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RESEARCH ARTICLE

Antibiotic resistome and associated bacterial communities in agricultural soil following the amendments of swine manure–derived fermentation bed waste

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Abstract

The practice of utilizing animal manures on land is widespread in agriculture, but it has raised concerns about the possible spread of antibiotic resistance genes (ARGs) and the potential risk it poses to public health through food production. Fermentation bed culture is an effective circular agricultural practice commonly utilized in pig farming that minimizes the environmental impact of livestock farming. However, this method generates a significant amount of fermentation bed waste (FBW), which can be turned into organic fertilizer for land application. The objective of this research was to examine the impacts of amending agricultural soil samples with swine manure–derived FBW on microbial communities, mobile genetic elements (MGEs), and ARG profiles over different periods. The study findings indicated that the amendment of swine manure–derived FBW significantly increased the diversity and abundance of ARGs and MGEs during the early stages of amendment, but this effect diminished over time, and after 12 months of FBW amendments, the levels returned to those comparable to control samples. The shift in the bacterial communities played a significant role in shaping the patterns of ARGs. Actinobacteriota, Proteobacteria, and Bacteroidetes were identified as the primary potential hosts of ARGs through metagenomic binning analysis. Furthermore, the pH of soil samples was identified as the most important property in driving the composition of the bacterial community and soil resistome. These findings provided valuable insights into the temporal patterns and dissemination risks of ARGs in FBW-amended agriculture soil, which could contribute to the development of effective strategies to manage the dissemination risks of FBW-derived ARGs.

Keywords Fermentation bed waste · Temporal variability of amendments · Antibiotic resistance genes · Host bacteria · Metagenomic and binning

Introduction

The prevalence of antimicrobial usage in intensive animal production systems is primarily for disease prevention, for treatment, and to meet the global demand for animal protein (Van Boeckel et al. 2015, 2019). Approximately 73% of global antimicrobial usage is attributed to animals, leading to the emergence of antibiotic resistance (Mulchandani et al. 2023; Van Boeckel et al. 2017). After administration, antibiotics are poorly absorbed by animals, with a significant proportion of 70–90% of antibiotics being excreted (Kumar et al. 2005). This excretion can lead to the emergence of antibiotic-resistant genes (ARGs) and antibiotic-resistant bacteria (ARB) in manure-affected environments, including those containing antibiotic residues and metabolites (Chen et al. 2019; Van den Meersche et al. 2020; Zhou et al. 2020). The application of manure to land, in particular, has facilitated the spread of ARGs from agricultural sources to surrounding ecosystems (Christou et al. 2017; Williams-Nguyen et al. 2016; Zhou et al. 2019).

Fermentation bed culture is a circular agriculture method commonly used in pig farming, referred to as breeding pig on litter, deep-litter-system, or in situ decomposition of pig manure (Morrison et al. 2007). Pigs are raised on a

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fermentation bed, and the manure is decomposed in situ. While this method reduces the environmental impact of livestock farming, it generates a large amount of fermentation bed waste (FBW), which comprises both manure and fermentation bed materials, such as agricultural straw, saw powder and rice husk (Pérez et al. 2022). FBW can be utilized as organic fertilizer in agriculture, enhancing microbial diversity and activity in soil, and improving soil nutrient availability and soil organic matter content for plant health and growth (Chen et al. 2017; Xu et al. 2022). However, the use of FBW can also contribute to the spread of ARB and ARGs. ARB can transfer genetic information via horizontal gene transfer mechanisms facilitated by mobile genetic elements (MGEs), including integrative conjugative elements, integrons, plasmids and insertion sequences (Ezugworie et al. 2021; Mbanga et al. 2021). Although numerous studies have examined the effects of manure application on ARG profiles and ARB, there is a scarcity of research on the impact of FBW, which is applied after the in situ decomposition of manure (Checcucci et al. 2020; Chen et al. 2016; He et al. 2020; Yu et al. 2023; Zou et al. 2020).

To address this research gap, we sampled three farmland plots with varying periods of FBW amendments near the swine feedlots equipped with fermentation beds. Our objective was to assess the influence of FBW on the microbial communities and ARG profiles in amended soil and identify the potential bacterial hosts of ARGs through metagenomic binning analysis.

Materials and methods

Sampling site

A total of 12 soil samples were collected in April 2021 from the main provinces in China for fermentation bed culture. Specifically, three soil samples were collected from Jilin (Jilin farmland), three from Shaanxi (Shaanxi farmland), three from Guizhou (Guizhou farmland), and three soil samples for control. The experimental stations were selected based on their continuous amendments of swine manure–derived FBW from nearby small-scale fattening pig production feedlots. These feedlots were equipped with fermentation beds managed in a similar manner, and the fermentation bed materials had been used for more than two years. The FBWs were collected from the surface layer of the fermentation bed, which comprised both swine manure and fermented bed materials, such as agricultural straw, saw powder and rice husk.

Soil amendments with FBW were applied to the farmland at a rate of approximately 15 tons per hectare once every two years. Soil samples were collected from specific locations with different amendment periods at each experimental station. Specifically, the soil samples from Jilin farmland were collected 6 months after the FBW amendment (designated JLF6); The farmland had not been cropped during this period. The soil samples from Guizhou farmland were collected 21 months after the FBW amendment (designated GZF21), and the farmland had been cropped with cabbage and green bean under rotation during this period. The soil samples from Shaanxi farmland were collected 12 months after the FBW amendment (designated SXF12), and the farmland had been cropped with apple trees during this period. The sampling site followed typical local practices, which usually involved the application of chemical fertilizer.

Control samples were collected from a pristine forest that had not been subjected to any amendment of FBW, swine manure, or anthropogenic antibiotics. These control samples enable a more comprehensive assessment of the potential risks associated with the application of FBW in agricultural settings, particularly the antibiotic resistome risk. Thus, we designated these soil samples as the control samples (CK).

Soil sampling and physicochemical properties analysis

For soil sampling, five surface soil cores (0-20 cm) were randomly collected from each site and combined into one plot sample. Three replicates were taken from each farmland. After sieving through a 2 mm mesh and thoroughly mixed, one portion was air-dried for analysis of soil physicochemical properties, while the other portion was stored at -80 ℃ for further DNA extraction.

Soil physicochemical properties, including electrical conductivity (EC), soil pH, total organic carbon (TOC), total nitrogen (TN) and C/N ratio were analyzed using the methods outlined by Cheng et al. (Cheng et al. 2019). Soil pH was measured in suspensions with a 1:5 soil/distilled water ratio (w/v) using a pH meter. Soil EC was measured in the water extracts with a 1:5 soil/distilled water ratio (w/v). TN content was measured by the Kjeldahl method, while soil TOC was measured through dichromate oxidation and titration with a ferrous sulfate solution.

DNA extraction

The genomic DNA of each soil sample was extracted by utilizing the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA), following the manufacturer's instructions, from 0.25 g of freeze-dried soil samples. The purity and quality of the extracted DNA were assessed on 1% agarose gels, and then, the DNA was stored at -20 ℃ for further experiments.

Library preparation, sequencing, and assembly

The extracted DNA was transported to Allwegene Company (Beijing, China) on dry-ice for library preparation and shotgun metagenomics sequencing using the Illumina Novaseq platform. Trimmomatic (v0.36) was used to remove adapter sequences and low-quality reads from the raw metagenomic reads (Chen et al. 2018). MEGAHIT (v1.1.2) was then used to assemble quality-controlled reads into contigs, with contigs over 500 bp as the final assembling result (Li et al. 2015). The raw metagenomic sequence data were publicly available in the National Center for Biotechnology Information (NCBI) BioProject database with the accession number of PRJNA953804.

Gene prediction, taxonomy, and ARG and MGE analysis

Open reading frames (ORFs) were identified by Prodigal (Hyatt et al. 2010). The predicted ORFs with a length of 100 bp or greater were selected and translated into amino acid sequences. CD-HIT was then used to construct the non-redundant gene with 95% sequence identity (Fu et al. 2012). Bowtie (Langmead and Salzberg 2012) was employed to align the sequences with the non-redundant gene set, and gene abundance information was counted. The high-quality sequences were analyzed using Diamond (v 0.8.35) to be blasted against the NCBI NR database for taxonomic annotations.

ARG-like sequences were annotated from the metagenomic sequencing reads using an online analysis pipeline, ARGs-OAP, with default settings (Yang et al. 2016). The plasmids, integrons, insertion sequences and integrative conjugative elements were identified based on NCBI RefSeq, INTEGRALL, ISfinder and ICEberg database by Diamond (v 0.8.35) with the following parameters: under a cutoff of E value of $< 10^{-5}$, identify $\geq 90\%$, and over an alignment of at least 50 bp or 50 amino acids (Zhu et al. 2023). The abundance of MGEs was conducted using gene abundance information.

Metagenomic binning and function annotation

Metagenome-assembled genome (MAG) was recovered from assembly contigs of metagenomic data in soil samples by MetaBAT2 (v2.12.1) with default parameters (Kang et al. 2019). The completeness and contamination of MAGs were evaluated with CheckM (v1.1.3) (Parks et al. 2015). MAGs with completeness $\geq 50\%$ and contamination $\leq 10\%$ were selected for further refinement by secondary assembly. The relative abundance of each MAG was calculated by Salmon v0.9.1 (Patro et al. 2017), and the taxonomy of MAG was assigned by the GTDB-Tk (v1.3.0) with default options (Chaumeil et al. 2020). ARGs carried by MAGs were identified using Diamond (V0.8.35) against the SARG database (v2.2) with an e value cutoff of 10^{-5}, 70% query coverage, and 70% similarity (Yin et al. 2018). MGEs carried by MAGs were identified by matching the results of NR annotation to keywords: plasmid, relaxase, resolvase, transposase, transposon, conjugal, recombinase, mobilization, conjugative, integrase, integron, recombination, invertase, and translocase (Li et al. 2017).

Results

Physicochemical properties of soil samples

The physicochemical properties of soil samples with varying periods of FBW amendments were assessed through one-way ANOVA and least significant difference (LSD) analysis. The results revealed that there were significant variations in the physicochemical properties among the different soil samples ($P < 0.05$, Table 1). The JLF6 soil samples showed the lowest pH value, while GZF21 soil samples displayed the highest levels of TN, TOC, and C/N ratio. As for CK soil samples, they showed the highest soil pH and EC but the lowest TN content.

Diversity of the microbial community

Metagenomic taxonomic annotation was performed to investigate the diversity of the microbial community. Bacteria were found to be the dominant domain across all soil samples, with a mean value of 98.44% of total sequences (Table S1). Principal component analysis (PCA) indicated significant separation of microbiota composition across soil samples with varying periods of FBW amendments (Fig. 1a). A total of 86 bacterial phyla were detected. The most common bacterial phyla

Table 1 Physicochemical properties of the soil samples

	pH	EC (ms/cm)	TOC (%)	TN (%)	C/N ratio
JLF6	6.27 ± 0.05^{d}	0.21 ± 0.06^{a}	0.79 ± 0.07^{b}	0.09 ± 0.01^{b}	9.12 ± 0.18^{bc}
SXF12	7.98 ± 0.12^{b}	0.2 ± 0.03^{a}	0.72 ± 0.21^{b}	0.07 ± 0.01^{c}	10.69 ± 2.12^{ab}
GZF21	7.18 ± 0.05^{c}	0.13 ± 0.01^{b}	2.77 ± 0.17^{a}	0.24 ± 0.01^{a}	11.72 ± 0.47^{a}
CK	8.33 ± 0.03^{a}	0.23 ± 0.03^{a}	0.4 ± 0.02^{c}	0.05 ± 0.01^{d}	8.19 ± 0.27^{c}

*Data with different letters indicate significant differences ($p < 0.05$)

present in all soil samples were Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria, Planctomycetes, Chloroflexi, Gemmatimonadetes, Firmicutes, and Verrucomicrobia (Fig. 1b). Among these, Proteobacteria, Actinobacteria, and Bacteroidetes were the dominant phyla in all soil samples including CK samples. In JLF6, the relative abundances of Proteobacteria and Bacteroidetes were much higher than in other soil samples, accounting for 50.3% and 14.3% of the community, respectively. Conversely, the relative abundances of Planctomycetes, Acidobacteria, and Chloroflexi were lower in JLF6 than in other soil samples.

At the genus level, heatmap analysis displayed significant differences in the top 30 abundant genera among different soil samples (Fig. 1c). For instance, the relative abundances of *Mycobacterium*, *Pseudomonas, Rhizobium, Devosia, Mesorhizobium, Rhodanobacter, Flavobacterium*, and Pedobacter were more abundant in JLF6 compared to the GZF21, SXF12, and CK samples. In contrast, the relative abundances of *Pyrinomonas, Streptomyces, Solirubrobacter*, and *Conexibacter* were highest in CK samples, followed by SXF12 and GZF21, while JLF6 had the lowest abundance. An interesting observation was that the relative abundance of *Arthrobacter, Sphingomonas*, and *Nocardioides* in JLF6, SXF12, and GZF21 gradually decreased with the increasing amendment period of FBW (Fig. 1c).

Variations in the abundance and diversity of ARGs

In terms of ARGs, a total of 129 unique ARGs were detected across all soil samples (Table S2). The FBW-amended soils (JLF6, SXF12, and GZF21) exhibited a significantly higher number of identified ARGs compared to the control samples (CK), showing a seven-fold increase (Fig. 2a). Kruskal–Wallis ANOVA confirmed a significant difference in the number of detected ARGs among soil samples with different durations of FBW

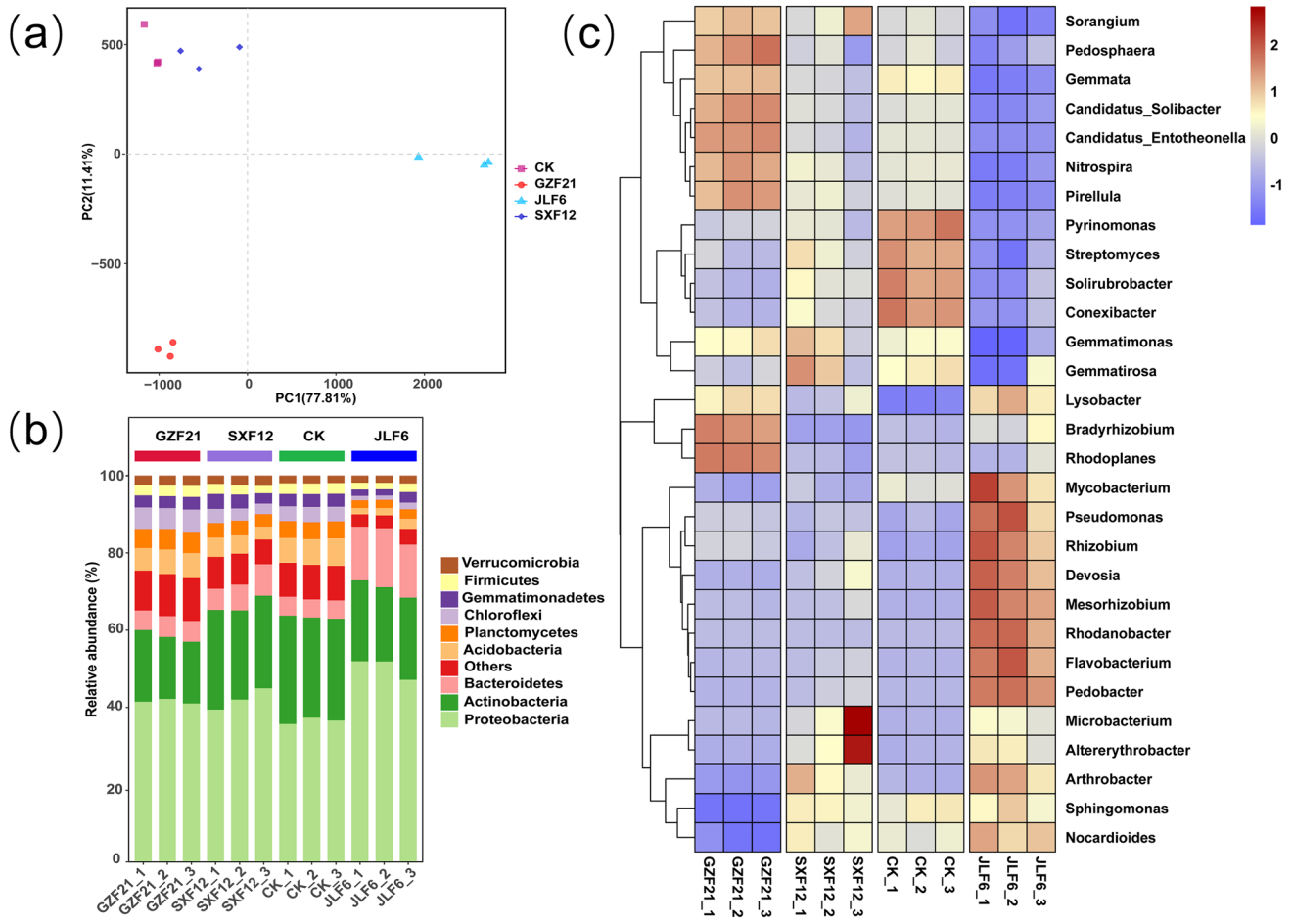

Fig. 1 The principal component analysis (PCA) of bacterial community in different soil samples (**a**). Relative abundance (%) of the main bacterial community at phylum level in different soil samples (**b**). The heatmap analysis of top 30 microbial genera in different soil samples (**c**)

amendments. The range of detected ARGs in FBW-amended soil samples was 12 to 87, with JLF6 having the highest number, accounting for 69% of the total number of detected ARGs. SXF12 had the second-highest number, which was significantly greater than that of CK ($P < 0.05$). However, GZF21 exhibited the lowest number of detected ARGs, similar to that of CK (Fig. 2a). An inverse linear relationship was observed between the number of detected ARGs in the soil samples and the duration of FBW amendments (Fig. 2a). The most frequently encountered ARGs were those conferring resistance to multidrug (21.11%), tetracycline (20.58%), and aminoglycoside (15.04%), followed by macrolide-lincosamide-streptogramin (MLS) (13.98%) and trimethoprim (6.07%) (Fig. 2b).

The relative abundance of ARGs (normalized to 16S rRNA) followed a similar trend as the number of detected ARGs, decreasing as the duration of FBW amendments increased. The highest abundance of ARG was observed in JLF6, 6 months after FBW amendments without any cropped, which was 20-fold higher than that in CK (Fig. 2c). However, over the course of 12 months of FBM amendments (SXF12 and GZF21), the relative abundance of ARGs decreased and returned to a level comparable to that of CK, with no significant difference observed ($P > 0.05$) (Fig. S1). Additionally, the principal coordinates analysis (PCoA) based on the Barry Curtis distances of ARGs also indicated that SXF12, GZF21, and CK exhibited greater similarity to each other than to JLF6 (Fig. S2). In JLF6, the most prevalent types of ARGs were those conferring resistance to sulfonamide (37.8%, 0.04

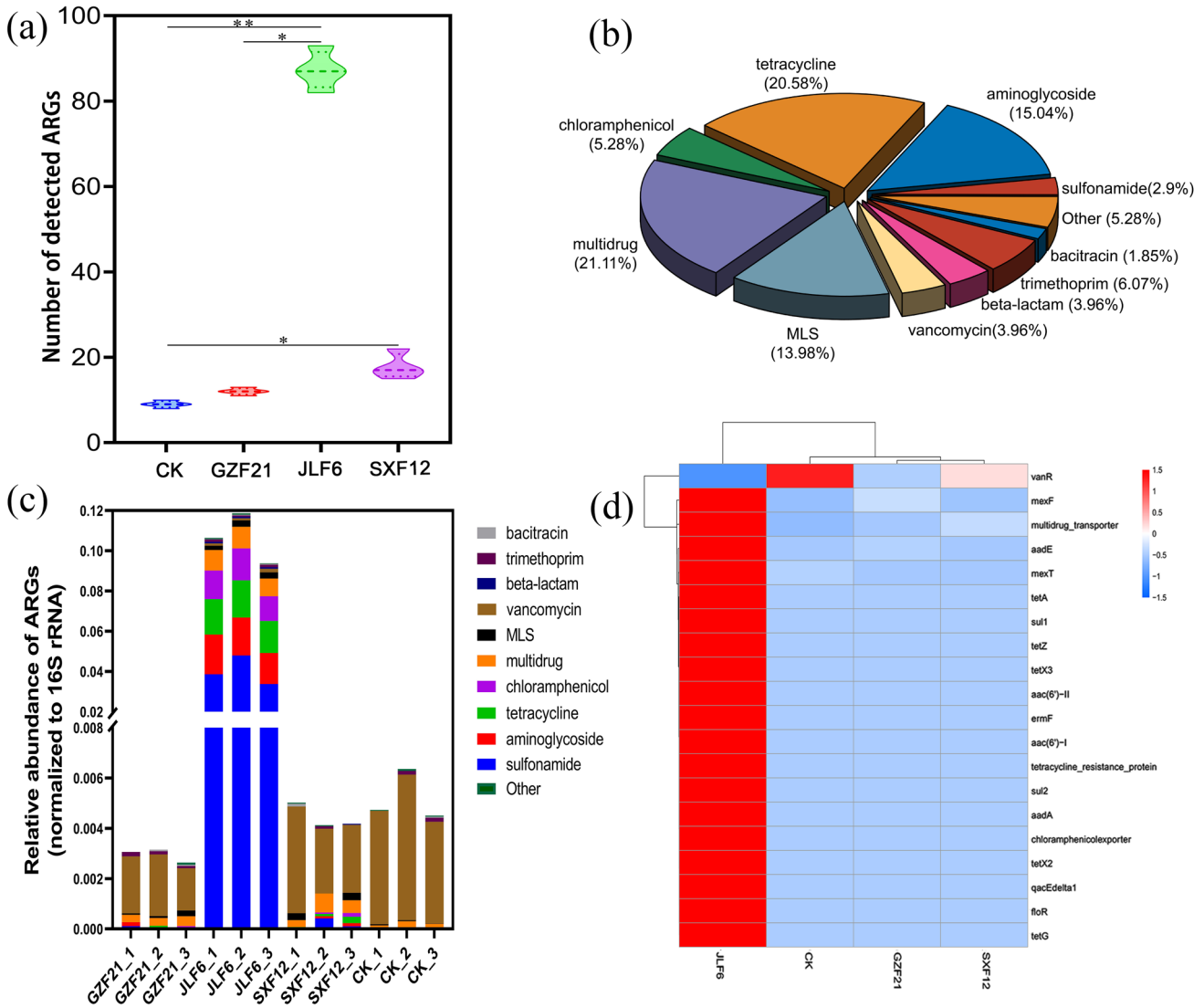

Fig. 2 The number of detected ARG types in different soil samples (Kruskal–Wallis ANOVA test: $*p < 0.05$, $**p < 0.01$) (**a**). The percentage in the number of detected ARG types in all soil samples (**b**). The relative abundance (normalized to 16S rRNA) of ARG types in different soil samples (**c**). The heatmap analysis of top 20 ARG subtypes in different soil samples (**d**)

copies per 16S rRNA gene), aminoglycoside (16.9%, 0.018 copies per 16S rRNA gene), tetracycline (16.32%, 0.017 copies per 16S rRNA gene), and chloramphenicol (13.3%, 0.014 copies per 16S rRNA gene). In contrast, these ARGs were present at very low abundance (below 0.00025 copies per 16S rRNA gene) in SXF12, GZF21, and CK. Instead, the most abundant types of ARG in SXF12, GZF21, and CK were vancomycin and multidrug (Fig. 2c and Fig. S3). Furthermore, the heatmap analysis of top 20 ARGs revealed significant enrichment of sulfonamide resistance genes (*sul1* and *sul2*), aminoglycoside-resistant genes (*aadA*, *aac(6')-I*, *aac(6')-II*, and *aadE*), and tetracycline resistance genes (*tetG*, *tetX2*, *tetA*, and *tetZ*) in JLF6, which were decreased significantly in SXF12, GZF21, and CK. Conversely, the vancomycin-resistant gene (*vanR*) showed a high level of enrichment in SXF12, GZF21, and CK (Fig. 2d).

The presence, abundance, and diversity of MGEs

We aimed to investigate the abundance, diversity, and presence of mobile genetic elements (MGEs) among all soil samples, including integrative conjugative elements (ICEs), insertion sequences, plasmids, and integrons. MGEs played a pivotal role in facilitating the horizontal transfer of ARGs among microorganisms. Similar to the patterns of ARG distribution in different soil samples, we found that MGEs including plasmids, ICEs, insertion sequences, and integrons were most abundant in JLF6 compared to SXF12, GZF21, and CK (Fig. 3). The number of gene counts of JLF6 matched to plasmids was 3780, while SXF12, GZF21, and CK exhibited lower gene counts with 1883, 2753, and 2541 gene counts, respectively (Table S3). Integrons were also analyzed by aligning them against the INTEGRALL database, and JLF6 had the highest number of gene counts, with *intI1* being the most abundant. In terms of insertion sequences, JLF6 had a higher count of 105 compared to CK, SXF12, and GZF21 (Table S3). The dominant insertion sequences in JLF6 were *IS1247*, *IS1245*, and *ISMlu5*, while *ISBj17*, *ISSsp2*, and *ISBj5* were dominant insertion sequences in CK, SXF12, and GZF21, respectively (Fig. S4). Furthermore, we evaluated the presence of ICEs in soil samples with different durations of FBW amendments. JLF6 had a total of 212 gene counts matched to ICEs, with the dominant ICEs being *ICE6440*, *ICEPaePA14-1*, and *ICEEa1*. In contrast, gene counts matched to ICEs in SXF12, GZF21, and CK were below 23, and the dominant ICEs were *ICEMloMAF-1*, *ICEMaSym (WSM2073)-alpha*, and *ICEMcSym (1284)-alpha*, respectively (Fig. S5). These results indicated that JLF6 exhibited greater MGE abundance and more diverse MGE profiles than SXF12, GZF21, and CK.

Relationships among soil physicochemical properties, ARG profiles, and bacterial communities

We further assessed the impact of soil physicochemical properties including EC, pH, TOC, TN, and C/N ratio on the composition of bacterial communities and ARGs through redundancy analysis (RDA). The result indicated that RDA1 and RDA2 elucidated 95.84% and 98.2% of the total variation in bacterial communities and ARG profiles, respectively (Fig. 4a and b). The RDA vectors indicated that pH played a more significant role in driving the composition of bacterial communities and ARGs, which aligned with the results of the Mantel test (Table S4). Additionally, Spearman's heatmap analysis showed that the relative abundance of Actinobacteria was positively correlated with pH and EC but negatively correlated with TOC, TN, and C/N ratio. In contrast, Proteobacteria and Bacteroidetes exhibited a negative correlation with pH, but a positive correlation with TN, TOC, and C/N ratio (Fig. 4c). Moreover, the relative abundance of vancomycin-resistant genes was positively correlated with pH, but negatively correlated with TOC and TN (Fig. 4d). These results suggested that soil physicochemical properties influence the distribution of soil bacterial communities and ARGs.

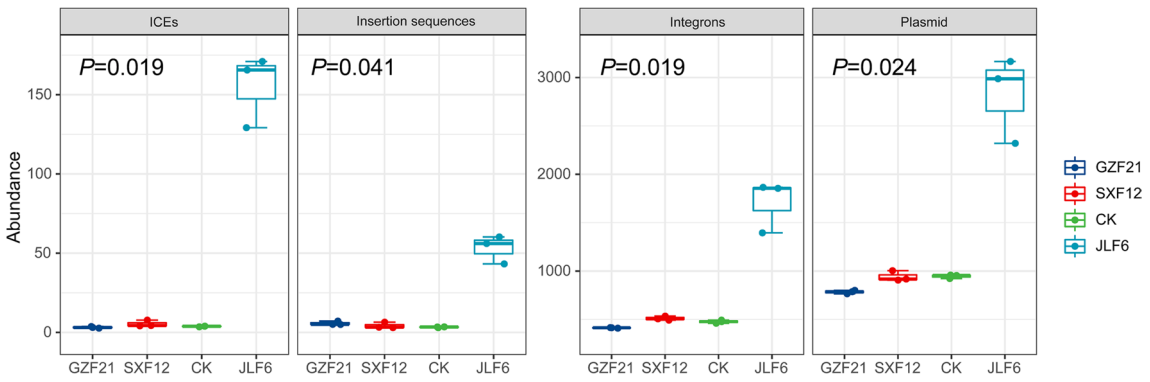

Fig. 3 The abundance of MGEs, including integrative conjugative elements (ICEs), insertion sequences, integrons, and plasmids in different soil samples (Kruskal–Wallis test: all significant difference $p < 0.05$)

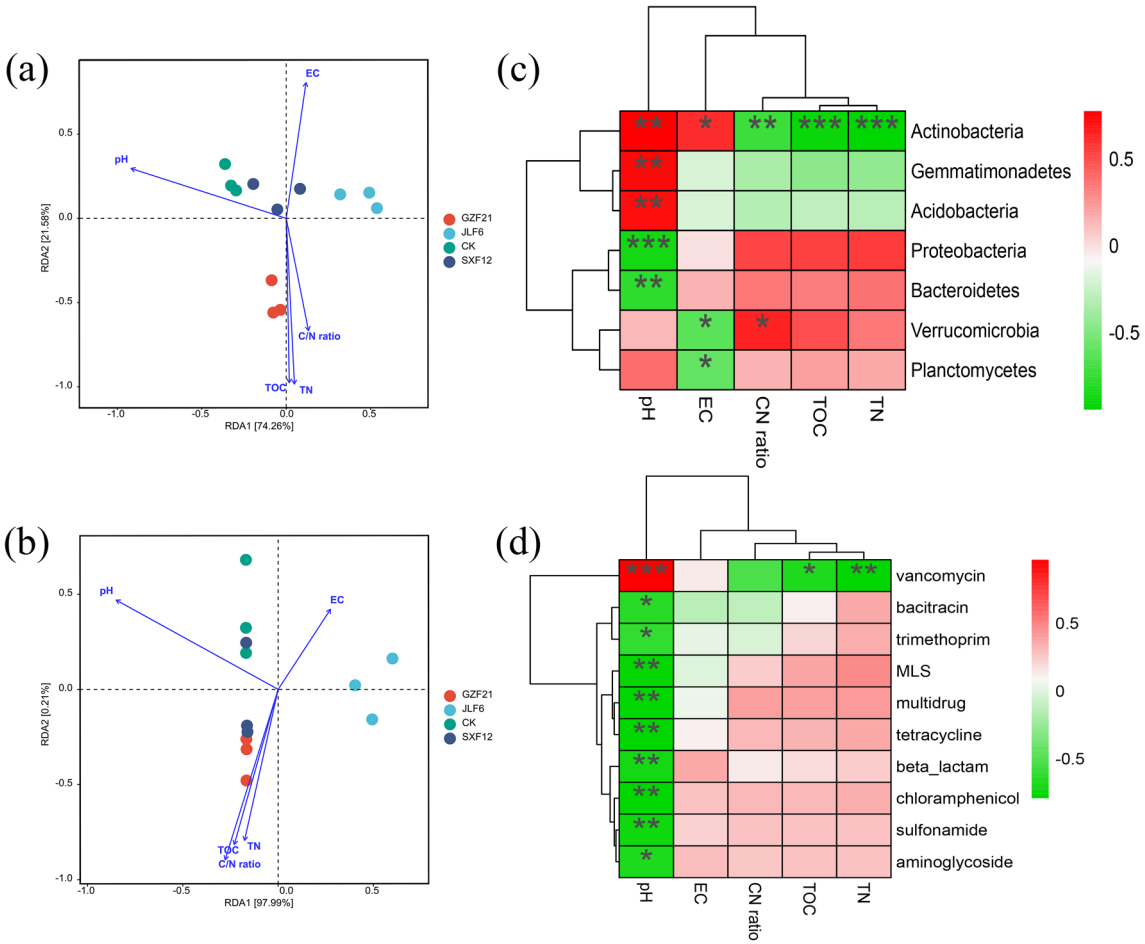

Fig. 4 Redundancy analysis (RDA) of quantitative correlation between bacterial communities (**a**) or ARG types (**b**) and soil chemical properties in different soil samples. Spearman heatmap of correlation between bacterial communities (**c**) or ARG types (**d**) and soil chemical properties (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$)

The recovered metagenome-assembled genomes (MAGs) binning and their distribution to ARGs and MGEs

Based on metagenomic binning analysis, a total of 281 MAGs were assembled from soil samples. After quality control, 55 high-quality MAGs were retained for downstream analysis, comprising 10 near-complete MAGs (≥ 90% completeness and ≤ 5% contamination), 20 medium-quality MAGs (≥ 70% completeness and ≤ 10% contamination), and 25 partial MAGs (completeness ≥ 50% and contamination ≤ 10%) (Fig. 5a). Among these 55 recovered MAGs, 12 MAGs were found to carry ARGs, with their relative abundance ranging from 1.0 to 35.2 cpm (copies per million) in each soil sample. Notably, the relative abundance of ARG-carrying MAGs in JLF6 was more than tenfold higher than that in CK, SXF12, and GZF21 (Fig. 5b and Table S5).

As shown in Fig. 6, these ARG-carrying MAGs predominantly harbored eight types of ARGs, with tetracycline, multidrug, and vancomycin being the most frequently detected types. Most ARG-carrying MAGs belonged to Actinobacteriota, Proteobacteria, and Bacteroidota, followed by Chloroflexota and Verrucomicrobiota. Many of these bacterial hosts exhibited potential for multi-antibiotic resistance. For example, one MAG (bins.30) belonging to genus *Mycobacterium* of the phylum Actinobacteriota could confer multiple resistance to tetracycline (*tetX2* and *tetV*), rifamycin (*ADP-ribosylating transferase_arr*) and quinolone (*mfpA*). Additionally, the genus *Herbaspirillum* affiliated to the phylum Proteobacteria and family *CSP1-4* were also found to harbor two or more ARG subtypes (Fig. 6 and Table S6). These bacterial hosts could potentially play a significant role in the dissemination and acquisition of ARGs in the environment. According to mobilome profile analysis, Acidobacteriota harbored the highest number of MGEs (accounting for 49.86%), followed

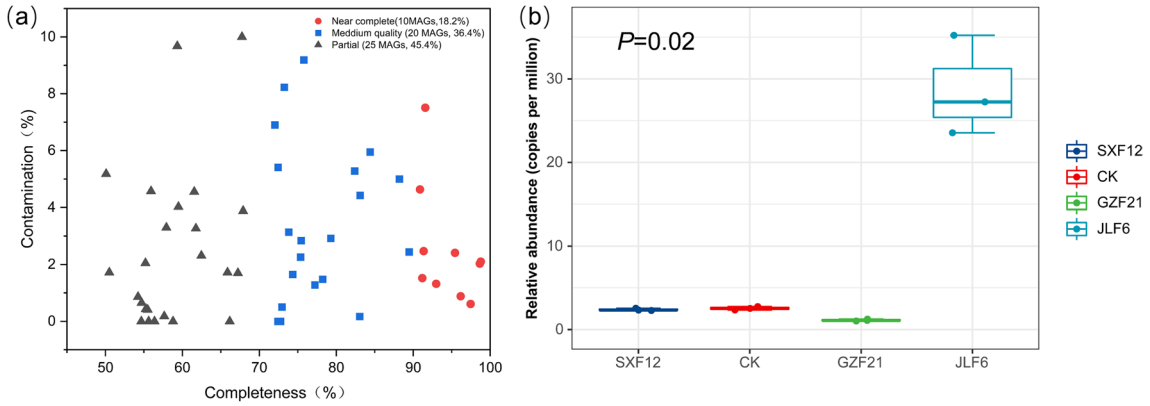

Fig. 5 Quality assessment of the recovered MAGs carrying ARGs (**a**). Genome quality was defined as completeness − 5 × contamination, and only genomes with quality of ≥ 50% were retained. Near-complete genomes (completeness ≥ 90%; contamination ≤ 5%) are shown in red, medium-quality genomes (completeness ≥ 70%; contamination ≤ 10%) in blue, and partial genomes (completeness ≥ 50%; contamination ≤ 10%) in black. The relative abundance of ARG-carrying MAGs in different soil samples (Kruskal–Wallis test: $p = 0.02$) (**b**)

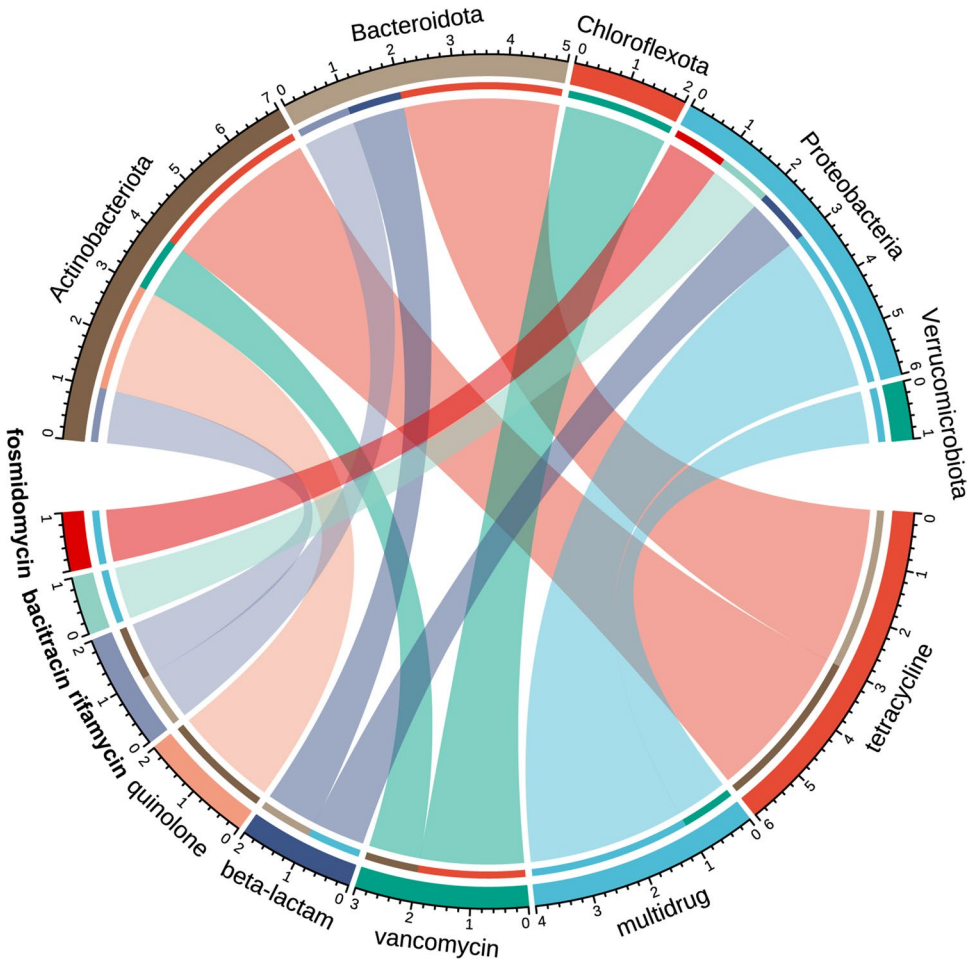

Fig. 6 Chord diagram between bacterial hosts of ARGs and the carried ARGs across all soil samples. The length of the bars on the ring correspond to the total number of ARGs or MAGs, respectively

by Patescibacteria (accounting for 23.04%), Bacteroidota (accounting for 22.49%), and Gemmatimonadota (accounting for 2.98%) (Fig. S6). These findings suggested that these bacterial hosts have a greater potential to facilitate the transfer of ARGs in the environment.

Discussion

Impact of swine manure–derived fermentation bed waste amendment on ARGs

The application of swine manure–derived fermentation bed waste (FBW) as an amendment in agricultural fields was found to have a significant impact on the diversity and detection frequency of ARGs. The number of detected ARGs in FBW-amended soil samples was significantly higher compared to control samples. However, as the duration of FBW amendments increased, the number of detected ARGs decreased, suggesting a reduction in the potential ARG risk associated with FBM amendments over time. The relative abundance of ARGs followed a similar trend, with an increase in their abundance following FBW amendments. Even after 6 months of FBW amendments without cropping, the relative abundance of ARGs remained higher in JLF6. The higher detected ARGs in JLF6 may be attributed to FBW from swine feedlot, with sulfonamide, aminoglycoside, and tetracycline resistance genes found to be the dominant types of ARGs (Fig. 2c). These three types of ARGs were universally distributed in the conventional concrete-floor swine feedlot and in agriculture soils that received swine manure (Ben et al. 2017; Wang et al. 2016). To further support this, initial FBW samples were collected from the same swine feedlots in Jilin province as in our previous study (referred to as JLW, data not yet published). The dominant types of ARGs in JLW were sulfonamide (32.3%)-, aminoglycoside (23.2%)-, and tetracycline (19.9%)-resistant genes, accounting for 75.4% of total abundance of ARGs (Fig. S7a). The ARG profiles in JLW were similar to those in JLF6, and the relative abundance of ARG increased significantly during the first 6 months after FBW amendments in agricultural fields (Fig. S7b). These results suggested that the amendment of FBW in agricultural soils could pose a risk of transferring ARGs from swine feedlots to the surrounding environment within the first 6 months. However, after more than 12 months of FBW amendments, the relative abundance of these three types of ARGs decreased significantly and the profiles of ARGs returned to a pristine level similar to that of control samples, indicating a decrease in the soil resistome risk over time. This finding was supported by the results of clustering analysis on heatmap and PCoA (Fig. 2d and Fig. S2). The most abundant types of ARGs were those conferring resistance to vancomycin and multidrug, after more than 12 months of FBW amendments (SXF12 and GZF21), which were similar to the control samples. These types of ARG were ancient and commonly found in soils worldwide. Extensive research had been conducted on these ARGs, supporting their widespread presence (D'Costa et al. 2011; Zheng et al. 2022). Specifically, *vanR* was consistently the most frequently detected ARG subtype in SXF12, GZF21, and CK. It was important to note that *vanR* was highly prevalent in "non-human-associated" environments such as soil and was not human-associated and the least likely to endanger human health, which was classified as low risk to humans (Rank IV) (Zhang et al. 2021).

The underlying mechanisms in the dissemination of ARGs

The addition of manure in agricultural soil could cause significant changes to the soil microbial community, potentially impacting the prevalence of ARGs (Guan et al. 2022; Li et al. 2022). In this research, we investigated the impact of swine manure–derived FBW amendment on the soil resistome. A mantel test revealed a strong correlation between bacterial communities and ARG profiles (Fig. S8). The significant differences in bacterial community structure were observed between early stage of FBW-amended soil samples (JLF6) and control samples (CK) or long-term FBW-amended soil samples (SXF12 and GZF21) (Fig. 1). These findings suggested that the shift in bacterial community might lead to the changes in soil resistome, which aligned with the impact of manure amendment on soil resistome (Gu et al. 2021). However, reports on the bacterial communities and ARG profiles of the early stage and long-term FBW-amended soil were scarce.

In this study, we found that early stage of FBW-amended soil samples (JLF6) had a higher proportion of Proteobacteria, Actinobacteriota, and Bacteroidetes, which were known to be the most abundant phyla in manures and could possibly carry ARGs (Han et al. 2018) (Fig. 1a). As these bacteria were derived from swine manure in FBW, they may potentially contribute to the dissemination of ARGs into agricultural soil during the early stage of the amendment. Additionally, metagenome-assembled genome binning revealed that Actinobacteriota, Proteobacteria, and Bacteroidota were the dominant bacterial hosts of ARGs in this study (Fig. 6). This finding was consistent with previous research which reported that Actinobacteriota, Proteobacteria, and Bacteroidetes were the most common hosts of ARGs in wastewater (Zhu et al. 2023). We also found that many bacterial hosts of ARGs showed possible multi-antibiotic resistance characteristics, indicating higher risks for the spread of ARGs. For instance, *Mycobacterium elephantis* was detected as the carrier of multiple antibiotic resistance, including *tetX2*, *tetV*, and *ADP-ribosylating transferase_arr* and *mfpA*, which had

previously been identified as a most important multi-drug resistant bacteria in wastewater treatment plants (Zhao et al. 2020). *Mycobacterium* were commonly found in soil and played a significant role in the spread and transfer of ARGs in soil. Some *Mycobacterium* species, including pathogenic species such as *M. tuberculosis*, would pose the risk of ARG from environment to human health (Peterson and Kaur 2018). Additionally, genus *Herbaspirillum* and family CSP1-4 harbored two or more ARGs, which were also enriched in JLF6 (Fig. S9).

In addition to changes in the bacteria community, MGEs were also prominently correlated with soil resistome, potentially facilitating the dissemination of ARGs (Zheng et al. 2022). Swine manure–derived FBW-borne bacteria can transfer into indigenous bacteria in agriculture soil via MGEs, providing an alternative mechanism for the soil resistome (Du et al. 2020). The largest number and abundance of MGEs were detected during the early stages of FBW amendment (JLF6) (Table S3, Fig. 3), indicating that MGEs originating from swine manure–derived FBW bacteria could establish themselves in soils and promote soil resistome more extensively (Heuer et al. 2011). The MAGs binning results further revealed that Acidobacteriota, Patescibacteria, and Bacteroidota were the main hosts of MGEs in soil samples, suggesting that these bacterial hosts possess better abilities to facilitate the transfer of ARGs in the environment. However, after more than 12 months of FBW amendments, the relative abundance of ARGs and MGEs decreased substantially, and profiles of bacteria community and ARGs were brought back to a level similar to those of CK. These results suggested that the risk of soil resistome decreases over time. The structure of the bacteria community may have a significant impact on the dissemination and acquisition of antibiotic resistance in the environment.

Conclusions

As a result of this study, it has been demonstrated that the amendment of soil samples with swine manure–derived FBW can initially increase the abundance and diversity of ARGs and MGEs, particularly within the first 6 months of amendment. However, the abundance of ARGs and MGEs declined over time, and after 12 months of FBW amendments, their levels returned to similar to control samples. The structure of the bacterial community played a critical role in shaping the ARG patterns, with Actinobacteriota, Proteobacteria, and Bacteroidota identified as the main potential hosts of ARGs. Furthermore, Acidobacteriota, Bacteroidota, and Patescibacteria were found to harbor more MGEs, potentially facilitating the transfer of ARGs. These findings provided insights into the temporal patterns and dissemination risk of ARGs in swine manure–derived FBW-amended agricultural soil. Further research about the dissemination risk of ARGs in swine manure–derived FBW-amended agricultural soil with different amendment periods could be conducted in one experimental station with or without cropping, in order to better verify the risk of ARG dissemination from swine manure-derived FBW.

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Data availability The raw metagenomic sequence data were publicly available in the NCBI BioProject database with the accession number of PRJNA953804.

Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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