk in soil ecosystem.

**Keywords** Manure · Antibiotics · Antibiotic resistance genes · Heavy metals · Community composition

## Introduction

Heavy metals are often added as additives to animal feed (Hashemi 2018). These metal elements cannot be fully utilized during the animal growth, which will increase their contaminative levels in environmental soil (Liu et al. 2020a, b).

Moreover, heavy metals may be accumulated in plants or meat (Hashemi 2018; Duan et al. 2019), and pose health risks to the human body through the food chain. Many studies have demonstrated the presence of heavy metals in manure and fertilizing soils. The emissions of heavy metals, Cu and Zn, in the discharged 240 million tons animal manures reached 7153 tons in 2010 (Xu et al. 2019a). The concentration of heavy metals in the animal manure compost ranged from 0.01 to 3692 mg/kg, and the amount of Cr reached up to 6603 mg/kg (Yang et al. 2017). Mean concentration of As in chicken manure from several states in USA was 15.7 mg/kg (Jackson et al. 2003). Therefore, for such a large amount of animal manure and high concentration of heavy metals, it is necessary to monitor the content of heavy metals in animal feces to understand the possible pollution.

Many countries, especially the European Union, have banned the use of antibiotics as growth promoter since 2006, but there are still many antibiotics added to feed in countries dominated by animal-produce (Ben et al. 2017; Carlota et al. 2021). Antibiotics are also often used as the animal feed additives in the livestock breeding, such as cattle, swine, and poultry, to prevent animal disease and promote animal growth (Kumar et al. 2005), which cover more than 16 and 80 million

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✉ Jianqiang Wu  
wujq@saes.sh.cn

✉ Changzheng Cui  
cuichangzheng@ecust.edu.cn

<sup>1</sup> State Environmental Protection Key Laboratory of Environmental Risk Assessment and Control on Chemical Process, School of Resources and Environmental Engineering, East China University of Science and Technology, Shanghai 200237, China

<sup>2</sup> Shanghai Institute of Pollution Control and Ecological Security, Shanghai 200092, China

<sup>3</sup> Shanghai Academy of Environmental Sciences, 508 Qinzhou Road, Shanghai 200233, China

<sup>4</sup> College of Environmental Science and Engineering, Donghua University, 2999 Renmin North Road, Shanghai 201600, China

kilograms every year in the USA and China, respectively (Ji et al. 2012). Most antibiotics cannot be metabolized completely by animal body, about 30–90% of which are discharged via the urine or feces excretion (Ajit et al. 2006; Zhang et al. 2021). Large amount of livestock and poultry manures are produced every year around the world, introducing a great deal of antibiotics into the environment via land-applied in rural area (Guo et al. 2018). As we all know, the livestock and poultry manure application can bring antibiotics into the soil system and introduce resistant microorganisms and antibiotic resistance genes (ARGs) (Macedo et al. 2020). Therefore, the potential risk of residual antibiotics in manure to farmland ecosystem should be paid attention to.

The land application of animal manures was considered as the primary original source of antibiotics into the environment (Cui et al. 2016). For example, seven antibiotics were remained in swine manure, with the concentration range of prioritized antibiotics (sulfadiazine and doxycycline) being from 55 to 7744.6  $\mu\text{g}/\text{kg}$  (Meersche et al. 2019), and the concentration of antibiotics in manure slurry still presented with the amount of 13.7–685.6  $\mu\text{g}/\text{L}$  (Chen et al. 2012). Antibiotic residues were detected in various farm plants, including corn, tomatoes, lettuce, and carrot planted on the farm-applied animal manures (Rahube et al. 2014; Mullen et al. 2019). What's more, antibiotics also induce the emergence and development of ARGs among soil microorganism (Zhao et al. 2021; Liu et al. 2021a). Previous study reported that the transfer of ARGs in manure was accelerated (Ngigi et al. 2019). Sulfonamide and tetracycline ARGs in bovine manures reached  $10^{-6}$ – $10^{-3}$  (relative concentration, ARGs/16S rRNA) (Lin et al. 2019). Xu et al. (2019b) found that the abundance of 146 ARGs belonging to four antibiotic types decreased during compost, while the half-life and persistence of ARGs increased. The risk induced by antibiotics and ARGs' spread was a great challenge for the ecosystem and food chain for human health (Rahube and Yost 2012; Zhang et al. 2015). However, few studies investigate the correlation between heavy metals, antibiotics, and ARGs in the manures as well as their potential risk. Therefore, in this study, we will systematically study it.

In order to discover the original source of heavy metals, antibiotics, and ARGs into the farmland, three different manures were collected to investigate the occurrence and the correlation among those contaminants in this study. The aim of this study is to carry out a comprehensive analysis of the potential pollution source of farmland ecosystem—manure—to provide the basis for blocking pollutants from manure into farmland ecosystem, then make better use of animal manure and reduce its harm to people and the environment. These results will provide basic data for the heavy metal, antibiotics, and ARG pollution in land-applied manures, illuminating the original source of antibiotic risk in soil ecosystem.

## Materials and methods

### Sample collection

Three kinds of manure samples were collected in April 2019 from a swine farm and a chicken farm in Jinshan and Chongming, Shanghai, China, respectively. The swine farm produced 26000 meat pigs a year, with the annual production of swine manure about 20000 tons. The chicken farm produced 48000 laying hens a year, producing chicken manure about 2100 tons per year. The animal manure produced by the two farms was stored in the cement storage tanks. The manure used in this experiment was fresh manure produced within 2 days. The organic fertilizer (OF) was prepared by mixing the swine manure (SM) and the mushroom growing base material through the composting. The moisture contents of SM, CM, and OF were 68.84%, 66.26%, and 42.28%, respectively, in Table 1. At each point, about 5 L of manure was collected in a plastic container. Each sample was performed in three parallels. All samples were shipped to the laboratory in a portable refrigerator and stored in  $-80\text{ }^{\circ}\text{C}$  refrigerator for subsequent experimental analysis.

### Conventional indices and heavy metals of manure

The tested indices including organic matter, total N,  $\text{NH}_4^+\text{-N}$ , and  $\text{NO}_3^-\text{-N}$ . Nine heavy metals (Cd, Pb, Cu, Ni, Zn, As, Ag, Hg, and Cr) in manures were determined. After freeze-dried samples, 0.2 g of the sample was placed in a Teflon crucible with a mixture of HF,  $\text{HNO}_3$ , and  $\text{HClO}_3$  acid to perform a digestion reaction. The solution obtained from the reaction was diluted to 50 mL in a volume bottle, filtered, and stored at  $4\text{ }^{\circ}\text{C}$  for further analysis. The concentration of metal ions was quantified by inductively coupled plasma-mass spectrometry (ICP-MS) (Guo et al. 2018). All measurements were performed three times.

### Analysis of antibiotics

#### Chemicals and reagents

Sulfadiazine (SDZ), sulfamethazine (SMZ), sulfamethoxazole (SMX), chlortetracycline (CTC), oxytetracycline (OTC), tetracycline (TC), doxycycline (DOC), TC- $\text{D}_6$ , SMX- $^{13}\text{C}_6$ , and CIP- $\text{D}_8$  were obtained from Sigma (USA). Norfloxacin (NOR), ciprofloxacin (CIP), ofloxacin (OFL), and enrofloxacin (ENR) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The purity of all drugs was more than 98%. Acetonitrile (99.9%) and methanol (99.9%) were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Ultrapure water was prepared using a Milli-Q water system (Millipore, USA).  $\text{Na}_2\text{EDTA}$ -McIlvaine buffer solution was prepared by dissolving 12.9 g

**Table 1** Conventional index and heavy metals in different manures. Units: mg/kg

Index		Swine manure	Chicken manure	Organic fertilizer	Limit value
Conventional index	Moisture	68.84%	66.26%	42.28%	—
	TOC	316000.00 ± 3532.00	427000.00 ± 8230.73	616000.00 ± 1053.59	—
	NH <sub>4</sub> <sup>+</sup> -N	4770.00 ± 77.78	19800.00 ± 120.1	436.00 ± 6.36	—
	NO <sub>3</sub> <sup>-</sup> -N	613.00 ± 7.78	5400.00 ± 127.28	4770.00 ± 219.20	—
	TN	14600.00 ± 47.38	21400.00 ± 28.28	16900.00 ± 42.43	—
Heavy metals	Cd	0.31 ± 0.0	0.12 ± 0.007	0.38 ± 0.0	3
	Cr	4.60 ± 0.07	2.90 ± 0.14	7.60 ± 0.21	150
	Cu	311.00 ± 2.83	46.50 ± 0.35	248.00 ± 2.83	—
	Zn	1820.00 ± 42.43	397.00 ± 7.78	843.00 ± 12.73	—
	As	1.80 ± 0.07	0.90 ± 0.07	4.40 ± 0.14	15
	Pb	2.00 ± 0.14	2.00 ± 0.07	5.20 ± 0.14	50
	Hg	0.12 ± 0.0	0.06 ± 0.0	0.07 ± 0.0	2
	Ag	0.80 ± 0.07	1.40 ± 0.0	0.40 ± 0.0	—
	Ni	6.40 ± 0.01	3.90 ± 0.21	10.20 ± 0.07	—

Limit value from the standard of organic fertilizer (NY 525, based on manure, plant/animal residues, and/or by-products), Ministry of Agriculture of China, 2012

of citric acid monohydrate, 27.5 g of Na<sub>2</sub>HPO<sub>4</sub>, and 41.18 g of Na<sub>2</sub>EDTA·2H<sub>2</sub>O in 1.0 L of ultrapure water, with the pH adjusted to 4.00 ± 0.05 using HCl or NaOH. This solution was prepared daily and stored in a refrigerator until used.

HLB solid-phase extraction columns (500 mg/6 mL) were obtained from ANPEL Laboratory Technology, Shanghai. The residual antibiotic concentration was analyzed by high-performance liquid chromatographic tandem mass spectrometry (HPLC-MS-MS, Agilent, USA).

### Experimental operation

Before the experiment began, all samples were freeze-dried for 48 h. These samples were crushed and refined and then weighed 1 g of samples placed them in a 50-mL centrifugal tube. Add 10 mL of 1:1 mixture of methanol and Na<sub>2</sub>EDTA-McIlvaine buffer to the tube. Next, concussion of the centrifuge tube for 1 min (2500×g per minute), ultrasonicated for 20 min (50 KHz, 300 W), 5000×g centrifugation for 10 min. After the supernatant was collected, the sample was repeat extracted twice. After three consecutive extracts, a total of 30 mL supernatant was obtained. Dilute it to about 300 mL in a 1000-mL glass bottle with ultrapure water to reduce the methanol content to ≤ 5%.

The diluted extraction of manure was concentrated and purified by HLB columns. The SPE solid-phase extraction column was activated by adding 5 mL methanol and 5 mL ultrapure water in turn (natural deposition of methanol and ultrapure water in columns). Then connected the diluent to the columns and adjusted the flow rate to about 5 mL/min. Elution of impurities with 5% methanol-water solution after

extracting the solution. Finally, 10 mL methanol was used to elute the enriched antibiotics on the columns and collected in the test tube. Blew the eluent close to dry with nitrogen and fixed the volume with 1 mL of 50% methanol-water. Filter the above solution with a 0.22-μm needle filter and transfer to a brown injection bottle to be stored at - 20 °C, waiting to be detected by HPLC/MS/MS system.

The chromatographic separation of antibiotics was carried out by an Agilent Technologies 1260 HPLC system, which was composed of a binary solvent manager and a sample manager. The separation of compounds was performed using an Agilent Zorbax Eclipse Plus C18 column (1.8 m, 2.1 mm × 100 mm). The detection conditions of liquid chromatography are as follows: mobile phase A: acetonitrile solution containing 0.1% formic acid; mobile phase B: pure water containing 0.1% formic acid; flow rate: 0.4 mL/min; injection volume: 10 μL; chromatographic column: Agilent Zorbax Eclipse Plus C18 and column temperature: 35 °C; gradient eluting program: the eluting gradient began with 85% B, decreased to 75% at 0–5 min, and further decreased to 55% at 5–10 min. Finally, the linearity returned to 85% within 4 min and maintained 1 min, until the next injection (Cui et al. 2019). The detailed process of mass spectrometry refers to the literature previously reported (Cheng et al. 2015).

### Analysis of ARGs

#### DNA extraction

DNA was extracted from 0.5 g of fresh manures with three replicates using the FastDNA Spin Kit for Soil (MP

Biomedicals, USA). The steps were carried out according to the instruction provided by the manufacturer. The quality and concentration of the purified DNA were measured by gel electrophoresis and spectrophotometer (NanoDrop ND 1000, Thermo, USA) (Hu et al. 2019). The separated DNA was stored at  $-20\text{ }^{\circ}\text{C}$  until qPCR analysis was performed.

### Quantification of ARGs (qPCR)

According to previous studies (Liu et al. 2021b; Eric et al. 2020; Wang et al. 2019a), the abundances of thirteen ARGs belonging to five different antibiotic classes (*tetA*, *tetW*, *tetQ*, *sul1*, *sul2*, *qnrD*, *qnrS*, *qnrA*, *qepA*, *strA*, *strB*, *bla<sub>OXA-1</sub>*, and *bla<sub>TEM-1</sub>*) were investigated in this study. The 16S rRNA and *intI1* gene were also quantified in each sample using qPCR. The construction of standard plasmid, the establishment of standard curve, and other experimental processes were carried out with reference to the previous reports (Ji et al. 2012; Hu et al. 2018). The establishment of standard curve and the quantification of target ARGs were carried out on LightCycler 480 II instrument. The primer sequence, annealing temperature of each ARG subtypes, and the manipulation of qPCR referenced to Hu et al. (Hu et al. 2019). All samples were tested in triplicate and ddH<sub>2</sub>O was added as the negative control. More information about the qPCR reactions was in Table S4 and Table S5.

### Analysis of community structure

Based on high-throughput sequencing, the distribution characteristics of microbial community structure in different manure were analyzed. The raw data were obtained from Sangon Biotech (Shanghai) Co., Ltd. and paired-end sequenced (2 × 300 bp) using the Illumina HiSeq sequencing platform, then follow-up analysis was made. The V3–V4 hypervariable regions were amplified using the primers 341F (5'-CCTA CGGGNGGCWGCAG-3') and 805R (5'-GACT ACHVGGGTATCTAATCC-3'). The distribution of community structure at genus level of different samples was analyzed. USEARCH (version 7.1) was employed to assign cluster Operational Taxonomic Units (OTUs) at the 97% similarity. In brief, setting a window with length of 10 bp, and removing the bases with reads tail mass value below 20 in each sample. The sample files were then merged in a fastq file, the primer sequences were removed using cutadapt (version 1.2.1), and the Prinseq (with parameters `-lc_method dust -lc_threshold 40 -min_len 200`) was used to filter the quality of each sample data. Using RDP for classification analysis, the database is <http://rdp.cme.msu.edu/misc/resources.jsp>. Chao1 index, Shannon index, and other indexes were obtained to access the alpha diversity. The raw reads were uploaded into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA703060).

### Statistical analysis

Microsoft Excel was used to filter and calculate all raw data. All related graphics were generated using Origin Pro 9.0. The correlation analysis was performed using SPSS, and *p* values < 0.05 were considered statistically significant.

## Results and discussion

### Conventional indices of SM, CM, and OF

Table 1 summarized the conventional indices and heavy metals in three different manures (SM, CM, and OF). The amounts of total organic carbon (TOC) in SM and CM were detected with the concentrations being  $3.16 \times 10^5$  and  $4.27 \times 10^5$  mg/kg, respectively, which held the same level as those in SM ( $3.5 \times 10^5$  mg/kg) and CM ( $3.3 \times 10^5$  mg/kg) from Guangdong (Zhang et al. 2019). However, the TOC in OF was slightly higher than that in SM, which might be due to the additional material during the composting process, the amounts of NH<sub>4</sub><sup>+</sup>-N ( $4.77 \times 10^3$ – $1.98 \times 10^4$  mg/kg) and NO<sub>3</sub><sup>-</sup>-N ( $6.13 \times 10^2$ – $5.40 \times 10^3$  mg/kg) in SM and CM were also higher than those in the manures from Guangdong ( $3.50 \times 10^3$ – $1.90 \times 10^4$  mg/kg and 103–124 mg/kg) (Zhang et al. 2019). In this study, the concentration of NH<sub>4</sub><sup>+</sup>-N in OF was lower than that in SM, but the content of the NO<sub>3</sub><sup>-</sup>-N was the opposite. This might be due to the addition of mushroom substrate that can increase the great bioactivity of nitrifying microorganism during composting that NH<sub>4</sub><sup>+</sup>-N can be converted to NO<sub>3</sub><sup>-</sup>-N and released NH<sub>3</sub> (Meng et al. 2017).

The residual heavy metals in animal manures were also monitored. The concentrations of Cu and Zn in three manures were higher than other heavy metals, with the concentrations 46.5–311 mg/kg and  $3.97 \times 10^2$ – $1.82 \times 10^3$  mg/kg, respectively, which was greatly higher than the limit values of the compost/organic fertilizer standards from Australia and the EU (70 and 200 mg/kg) (Yang et al. 2017). This result gave a warning of the residual of Cu and Zn. The main reason for the high concentration of Zn and Cu in manure was their misuse in feed (Hasman et al. 2006). For example, the dosage of Cu and Zn in feed from seven provinces and cities in China were 1017–1591 and 7113–8710 mg/kg, respectively (Zhang et al. 2005). Wang et al. (2013) detected 427.2 mg/kg and 132.3 mg/kg Zn, and 113.7 mg/kg and 141.7 mg/kg Cu in pig manure and chicken manure, respectively. The concentrations of other seven heavy metals were between 0.06 and 10.2 mg/kg. Among the nine detected heavy metals, the amounts of As, Cr, Cd, Pb, and Hg were all lower than the limit values of the Standard of Ministry of Agriculture of China (Yang et al. 2017). The level of Hg, Pb, Cd, and Cr in this study (0.06–7.6 mg/kg) were similar to those in a previous study (below 10 mg/kg) (Wang et al. 2013).



As shown in Table 1, the contents of heavy metals in CM were lower than those in SM, especially for the contents of Zn and Cu. This might be due to that the amount of heavy metals added in the feed of chickens and pigs were greatly different (Wang et al. 2013). The concentrations of Zn and Cu detected in a previous report in pig and chicken feeds were 15.9–2041.8 and 0–392.1 mg/kg, and 5.6–296.3 and 3.8–198.7 mg/kg (Wang et al. 2013). The contents of Zn and Cu in OF were lower than that in SM. The morphology of Cu and Zn can be changed during composting. The composting process reduced the activity and mobility of Zn and Cu, resulting lower contents of Zn and Cu in the OF than those in the SM (Li et al. 2019a). The concentration range of Zn, Cu, Cr, Ni, Pb, and Cd in the OF were from 0.07 to 843 mg/kg, which was higher than those in the composition process (0.012 to 6629 mg/kg) (Yang et al. 2017), showing that the heavy metal content of the compost samples in this study was at a lower level.

### Community structure of SM, CM, and OF

Livestock and poultry manures contained various animal intestinal microorganisms, which might further impact the soil ecosystem, so the microorganism community structure of these three manures was analyzed. Alpha diversity indexes of samples were summarized in Table 2 and the Venn analysis for bacterial species of samples was presented in Fig. S1. All samples' coverages were higher than 94%. SM, CM, and OF samples covered 1533, 4222, and 2695 OTUs, and only 81 OTUs were shared in three manures, only accounting for 1.9–5.3% of all manures, indicating that three manures had great difference. The Shannon index order of SM, CM, and OF ranked as SM (5.64) > OF (5.15) > CM (3.54), which indicated the most abundant species composition of SM. The Simpson index order was as follows: CM (0.09) > SM (0.02) = OF (0.02), showing the lowest sample diversity in CM. During composting, undegradable organic matter was degraded and utilized microorganisms, which might lead to higher species abundance in OF than CM (Wang et al. 2019b).

The composition of microorganisms in different manure at genus level was studied in this study. There were significant differences in the diversity of bacterial genus level in Fig. 1. The highest abundance of genera in CM, SM, and OF was *Sphingobacterium* (44.81%), *Arcobacter* (6.60%), and *Nonomuraea* (4.35%), respectively. *Sphingobacterium* was a

common dominant bacterium in animal manure, compost, or manured soil (Wang et al. 2019b; Wang et al. 2018; Dong et al. 2018). *Sphingobacterium* showed tolerance to high temperature, converted cellulose and lignin into stable humus (Wang et al. 2019b). *Arcobacter* was reported to be a dominant pathogen in aquaculture (Liu et al. 2020a, b), indicating that the SM should not be land-applied directly, especially for the rice fields. *Nonomuraea*, the dominant bacteria of OF in this study, was reported to be significantly related to the degradation rate of aromatic C and olefin C during composting ( $R^2 = 0.976, p < 0.05$ ) ( $R^2 = 0.976, p < 0.05$ ) (Jiang et al. 2019). *Myroides* (8.24%) and *Luteimonas* (7.56%) were the other two abundant genera in the CM. *Comamonas* (5.94%), *Kurthia* (5.87%), *Acinetobacter* (5.82%), *Clostridium sensu stricto* (5.21%), and *Ruminococcus* (5.15%) had higher abundance in SM, indicating that multiple microorganisms survive together in the SM. After composting, nearly 60% genera in OF were unclassified. *Acinetobacter* was reported to be the dominant genus in compost (Jiang et al. 2019; Tortosa et al. 2017). Figure 2 shows that the abundance of the same bacterial genus in the three manures were significantly different, and most of the species in SM had higher abundance. Table S1 also demonstrated significant differences in bacterial community composition among the three manures. When animal manure is used as fertilizer to farmland, there is concern about the spread of zoonotic bacteria, which may contain pathogenic microorganisms (Bui et al. 2011). *Luteimonas* was the dominant genus in the soil of chicken manure improvement and been widely detected in food waste, but the detection of it in the soil of non-livestock manure improvement was rarely reported (Wang et al. 2018). Therefore, attention should be paid to whether *Luteimonas* can be used as indicator bacteria in the soil of chicken manure improvement in the future, which will be of great significance to the pollution sources of livestock and poultry manure in the soil.

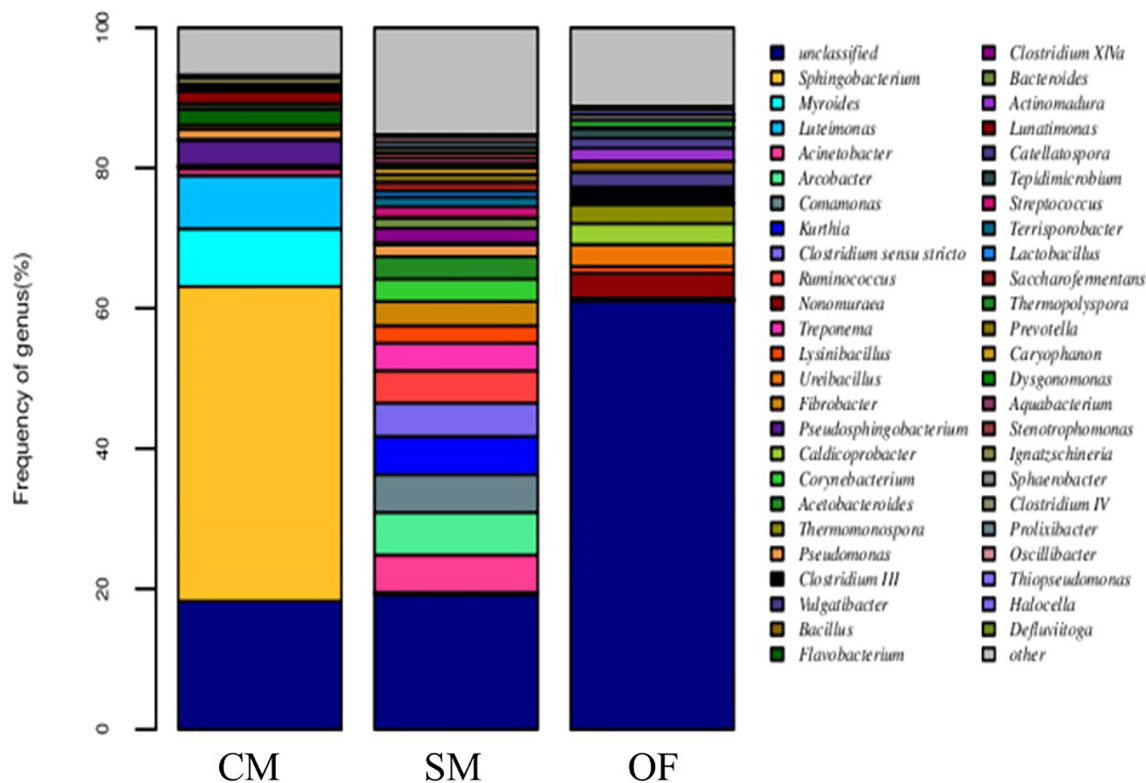
### Occurrence of antibiotics in different manures

Sulfonamides (SAs), tetracyclines (TCs), and quinolones (QNs) are three types of antibiotics frequently used in livestock and poultry industry (Tang et al. 2015; Pan and Chu 2016; Pan and Chu 2017; Louise et al. 2021; Sameer et al. 2020). Figure 3 summarizes the concentration of antibiotics in the three manures. There was a great difference in the residual

**Table 2** Alpha diversity index of manure samples

Sample	Coverage	Chao 1	ACE	Shannon	Simpson	OUT Num.
CM	0.97	8855.21	22575.56	3.54	0.09	1533
SM	0.94	12523.36	19705.95	5.64	0.02	4222
OF	0.96	14841.90	29794.57	5.15	0.02	2695

CM, chicken manure; SM, swine manure; OF, organic fertilizer



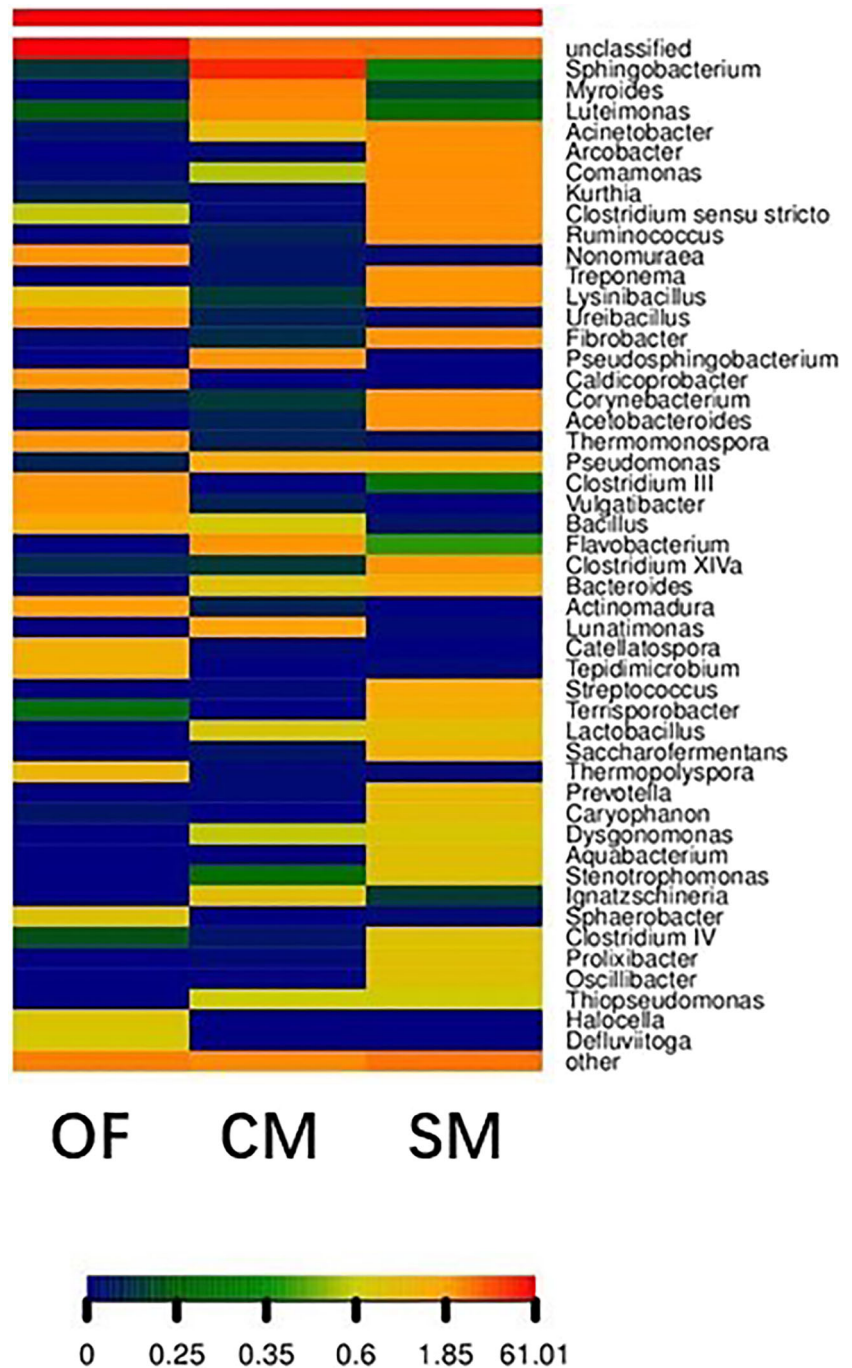
**Fig. 1** Distribution of community structure at genus

kinds and concentration of antibiotics in different manures (Qian et al. 2018). The recoveries of the target antibiotics in different manures ranged from 72.82 to 104.70% on average. The detection frequency of the target antibiotics in manures were 33.30%–100.00%. CTC and OTC were detected at a highest detection frequency of 100% in all samples. The total concentrations of detected antibiotics in SM, CM, OF were  $2.15\text{--}6.61 \times 10^3 \mu\text{g/kg}$ ,  $3.53\text{--}89.74 \mu\text{g/kg}$ , and  $7.58\text{--}79.03 \mu\text{g/kg}$ , respectively. Eleven antibiotics were detected in SM, ENR was not detected in CM, DOC and QNs were not detected in OF. Generally, the amount of SAs was not so high as that of TCs and QNs. The organic carbon-normalized partition coefficients ( $K_{oc}$ ) of SAs were lower than TCs and QNs, which indicated that the persistence of SAs in environment was lower than TCs and QNs (Zhang et al. 2015). In addition, SAs are not inclined to be adsorbed into solid-phase medium, so it may be further released into wastewater or leaching (Wegst-Uhrich et al. 2014). Antibiotics, CIP, and OTC, in the SM, were detected with the concentrations being three orders of magnitude higher than other antibiotics, reaching up to milligram per kilogram level (6.61 and 6.53 mg/kg, respectively). However, antibiotics with highest concentrations in the CM and OF were DOC and SDZ, reaching 89.74 and 79.03  $\mu\text{g/kg}$ , respectively.

Previous studies showed that the concentrations of residual antibiotics in livestock manure were fairly high.

For example, the concentration of OTC and CIP in swine manures reached 59.06 and 33.98 mg/kg, respectively (Zhao et al. 2010). In the report of Pan et al. (2011), the maximum concentration of residual antibiotics in swine manure was 764.4 mg/kg (CTC). The OTC, TC, SDZ, and SMZ in manure samples from two chicken farms detected by Ji et al. (2012) were 21.96 and 3.41 mg/kg, 10.31 and 2.45 mg/kg, and 8.62 and 2.21 mg/kg, respectively. In a previous study, the highest residual concentration of common veterinary antibiotics detected in chicken manure was OTC 416.8 mg/kg (Zhang et al. 2015). Furthermore, Qian et al. (2016) detected 3.1–16280  $\mu\text{g/kg}$  of the antibiotic concentration in pig manure-based organic manure. Their highest levels of antibiotics in different manures were much higher than those in this study. Nine fluoroquinolone (FQ) concentrations of antibiotics in various manure, such as horse manure, chicken manure, and swine manure, were detected from 18.20 to 104.55  $\mu\text{g/kg}$  (Gu et al. 2019). The residues of SAs, TCs, and QNs in chicken and swine manure were detected; the concentrations were 3.1–185.9 and 4.6–202.2  $\mu\text{g/kg}$ , respectively (Xie et al. 2016). Zhou et al. (2020) collected the feces of pig, chicken, cattle, and duck, and obtained the concentration of SAs, FQs, and TCs ranging from 0 to  $5.66 \times 10^3 \mu\text{g/kg}$ . Indicating that in recent years, the strict management of antibiotic use in livestock and poultry

**Fig. 2** Heatmap showing the 50 most abundant genera in different manures



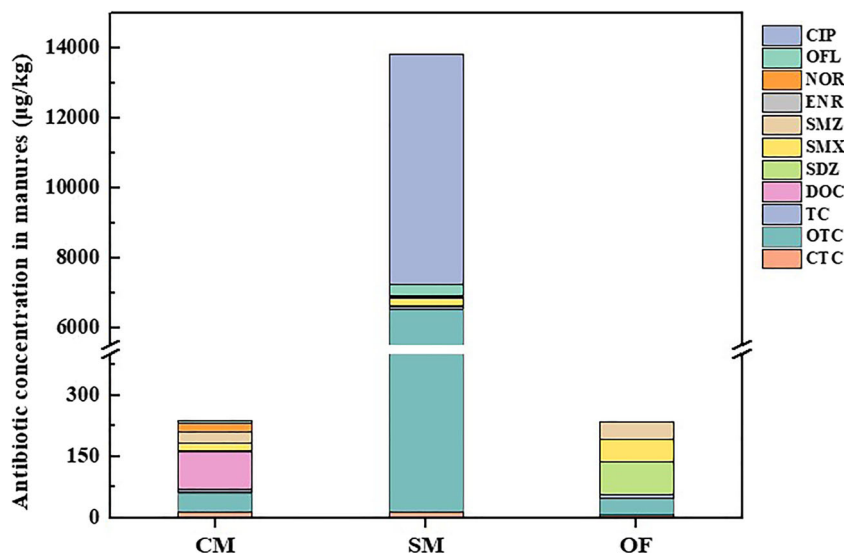
breeding industry is effective. At least, the concentration of antibiotics in manure from livestock farms in this study was lower than in many previous studies (Zhang et al. 2015; Pan et al. 2011; Zhao et al. 2010).

**Abundance of ARGs in different manures**

Figure 4 shows the concentrations of ARGs detected in manure samples. Thirteen ARGs showing resistance to five antibiotic classes (tetracyclines, quinolones, sulfonamides,

aminoglycosides, and  $\beta$ -lactams) as well as one class I integrase gene *intI1* were detected in all three manures. The absolute abundance of 16S rRNA gene was at  $10^{11}$ – $10^{13}$  copies/g manure. SM had the highest concentration of 16S rRNA, reaching  $3.78 \times 10^{13}$  copies/g. The order of ARGs’ total amount was CM ( $7.35 \times 10^{11}$  copies/g) > SM ( $3.22 \times 10^{10}$  copies/g) > OF ( $2.30 \times 10^8$  copies/g). The amount of ARGs detected in this study was lower than  $3.01 \times 10^8$  to  $7.18 \times 10^{14}$  copies/g in a previous report (Wen et al. 2019). The contents of most ARGs in CM were higher than that in

**Fig. 3** Concentration of antibiotics in different manure samples

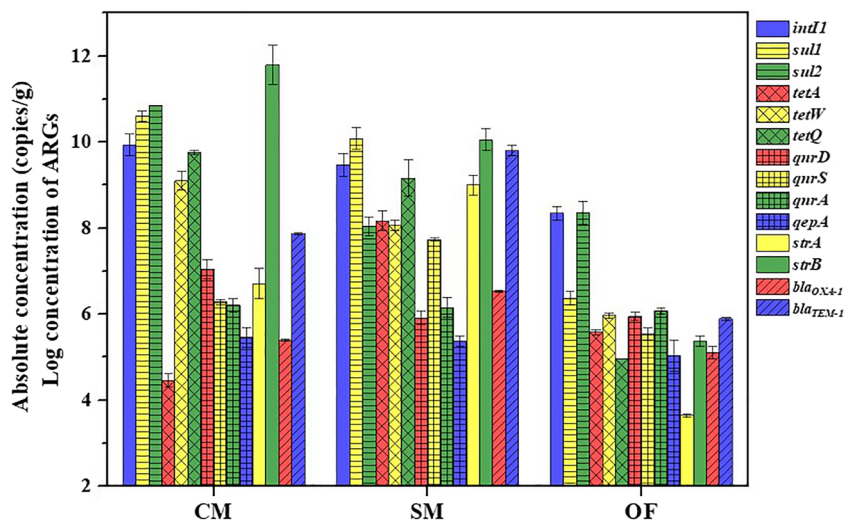


SM; this trend was similar to the reported literature (Wang et al. 2016); this might be due to that the high transferability of the ARGs of chicken microbes has led to the popularity of ARGs in chicken manure (Mu et al. 2015). The contents of ARGs in OF were lower than in SM, but the contents of *sul2* was higher than that in SM. This might be due to significant changes in bacterial community composition during composting, such as *Ureibacillus* in OF, which can lead to ARGs enrich during composting (Su et al. 2015; Zhou et al. 2019).

The results showed that *sul*, *tet*, and *strB* were the dominant ARGs in these three manures. Zhou et al. (2016) reported *strB*, *strA*, and *sul1* genes are the most abundant ARGs of soil samples, so it is worth paying attention to these ARGs that may accumulate a large amount in the soil after manure returned to the field. In this study, *sul1* and *sul2* were the dominant ARGs in CM; this result was similar to Wang et al. (2018). In addition, Wang et al. (2018) proposed that

manure should be effectively treated to reduce *sul1* and *sul2* before they enter the soil, rather than waiting for them to decay after entering the environment. The amount of *sul2* in CM was significantly higher than that in SM and OF. Previous study showed that the content of *sul* ARGs in chicken manure was obviously higher than those in pig manure (Cheng et al. 2013). Meersche et al. (2019) reported *sul2* was a representative gene in swine manure, but the abundance of *sul2* in SM in this study was much lower than *sul1*, which was due to their different diffusion mechanisms. *sul1* is usually located in the conserved region of the *int11* gene cassette, but *sul2* is usually located in small IncQ family plasmids, so there is more extensive host bacteria and larger space for *sul1* to transfer and diffusive than *sul2*, and thus, the abundance of *sul1* is often higher than that of *sul2* (Duan et al. 2019; Wang et al. 2019a). Many studies have shown that the presence of *int11* in manure may promote ARG accumulation and persistence through horizontal gene transfer (Li et al. 2017; Qian et al. 2018; Mazhar et al. 2021).

**Fig. 4** Abundance of ARGs in different manure samples





In summary, ARG abundance in different animal manure varies greatly, the addition of different antibiotics to the feed consumed by different animals, and the different digestion and absorption in animal body resulting in different levels of ARGs.

### Correlation analysis

To explore the impact of common indices and heavy metals to the occurrence of ARGs in manures, the correlation analysis of ARGs among them were analyzed in Table S2. There was a strong correlation between TOC with *sul2* and *bla<sub>TEM-1</sub>* ( $p < 0.01$ ), which was consistent with a previous study (Wang et al. 2016). This might be due to that the biological effect of TOC in the growth of ARG host bacteria, affecting the occurrence of ARGs (Li et al. 2020). In this study, *sul1*, *sul2*, *strB*, and *bla<sub>TEM-1</sub>* all had a significant correlation ( $p < 0.01$ ) with Zn and Cu. It had been reported that *sul1* and *sul2* were closely related ( $p < 0.01$ ) to the contents of Zn and Cu (Guo et al. 2018). Zhou et al. (2016) also reported that the contents of Zn and Cu were significantly correlated with many ARGs ( $p < 0.01$ ). Usually, the abundance of ARGs could be increased under long-term co-selection pressures of heavy metals with metal resistance genes (MRGs) and mobile genetic elements (MGEs), etc. (Zhou et al. 2016; Li et al. 2019b; Zhao et al. 2019). It was reported that tetracycline antibiotics were positively correlated with heavy metals. This phenomenon occurs because it is easy to chelate with heavy metals (Zhang et al. 2015). However, some reports showed that there was a correlation between the use of antibiotics and the abundance of ARGs, while others reported that there was no significant correlation between antibiotics and ARGs (Zhang et al. 2015; Wang et al. 2016; Guo et al. 2018). It requires more and more in-depth research in the future to explore the reasons.

### Conclusions

The occurrence of heavy metals, antibiotics, and ARGs in SM, CM, and OF were studied in this research. The amount of TCs, SAs, and QNs remaining in three manures was low, while two antibiotics (OTC and CIP) in SM should be paid more attention to. The *sul1*, *sul2*, and *strB* were the dominant ARGs in animal manures. The abundance of most ARGs was significantly correlated with the concentration of Zn and Cu ( $p < 0.01$ ). The *Luteimonas* in CM might be used as an indicator of the source of manure in the soil. Overall, to reduce the risk of pollution from manure to the farmland soil and even agricultural products, here are two suggestions: the addition of antibiotics and heavy metals in animal feed must be strictly

controlled. The other one is that the animal manure should be effectively treated before applying into the soil. Based on this study, the migration of pollution from livestock and poultry manure in farmland soil should be deeply studied further.

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**Author contribution** The manuscript was reviewed and approved for publication by all authors. CC and JW conceived and designed the experiments. JX, JW, and YH performed the experiments. JX, CS, and SY analyzed the data. JX and CC wrote the paper. JX, JW, YH, CS, SY, PL, KL, and CC reviewed and revised the paper.

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**Data and materials availability** The availability of data and materials is on the base of personal request.

### Declarations

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### References

- Ajit KS, Michael TM, Alistair BAB (2006) A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere* 65(5): 725–759
- Ben LA, Haas B, Gottschalk M, Grenier D (2017) Antimicrobial potential of bacteriocins in poultry and swine production. *Vet Res* 48:1–12
- Bui XT, Wolff A, Madsen M, Bang DD (2011) Fate and survival of *Campylobacter coli* in swine manure at various temperatures. *Front Microbiol* 2:1–8
- Carlota GC, Sílvia B, Sofia VPA, Jorge B, Andreia F, Fernando R (2021) Assessing antibiotic residues in piglet liver and kidney samples: how to manage the results obtained. *Food Control* 122:107819
- Chen YS, Zhang HB, Luo YM, Song J (2012) Occurrence and dissipation of veterinary antibiotics in two typical swine wastewater treatment systems in east China. *Environ Monit Assess* 184:2205–2217
- Cheng WX, Chen H, Su C, Yan SH (2013) Abundance and persistence of antibiotic resistance genes in livestock farms: a comprehensive investigation in eastern China. *Environ Int* 61:1–7
- Cheng WH, Jiang L, Lu N, Ma L, Sun XY, Luo Y, Lin KF, Cui CZ (2015) Development of a method for trace level determination of antibiotics in drinking water sources by high performance liquid chromatography-tandem mass spectrometry. *Anal Methods* 7(5): 1777–1787

- Cui EP, Wu Y, Zuo YR, Chen H (2016) Effect of different biochars on antibiotic resistance genes and bacterial community during chicken manure composting. *Bioresour Technol* 203:11–17
- Cui CZ, Cao Z, Zhang SP, Hu YR, Jiang L, Yao SJ, Ye H, Zhou YB, Hu J, Lin KF, Zhang TY (2019) Application of a novel diol-based porous organic polymer to the determination of trace-level tetracyclines in water. *Anal Methods* 11(18):2473–2481
- Dong LL, Xu J, Liu Y, Fang HL, Niu WH, Li XW, Zhang YJ, Ding WL, Chen SL (2018) Manipulation of microbial community in the rhizosphere alleviates the replanting issues in *Panax ginseng*. *Soil Biol Biochem* 125:64–74
- Duan ML, Gu J, Wang XJ, Li Y, Zhang RR, Hu T, Zhou BB (2019) Factors that affect the occurrence and distribution of antibiotic resistance genes in soils from livestock and poultry farms. *Ecotoxicol Environ Saf* 180:114–122
- Eric TA, Mike OO, Omobola OO, Anthony IO (2020) A critical review on the occurrence of resistomes in the environment and their removal from wastewater using apposite treatment technologies: Limitations, successes and future improvement. *Environ Pollut* 263:113791
- Gu DM, Feng QY, Guo CS, Hou S, Lv JP, Zhang Y, Yuan S, Zhao X (2019) Occurrence and risk assessment of antibiotics in manure, soil, wastewater, groundwater from livestock and poultry farms in Xuzhou, China. *Bull Environ Contam Toxicol* 103(4):590–596
- Guo T, Lou CL, Zhai WW, Tang XJ, Hashmi MZ, Murtaza R, Li Y, Liu XM, Xu JM (2018) Increased occurrence of heavy metals, antibiotics and resistance genes in surface soil after long-term application of manure. *Sci Total Environ* 635:995–1003
- Hashemi M (2018) Heavy metal concentrations in bovine tissues (muscle, liver and kidney) and their relationship with heavy metal contents in consumed feed. *Ecotoxicol Environ Saf* 154:263–267
- Hasman H, Franke S, Rensing C (2006) Resistance to metals used in agricultural production. In: Aarestrup FM (ed) *Antimicrobial resistance in bacteria of animal origin*. ASM Press, Washington, DC, pp 99–114
- Hu YR, Jiang L, Zhang TY, Jin L, Han Q, Zhang D, Lin KF, Cui CZ (2018) Occurrence and removal of sulfonamide antibiotics and antibiotic resistance genes in conventional and advanced drinking water treatment processes. *J Hazard Mater* 360:364–372
- Hu YR, Zhang TY, Jiang L, Luo Y, Yao SJ, Zhang D, Lin KF, Cui CZ (2019) Occurrence and reduction of antibiotic resistance genes in conventional and advanced drinking water treatment processes. *Sci Total Environ* 669:777–784
- Jackson BP, Bertsch PM, Cabrera ML, Camberato JJ, Seaman JC, Wood CW (2003) Trace element speciation in poultry litter. *J Environ Qual* 32(2):535–540
- Ji XL, Shen QH, Liu F, Ma J, Xu G, Wang YL, Wu MH (2012) Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai, China. *J Hazard Mater* 235–236:178–185
- Jiang ZW, Lu YY, Xu JQ, Li MQ, Shan GC, Li QL (2019) Exploring the characteristics of dissolved organic matter and succession of bacterial community during composting. *Bioresour Technol* 292:121942
- Kumar K, Gupta SC, Baidoo SK, Chander Y, Rosen CJ (2005) Antibiotic uptake by plants from soil fertilized with animal manure. *J Environ Qual* 34(6):2082–2085
- Li JJ, Xin ZH, Zhang YZ, Chen JW, Yan JX, Li HJ, Hu HW (2017) Long-term manure application increased the levels of antibiotics and antibiotic resistance genes in a greenhouse soil. *Appl Soil Ecol* 121:193–200
- Li R, Meng HB, Zhao LX, Zhou HB, Shen YJ, Zhang X, Ding JT, Cheng HS, Wang J (2019a) Study of the morphological changes of copper and zinc during pig manure composting with addition of biochar and a microbial agent. *Bioresour Technol* 291:121752
- Li Y, Wang XJ, Li J, Wang Y, Song JK, Xia SQ, Jing HP, Zhao JF (2019b) Effects of struvite-humic acid loaded biochar/bentonite composite amendment on Zn(II) and antibiotic resistance genes in manure-soil. *Chem Eng J* 375:122013
- Li HY, Cheng WM, Li BH, Xu Y, Zheng XQ (2020) The fate of antibiotic resistance genes during co-composting of swine manure with cauliflower and corn straw. *Bioresour Technol* 300:122669
- Lin H, Chapman SJ, Freitag TE, Kyle C, Ma JW, Yang YY, Zhang ZL (2019) Fate of tetracycline and sulfonamide resistance genes in a grassland soil amended with different organic fertilizers. *Ecotoxicol Environ Saf* 170:39–46
- Liu WR, Zeng D, She L, Su WX, He DC, Wu GY, Ma XR, Jiang S, Jiang CH, Ying GG (2020a) Comparisons of pollution characteristics, emission situations, and mass loads for heavy metals in the manures of different livestock and poultry in China. *Sci Total Environ* 734:139023
- Liu X, Wang H, Zhao HM (2020b) Propagation of antibiotic resistance genes in an industrial recirculating aquaculture system located at northern China. *Environ Pollut* 261:114155
- Liu N, Xu LK, Han LJ, Huang GQ, Ciric L (2021a) Microbiological safety and antibiotic resistance risks at a sustainable farm under large-scale open-air composting and composting toilet systems. *J Hazard Mater* 401:123391
- Liu WB, Ling N, Guo JJ, Ruan Y, Wang M, Shen QR, Guo SW (2021b) Dynamics of the antibiotic resistome in agricultural soils amended with different sources of animal manures over three consecutive years. *J Hazard Mater* 401:123399
- Louise KMC, Timothy MG, Michael RG (2021) A survey of sub-inhibitory concentrations of antibiotics in the environment. *J Environ Sci* 99(01):21–27
- Macedo G, Hernandez-Leal L, Maas PVD, Heederik D, Mevius D, Schmitt H (2020) The impact of manure and soil texture on antimicrobial resistance gene levels in farmlands and adjacent ditches. *Sci Total Environ* 737:139563
- Mazhar SH, Li XJ, Rashid A, Su JM, Xu JQ, Brejnrod AD, Su JQ, Wu YJ, Zhu YG, Zhou SG, Feng RW, Rensing C (2021) Co-selection of antibiotic resistance genes, and mobile genetic elements in the presence of heavy metals in poultry farm environments. *Sci Total Environ* 755:142702
- Meersche TV, Rasschaert G, Haesebrouck F, Coillie EV, Herman L, Van Weyenberg SV, Daeseleire E, Heyndrickx M (2019) Presence and fate of antibiotic residues, antibiotic resistance genes and zoonotic bacteria during biological swine manure treatment. *Ecotoxicol Environ Saf* 175:29–38
- Meng LQ, Li WG, Zhang SM, Wu CD, Lv LY (2017) Feasibility of co-composting of sewage sludge, spent mushroom substrate and wheat straw. *Bioresour Technol* 226:39–45
- Mu QH, Li J, Sun YX, Mao DQ, Wang Q, Luo Y (2015) Occurrence of sulfonamide-, tetracycline-, plasmid-mediated quinolone- and macrolide-resistance genes in livestock feedlots in Northern China. *Environ Sci Pollut Res* 22(9):6932–6940
- Mullen RA, Hurst JJ, Naas KM, Sassoubre LM, Aga DS (2019) Assessing uptake of antimicrobials by *Zea mays* L. and prevalence of antimicrobial resistance genes in manure-fertilized soil. *Sci Total Environ* 646:409–415
- Ngigi AN, Ok YS, Thiele-Bruhn S (2019) Biochar-mediated sorption of antibiotics in swine manure. *J Hazard Mater* 364:663–670
- Pan M, Chu LM (2016) Adsorption and degradation of five selected antibiotics in agricultural soil. *Sci Total Environ* 545–546:48–56
- Pan M, Chu LM (2017) Leaching behavior of veterinary antibiotics in animal manure-applied soils. *Sci Total Environ* 579:466–473
- Pan X, Qiang ZM, Ben WW, Chen MX (2011) Residual veterinary antibiotics in swine manure from concentrated animal feeding operations in Shandong Province, China. *Chemosphere* 84(5):695–700
- Qian MR, Wu HZ, Wang JM, Zhang H, Zhang ZL, Zhang YZ, Lin H, Ma JW (2016) Occurrence of trace elements and antibiotics in manure-based fertilizers from the Zhejiang Province of China. *Sci Total Environ* 559:174–181

- Qian X, Gu J, Sun W, Wang X, Su J, Stedfeld R (2018) Diversity, abundance, and persistence of antibiotic resistance genes in various types of animal manure following industrial composting. *J Hazard Mater* 344:716–722
- Rahube TO, Yost CK (2012) Characterization of a mobile and multiple resistance plasmid isolated from swine manure and its detection in soil after manure application. *J Appl Microbiol* 112(6):1123–1133
- Rahube TO, Marti R, Scott A, Tien YC, Murray R, Sabourin L, Zhang Y, Duenk P, Lapen DR, Topp E (2014) Impact of fertilizing with raw or anaerobically digested sewage sludge on the abundance of antibiotic-resistant coliforms, antibiotic resistance genes, and pathogenic bacteria in soil and on vegetables at harvest. *Appl Environ Microbiol* 80(22):6898–6907
- Sameer JP, Matthew W, Rohan MS, Matthew JF (2020) Antibiotic stewardship in food-producing animals: challenges, progress, and opportunities. *Clin Ther* 42(9):1649–1658
- Su JQ, Wei B, Ou-Yang WY, Huang FY, Zhao Y, Xu HJ, Zhu YG (2015) Antibiotic resistome and its association with bacterial communities during sewage sludge composting. *Environ Sci Technol* 49(12):7356–7363
- Tang XJ, Lou CL, Wang SX, Lu YH, Liu M, Hashmi MZ, Liang XQ, Li ZP, Liao YL, Qin WJ, Fan F, Xu JM, Brookes PC (2015) Effects of long-term manure applications on the occurrence of antibiotics and antibiotic resistance genes (ARGs) in paddy soils: evidence from four field experiments in south of China. *Soil Biol Biochem* 90:179–187
- Tortosa G, Castellano-Hinojosa A, Correa-Galeote D, Bedmar EJ (2017) Evolution of bacterial diversity during two-phase olive mill waste (“alperujo”) composting by 16S rRNA gene pyrosequencing. *Bioresour Technol* 224:101–111
- Wang H, Dong YH, Yang YY, Toor GS, Zhang XM (2013) Changes in heavy metal contents in animal feeds and manures in an intensive animal production region of China. *J Environ Sci* 25(12):2435–2442
- Wang N, Guo XY, Yan Z, Wang W, Chen B, Ge F, Ye BP (2016) A comprehensive analysis on spread and distribution characteristic of antibiotic resistance genes in livestock farms of southeastern China. *PLoS One* 11(7):e0156889
- Wang MZ, Liu P, Xiong WG, Zhou Q, Wang-Xiao JY, Zeng ZL, Sun YX (2018) Fate of potential indicator antimicrobial resistance genes (ARGs) and bacterial community diversity in simulated manure-soil microcosms. *Ecotoxicol Environ Saf* 147:817–823
- Wang LJ, Wang J, Wang JH, Zhu LS, Yang LL, Yang R (2019a) Distribution characteristics of antibiotic resistant bacteria and genes in fresh and composted manures of livestock farms. *Sci Total Environ* 695:133781
- Wang JQ, Liu ZP, Xia JS, Chen YP (2019b) Effect of microbial inoculation on physicochemical properties and bacterial community structure of citrus peel composting. *Bioresour Technol* 291:121843
- Wegst-Uhrich SR, Navarro DA, Zimmerman L, Aga DS (2014) Assessing antibiotic sorption in soil: a literature review and new case studies on sulfonamides and macrolides. *Chem Cent J* 8:1–12
- Wen X, Mi JD, Wang Y, Ma BH, Zou YD, Liao XD, Liang JB, Wu YB (2019) Occurrence and contamination profiles of antibiotic resistance genes from swine manure to receiving environments in Guangdong Province southern China. *Ecotoxicol Environ Saf* 173:96–102
- Xie WY, Yang XP, Li Q, Wu LH, Shen QR, Zhao FJ (2016) Changes in antibiotic concentrations and antibiotic resistome during commercial composting of animal manures. *Environ Pollut* 219:182–190
- Xu Y, Li J, Zhang XB, Wang LQ, Xu XB, Xu L, Gong HR, Xie HY, Li FD (2019a) Data integration analysis: heavy metal pollution in China’s large-scale cattle rearing and reduction potential in manure utilization. *J Clean Prod* 232:308–317
- Xu M, Stedfeld RD, Wang F, Hashsham SA, Song Y, Chuang YH, Fan JB, Li H, Jiang X, Tiedje JM (2019b) Composting increased persistence of manure-borne antibiotic resistance genes in soils with different fertilization history. *Sci Total Environ* 689:1172–1180
- Yang XP, Li Q, Tang Z, Zhang WW, Yu GH, Shen QR, Zhao FJ (2017) Heavy metal concentrations and arsenic speciation in animal manure composts in China. *Waste Manag* 64:333–339
- Zhang SQ, Zhang FD, Liu XM, Wang YJ, Zou SW, He XS (2005) Determination and analysis on main harmful composition in excrement of scale livestock and poultry feedlots. *Plant Nutr Fert Sci* 11:822–829
- Zhang HB, Luo YM, Wu LH, Huang YJ, Christie P (2015) Residues and potential ecological risks of veterinary antibiotics in manures and composts associated with protected vegetable farming. *Environ Sci Pollut Res* 22(8):5908–5918
- Zhang M, He LY, Liu YS, Zhao JL, Liu WR, Zhang JN, Chen J, He LK, Zhang QQ, Ying GG (2019) Fate of veterinary antibiotics during animal manure composting. *Sci Total Environ* 650:1363–1370
- Zhang N, Juneau P, Huang RL, He ZL, Sun B, Zhou JZ, Liang YT (2021) Coexistence between antibiotic resistance genes and metal resistance genes in manure-fertilized soils. *Geoderma* 382:114760
- Zhao L, Dong YH, Wang H (2010) Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. *Sci Total Environ* 408(5):1069–1075
- Zhao Y, Cocerva T, Cox S, Tardif S, Su JQ, Zhu YG, Brandt KK (2019) Evidence for co-selection of antibiotic resistance genes and mobile genetic elements in metal polluted urban soils. *Sci Total Environ* 656:512–520
- Zhao RX, Feng J, Huang J, Li XY, Li B (2021) Responses of microbial community and antibiotic resistance genes to the selection pressures of ampicillin, cephalixin and chloramphenicol in activated sludge reactors. *Sci Total Environ* 755:142632
- Zhou BR, Wang C, Zhao Q, Wang Y, Huo MJ, Wang JD, Wang SL (2016) Prevalence and dissemination of antibiotic resistance genes and coselection of heavy metals in Chinese dairy farms. *J Hazard Mater* 320:10–17
- Zhou X, Qiao M, Su JQ, Wang Y, Cao ZH, Cheng WD, Zhu YG (2019) Turning swine manure into biochar can effectively mitigate antibiotic resistance genes as organic fertilizer. *Sci Total Environ* 649:902–908
- Zhou X, Wang J, Lu C, Liao QH, Gudda FO, Ling WT (2020) Antibiotics in animal manure and manure-based fertilizers: occurrence and ecological risk assessment. *Chemosphere* 255:127006

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