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Virome and metagenomic sequencing reveal the impact of microbial inoculants on suppressions of antibiotic resistome and viruses during co-composting

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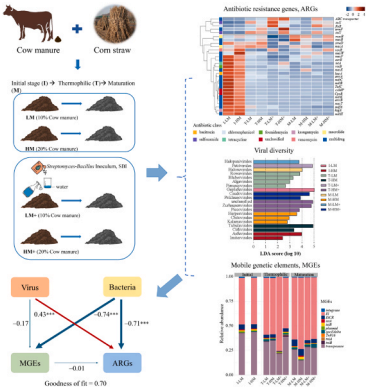
HIGHLIGHTS

- Co-composting with microbial inoculant suppressed ARGs and pathobiome risks.
- Widely-existed phytopathogens significantly decreased following co-composting.
- Inoculants and manure quantity modulates virus communities and ARGs.
- Virus was a significant positive factor influencing ARGs.
- *Gemycircularvirus* was the most influential taxon in shaping ARGs changes.

GRAPHICAL ABSTRACT



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ABSTRACT

Co-composting with exogenous microbial inoculant, presents an effective approach for the harmless utilization of livestock manure and agroforestry wastes. However, the impact of inoculant application on the variations of viral and antibiotic resistance genes (ARGs) remains poorly understood, particularly under varying manure quantity (low 10 % vs. high 20 % w/w). Thus, employing virome and metagenomic sequencing, we examined the influence of *Streptomyces-Bacillus* Inoculants (SBI) on viral communities, phytopathogen, ARGs, mobile genetic elements, and their interrelations. Our results indicate that SBI shifted dominant bacterial species from *Phenylobacterium* to thermotropic *Bordetella*, and the quantity of manure mediates the effect of SBI on whole bacterial community. Major ARGs and genetic elements experienced substantial changes with SBI addition. There was a higher ARGs elimination rate in the composts with low (~76 %) than those with high manure (~70 %)

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application. Virus emerged as a critical factor influencing ARG dynamics. We observed a significant variation in virus community, transitioning from *Gemycircularvirus*- (~95 %) to *Chlamydiamicrovirus*-dominance. RDA analysis revealed that *Gemycircularvirus* was the most influential taxon in shaping ARGs, with its abundance decreased approximately 80 % after composting. Collectively, these findings underscore the role of microbial inoculants in modulating virus communities and ARGs during biowaste co-composting.

1. Introduction

Due to the excessive of veterinary antibiotics for disease treatment, prevention, and as growth promoters, livestock manures has become a significant reservoir of antibiotic resistance genes (ARGs), posing considerable environmental and healthcare risks [1–3]. The overuse of antibiotics and the fluctuating prevalence of antibiotic resistance have resulted in long-term consequences, as antibiotics tend to accumulate in soil or water due to the improper disposal of manure waste [4]. Studies have demonstrated that co-composting of manure and other agricultural biowastes, can alter bacterial community structure through high temperatures and reduced water content. This process results in decreased diversity and abundance of ARGs in livestock manure following the thermophilic phase of composting [5,6]. However, the efficiency of antibiotic removal through microbial degradation, pyrolysis, and hydrolysis remains notably low, and traditional aerobic composting methods often fail to efficiently eliminate accumulated ARGs in livestock manure [7]. Consequently, researchers have focused on composting processes supplemented with microbial agents to enhance the efficacy of ARG removal[8–10].

In our previous work, we observed that *Streptomyces-Bacillus* Inoculation (SBI) significantly shortened the compost maturation time, and the high temperature period from 15d to 8d [11]. This suggests its potential to reduce pathogenic microorganisms and virus-associated ARGs [3,12,13]. Viruses are the most abundant biological entities on Earth, influencing microbial death through cell lysis and significantly impacting ecosystem element cycling by releasing nutrients, thereby affecting microbial community composition, diversity, and overall microbial residues [14–16]. Despite the growing attention on the role of viruses in nutrient turnover and organic matter mineralization in compost, our understanding of virus and bacterial community changes during composting remains limited. It is now recognized that viruses play significant roles as carriers and disseminators of ARGs [2,17,18]. However, the impact of SBI addition on microbial community dynamics during the co-composting process and ARGs, remains unclear. Hence, investigating differences in ARG distribution during composting, along with the dynamic interplay between bacterial and viral communities, will provide insights into the crucial role of viruses in composting [11].

In this study, co-composting of cow manure and corn straw was conducted to investigate changes in ARGs under varied initial cow manure amounts, with or without the application of SBI. Specifically, our objectives were to assess: 1) the impact of SBI and manure quantity on the succession of dominant bacterial communities and abundance of potentially pathogenic microorganisms during co-composting; 2) the alteration of ARG elimination rates and transmission pathways; 3) the response of the viral community to SBI and manure quantity changes; and 4) the correlation between changes in ARGs and bacterial and viral communities.

2. Material and methods

2.1. Experimental setup and sample collection

Composting was conducted using corn cob, corn straw, and cow manure as the primary raw materials. Four treatment groups were established based on variations in cow manure content (10 % vs. 20 % by weight) and the presence or absence of *Streptomyces-Bacillus* inoculum (SBI) (Luoyang Biotechnology Co., LTD., *Streptomyces-Bacillus* 1:1). Thus, the treatments comprised: Low cow manure addition of 10 % (L), low cow manure with SBI (LM), high cow manure addition of 20 % (H), and high cow manure with SBI (HM). In the low cow manure treatment, the ratio of cow manure, corn cob, and corn straw was 10 %, 3 %, and 86 %, respectively, while in the high cow manure treatment, the ratio was 20 %, 3 %, and 76 %, respectively. The additional SBI content in both LM and HM treatments was 10 $g{\cdot}L^{-1}$ (SBI is dissolved in water and stirred well). After thorough mixing of the raw materials in the specified proportions, 1 % quick lime (CaO) was added to each treatment and thoroughly mixed. Agricultural biowastes, such as crop straw and livestock manure, produce acids after decomposition, creating environments unsuitable for the survival of important microorganisms and fungi. To neutralize these acidic conditions, quicklime (CaO) was applied. The addition of quicklime (CaO) to the compost aids in sterilization, disease prevention, and the neutralization of the acidic environment in the initial compost stage [19]. A total of four piles were set. Samples from different heights of the pile were mixed and divided into three replicates. The mixed compost pile for each treatment had a volume of approximately 2 m^3, moisture content of 60–70 %. Piles were turned manually with a shovel at 3-day intervals to ensure adequate aeration. Sampling intervals were based on pile's temperature data from the outdoor HOBO sensor (Micro Station, H21-USB), during the initial (~20 ℃), thermophilic (>60 ℃), and maturation (~20 ℃) phases of composting [11]. At each sampling point, we collected samples from the upper, middle, and lower layers of each pile and mixed as a representative sample, and stored at –80 ℃ for metagenomic and virome sequencing.

2.2. Metagenome analysis

Samples (~0.5 g) were freeze-dried and subjected to DNA extraction using a Fast DNA Spin Kit following the manufacturer's instructions. The concentration and purity of the extracted DNA samples were assessed using an ultraviolet spectrophotometer (Quawell Q3000), with an A260/A280 ratio of 1.8–2.0 indicating satisfactory nucleic acid purity. Total DNA sequencing was performed on the HiSeq 2500 platform (Illumina, PE150 mode, Guangdong Meige Gene Technology Co., Ltd). Following metagenomic sequencing, quality control was conducted using Trimmomatic software to eliminate low-quality data. A total of 2553,884,960 reads were obtained from 48 samples. Metagenomic assembly was performed using MEGAHIT (v1.2.9) on clean reads after quality control. ORFs were predicted for each sequence using Prodigal (v2.6.3) based on Scaftigs longer than 500 bp, with ORFs shorter than 90 bp discarded. CD-HIT was employed to cluster sequences with $\geq$ 95 % sequence similarity and $>$ 90 % reads coverage, with the longest sequence in each cluster selected as the representative sequence (Unigene). Non-redundant Unigene sequences were blasted against the NCBI NR database using DIAMOND software (v0.9.10) with an e-value $\leq 1 \times 10 -5$. Metagenomic sequences were also blasted against the Pathogen Host Interactions database (PHI-base) for pathogen annotation [20]. ARG annotation was conducted against the SARG database using BLASTX via ARGs-OAP online [21]. Additionally, clean data were searched for MGEs against an offline database comprising integrons, gene cassettes, insertion sequences, and plasmids obtained from INTEGRALL, ISfinder, and the NCBI RefSeq database [22]. The data has been deposited in the NCBI Sequence Read Archive (SRA) database under accession number PRJNA996558.

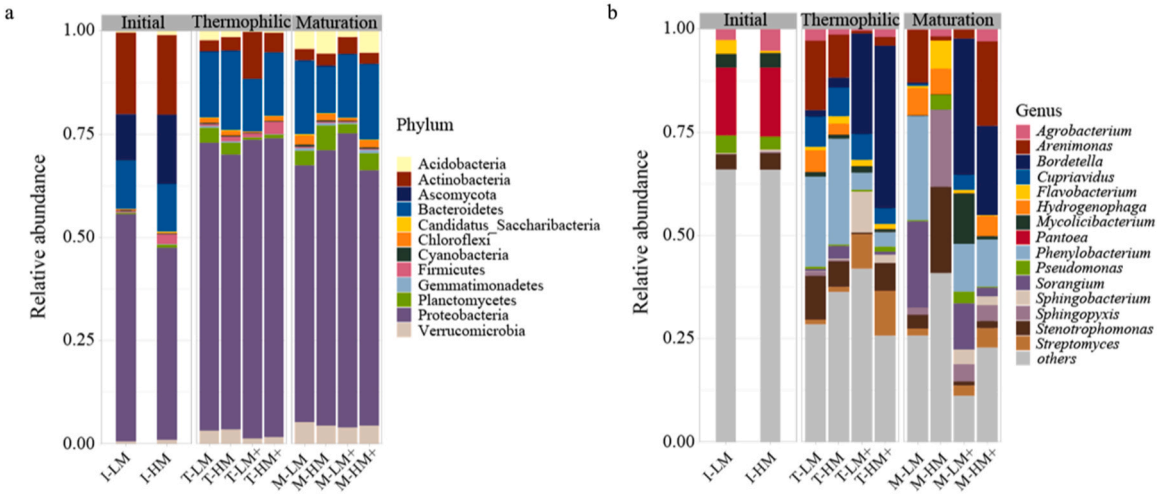

Fig. 1. Dynamics of dominant microbial phyla (a) and genera (b) during cow manure (10 % w/w, low manure amount (LM); and 20 % w/w high manure amount addition (HM) and corn straw co-composting. The initial stage of the two co-composts with varied cow manure (I-LM, I-HM); the thermophilic co-composting stage without SBI (T-LM, T-HM) and with SBI (T-LM+, T-HM+); the maturation stage of the composts without (M-LM, M-HM) and with (M-LM+, M-HM+) SBI. The specific sampling time and maximum temperature of each composting stage and the corresponding treatment of each sample name in the corresponding figure are shown in Table S3. Significant differences of bacterial taxa among different treatments were included in Table S6 and Table S7.

2.3. Virome sequencing, assembly, and annotation

Viral particle isolation and nucleic acid extraction techniques were employed to selectively remove host cell and ribosomal interference, enrich viral-like particles (VLP) in samples, and prepare a virus genome library. Following the acquisition of pure VLP samples, nucleic acids were extracted, encompassing double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), and RNA (ssRNA, dsRNA). Qualified DNA samples or amplified DNA samples underwent random fragmentation via ultrasonic disruption, producing short DNA fragments suitable for sequencing library construction. Sequencing libraries of qualified DNA were then subjected to sequencing on the Illumina platform with paired-end 150 bp reads. Subsequently, the sequencing data from each sample's virus group underwent quality assessment and removal of low-quality data using Trimmomatic software [23]. The clean reads were then assembled using MEGAHIT software (v1.1.2) with default parameters [24]. BWA software (v0.7.17) was employed to align clean reads to the assembly results and calculate the read utilization rate [25]. The assembled contigs were annotated using the Prodigal package within the CheckV software [26]. Genes annotated by these fragments were compared with Hidden Markov models (HMMs) constructed from seven major reference databases (KEGG, VOGDB, PfamA, PfamB, IMG/VR, TIGRFAM, RVDB) to identify microbial and viral genes. Virus sequences were identified using CheckV and Virsorter 2 software [27]. Based on the results of virus sequence identification and classification annotation, RNA genome type virus sequences were filtered, and subsequent analyses were conducted on the filtered virus sequences. Following quality control, clean reads accounted for 75.4 % (29,721,331; Supplementary Table S3). MEGAHIT software (v1.2.9) was employed for metagenomic assembly, yielding 4294,818 contigs with an average length of ~300 bp. Species annotation of virus contigs identified 362,389 potential virus contigs (>300 bp). The data has been deposited in the NCBI Sequence Read Archive (SRA) database under accession number PRJNA1088895.

2.4. Statistical analyses

One-way and Two-way ANOVA analysis were performed using SPSS 26.0 (IBM, Armonk, NY, USA) software. Stack diagrams of bacteria, pathogens, MGEs, and viruses were plotted using the "ggplot2″ package in the R program (v4.3.3). Heatmaps of ARGs were generated using the "ComplexHeatmap" and "circlize" packages in the R program (v4.3.3). We used R program (v 4.3.3) to conduct principal component analysis on ARGs and MGEs datasets and selected the first principal components (PC 1) for ARGs and MGEs. Furthermore, a Two-way ANOVA analysis was performed to determine the interactive effects of cow manure amount and SBI addition on the changes of ARGs and MGEs in compost by using the PC1 data of ARGs and MGEs, respectively. A box plot of the virus Shannon index was created using the "ggplot2″ package in R (v4.3.3). Non-metric multidimensional scaling (NMDS) with Bray-Curtis distance was employed to illustrate variations in virus community structure. NMDS analysis was performed using the "vegan," "ggplot2," "ape" and "RColorBrewer" packages in R (v4.3.3). Microbial biomarkers were identified using LDA effect size with a threshold of LDA score > 2.0 and $p < 0.05$ in virus communities. Alpha diversities of bacteria and viruses were calculated. The "randomForest" package in R (v4.3.3) was used to identify significant ARGs and MGEs. Redundancy analysis (RDA) of bacterial community, viral community, MGEs, and ARGs was plotted using the "ggplot2," "vegan," "ggrepel," and "ggpubr" packages in R (v4.3.3). Mantel-test analysis of the relationships between bacteria, virus, MGEs and ARGs was using the "Hmisc", "devtools", "linkET", "tidyverse", "RColorBrewer" and "geosphere" packages in R (v4.3.3). Partial least squares path modelling (PLS-PM) analysis of the relationships between bacteria, virus, MGEs and ARGs was using the "vegan", "ggplot2," "ape", and "plsm" packages in R (v4.3.3).

3. Results and discussion

3.1. Dynamics of dominant bacteria during composting

Throughout the composting process, Proteobacteria (averaging ~48.4 %) and Bacteroidetes (~11.1 %) emerged as the dominant bacterial phyla, followed by Actinobacteria (~5.5 %), Acidobacteria (~1.6 %), Planctomycetes (~1.8 %), and other minor taxa (relative abundances < 1 %; Fig. 1a). Notably, the addition of high cow manure (20 % w/w) led to pronounced variations in bacterial communities compared to low manure addition (10 % w/w) practices, manifesting as significantly increased Firmicutes and *Agrobacterium* ($p < 0.05$), and substantially decreased *Flavobacterium* ($P < 0.05$) (Table S5 and S6). Cow manure is rich in lignocellulosic components and organic nitrogen nutrients, making it a key factor in influencing the growth and metabolism of microorganisms that target these carbon and nitrogen elements [28]. Aerobic composting is primarily driven by microbial metabolic

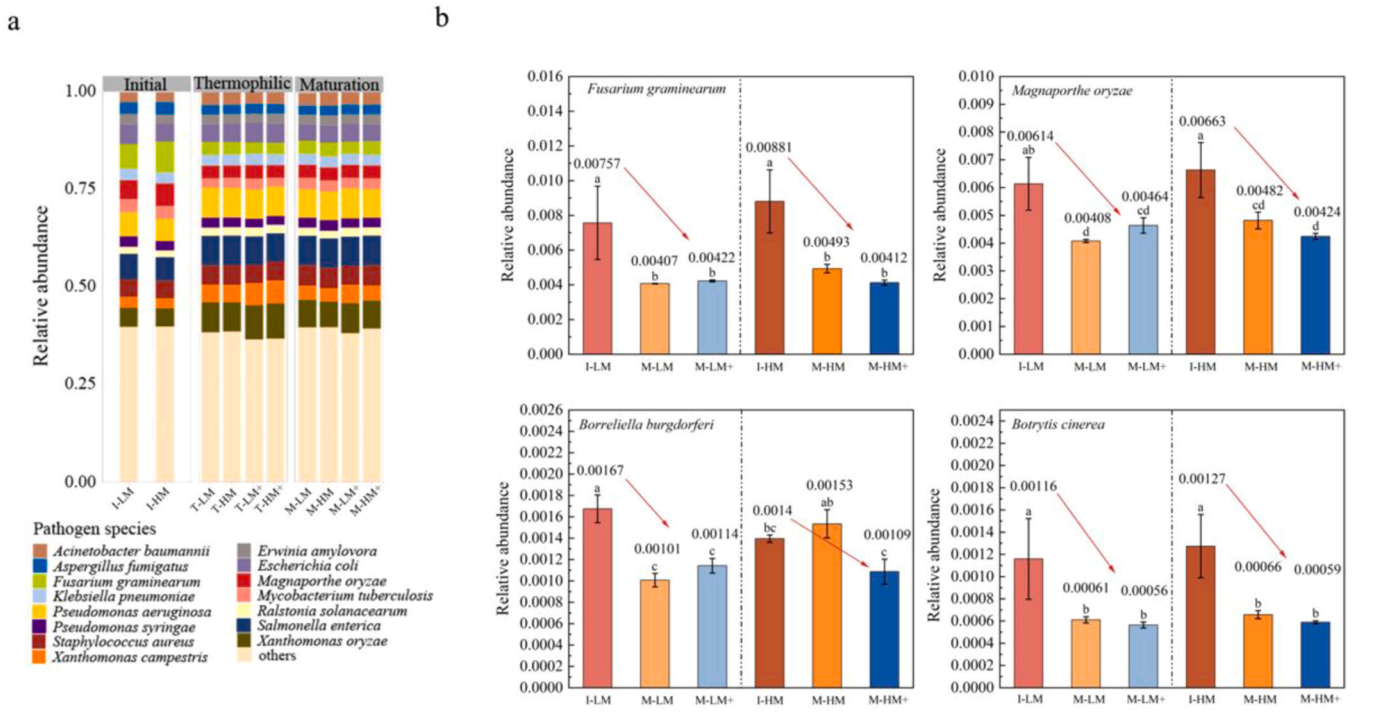

Fig. 2. Variations in overall pathogenic species (a) and significantly decreased (b) pathogens during cow manure and straw co-composting. Abbreviations are explained in Fig. 1.

activities. Carbon-degrading microbes play a significant role in the decomposition and transformation of organic matter during the composting process. Consequently, variations in the initial amounts of biowaste can lead to corresponding changes in the composition of the microbial community [28,29].

The interactions between exogenous microbial inoculants and indigenous microbes within composts can directly lead to changes in microbial diversity, structure and composition [30,31]. Microbial inoculants are increasingly attractive solutions to promote compost maturation. Among them, the *Streptomyces-Bacillus* Inoculant (SBI) has been proved to enhance C and N turnover [32], due to the characteristics of SBI in surviving in diverse conditions, and secreting broad-spectrum enzymes that capable of degrading organic matter [33]. Our results showed that the application of SBI altered the dominant bacterial taxa from *Phenylobacterium* to *Bordetella* during the thermophilic stage, regardless of initial cow manure amounts (Fig. 1b). *Phenylobacterium*, belonging to the Caulobacteraceae family of Proteobacteria (α-Proteobacteria), dominates in the biodegradation of polycyclic aromatic hydrocarbons [34] and plays a role in nitrogen conversion processes [35]. *Bordetella*, a member of the Alcaligenes family of Proteobacteria (β-Proteobacteria), elevates fermentation temperatures in bulk and effectively degrades oxytetracycline [36]. Furthermore, the effect of SBI closely correlates with the quantity of initial cow manure in co-composting. For instance, in low manure addition treatments, *Phenylobacterium* and *Bordetella* remained dominant during the maturation stage, while high manure treatments exhibited co-dominance by *Bordetella* (~30 %) and *Arenimonas* (~30 %). *Arenimonas*, affiliated with the *Xanthomonas* family of Proteobacteria (γ-proteobacteria), thrives in eutrophic environments such as reservoirs, estuarine sediments, and compost samples [37–40], suggesting its proliferation under high initial cow manure conditions.

In contrast, non-SBI-treated high cow manure composting was dominated by *Sphingopyxis* (~25 %) and *Stenotrophomonas* (~25 %), indicative of a community function aimed at suppressing hazardous substances. Notably, Stenotrophomonas secretes anti-fungal compounds and synthesizes anti-pathogenic extracellular enzymes [41]. *Sphingopyxis*, belonging to the family *Sphingomonadaceae* Proteobacteria (α-Proteobacteria), commonly inhabits agricultural soils, demonstrating versatility in diverse ecological niches [42] and capability in environmental pollutant degradation [43,44].

Proteobacteria (from 35.4 to 46.9 %), Planctomycetes (0.5–2.8 %), Verrucomicrobia (0.5–3.2 %), Acidobacteria (0.5–2.9 %), Chloroflexi (0.2–1.1 %), Gemmatimonadetes (0.04–0.4 %), and Cyanobacteria (0.1–0.2 %), while the Actinobacteria dramatically decreased form 13.6 % (at the initial stage) to 2.3 % (maturation stage) (Fig. 1a; Table S6). At the genus level, substantial changes were observed, with notable increases in *Pseudoxanthomonas, Chryseolinea, Steroidobacter, Mesorhizobium, Hydrogenophaga, Phenylobacterium*, and *Sphingopyxis*, while *Sphingomonas, Pseudomonas, Stenotrophomonas,* and *Microbacterium* experienced relative abundance decreases (Fig. 1b; Table S7). These substantial shifts align with previous co-composting studies using similar cow manure and corn straw substrates [45], possibly driven by temperature-based substrate alterations and the subsequent succession from mesophilic to thermophilic bacterial taxa [5,46,47]. Notably, a significant increase in dominant Proteobacteria from the initial to the thermophilic stage suggests their trophic role in efficiently utilizing macromolecular organic materials at elevated temperatures [48–50].

3.2. Changes in potential pathogenic microorganisms during composting

Salmonella enterica emerged as the most abundant species, with a mean relative abundance of 0.96 %, followed closely by *Pseudomonas aeruginosa* (~0.95 %), *Xanthomonas oryzae* (~0.95 %), and *Staphylococcus aureus* (0.64 %). These four species collectively constituted half of the pathogenic bacteria sequences across all samples, exhibiting stable abundances regardless of variations in SBI application during composting stages (Fig. 2a). At the initial stage, the relative abundance of *Salmonella enterica*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Erwinia amylovora*, *Klebsiella pneumoniae*, and *Pseudomonas syringae*, in the low cow manure treatment (I-LM) were significantly lower than that in the high cow manure treatment (I-HM) (Table S7). During the thermophilic stage, the variation of cow manure

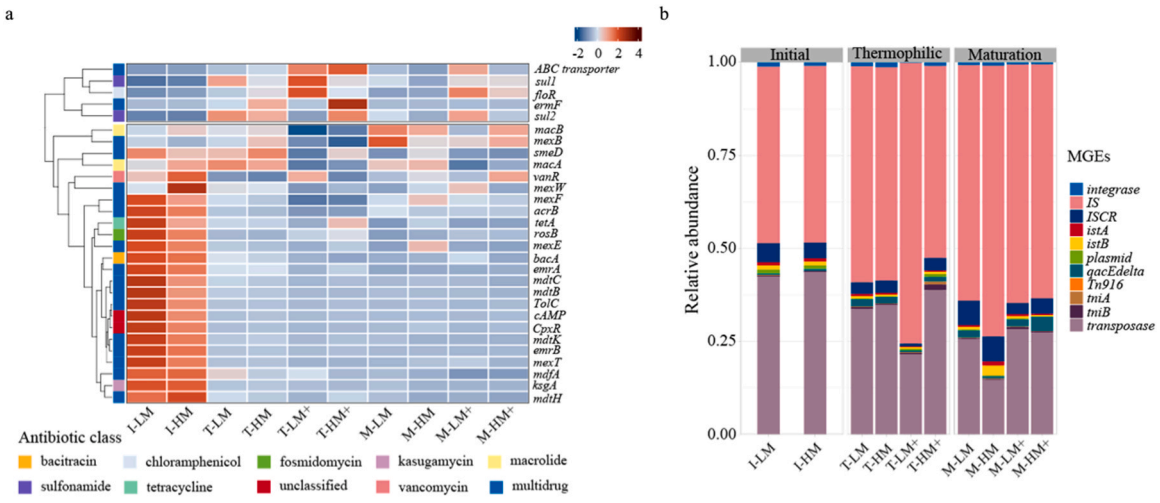

Fig. 3. The relative abundances of antibiotic resistance genes (ARGs; a) and mobile genetic element (MGEs; b) during composting. Interpretation of the abbreviations is presented in Fig. 1.

amount did not result in a significant difference in pathogenic bacteria abundance in the absence of SBI (T-LM vs. T-HM; $P > 0.05$; Table S7). However, the relative abundance of *Escherichia coli*, *Xanthomonas oryzae*, *Aspergillus fumigatus*, and *Xanthomonas campestris* significantly increased in the SBI-treated high-manure treatment (T-HM+; Fig. 2a). At the maturation stage of the low cow manure addition group, except for *Mycobacterium tuberculosis, Borreliella burgdorferi, Listeria monocytogenes*, and *Botrytis cinerea,* the abundance of other pathogens in SBI-treated compost (M-LM+) exceeded that in non-SBI compost (M-LM; Fig. 2a). Conversely, under the high cow manure condition, the abundance of major pathogenic microorganisms, except for *Xanthomonas oryzae, Klebsiella pneumoniae, Xanthomonas campestris,* and *Ralstonia solanacearum* was lower in SBI-treated compost compared to non-SBI-treated compost (Fig. 2a).

Among the pathogens, four taxa exhibited significant decreases during co-composting (Fig. 2b). *Fusarium graminaceum*, a member of the *Gibberella* ascomycetes, known for causing wheat scab, showed a significant decrease after composting, indicating reduced threats to human health given its potential to produce mycotoxins [51,52]. Additionally, two widely spread plant pathogenic fungi, *Magnaporthe oryzae* and *Botrytis cinerea* (both belonging to the phylum Ascomycota), were significantly reduced after co-composting ($P < 0.05$; Table S8), underscoring the co-composting's role in controlling crop diseases and mitigating potential economic losses. *Magnaporthe oryzae*, a major rice pathogen causing rice blast, annually leads to 10–30 % yield reductions [53,54]. Similarly, *Botrytis cinerea* infects over 200 plant species, including tomatoes, strawberries, and cucumbers, posing significant threats to plant growth and development [55]. Furthermore, the animal pathogen *Borreliella burgdorferi*, transmitted from ticks to humans and resulting in long-term organ dysfunction, also experienced substantial reduction after co-composting (Fig. 2b). Our results underscored the presence of diverse pathogenic types and their widespread existence in mature composts, posing potential pathogenicity risks [56–58]. Except for *Borreliella burgdorferi*, the other three pathogens (*Fusarium graminaceum, Magnaporthe oryzae*, and *Botrytis cinerea*) did not significantly differ between SBI and non-inoculant treatments (Fig. 2b), possibly due to the stronger influence of elevated temperature on pathogenic bacteria activities compared to that of exogenous bacterial inoculants, and fungi are less likely to survive the thermophilic phase. In manure-involved aerobic composting, SBI had no to little effect on potential pathogens (or the most abundant ones), high temperature primarily drives pathogen inactivation [59].

3.3. Changes in antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) during composting

Resistance to sulfonamides primarily involves genes *sul1, sul2*, and *sul3* encoding dihydropteroate synthases insensitive to sulfonamides [60]. During composting, genes associated with sulfonamides (such as *sul1/sul2*) exhibited increased abundance, especially at the thermophilic stage (Fig. 3a). This trend aligns with previous findings of highly enriched sulfonamide resistance genes during composting [61,62]. The thermophilic stage of composting (≥55 °C) is recognized as critical for antibiotic and ARG degradation [63]. While, for sulfonamide-resistant genes (*sul1* and *sul2*), they correlate positively with composting temperature [64]. Thus, the observed increase in *sul1* and *sul2* may relate to advantageous conditions for the proliferation and dissemination of these genes under aerobic thermal conditions [65]. Additionally, antibiotic selection pressure and horizontal transfer of dihydropteroate reductase coding genes via integrons may contribute to their increased abundance [66].

While the majority of highly abundant ARGs (18/29) exhibited decreasing trends during the thermophilic and maturation stages, ARGs associated with sulfonamide, macrolide, and vancomycin (*macA, smeD, sul2, ermF, floR, sul1,* and ABC transporter) increased during composting (Fig. 3a). This diverse trend in ARG changes is consistent with studies on livestock manure co-composting experiments [67,68], possibly due to diverse potential host bacteria for the same gene. For example, *tetA, tetX*, and *sul2* can be hosted by Actinobacteria, Bacteroides, Proteobacteria, and Firmicutes during composting [69]. In our study, *sul2* exhibited a high positive correlation with Firmicutes (Fig. S1), suggesting a potential hosting relationship between *sul2* and Firmicutes. Variations in host bacterial abundance with compost temperature may enhance the adaptability of these genes to different temperatures (Table. S9). Elevated resistance genes (*sul1* and *sul2*) introduced into the environment can alter microbial community structures, disrupting ecosystem stability and function and posing risks to human health through the food chain [70].

Comparing the elimination of abundant ARGs (top 6, with a relative abundance >90 %), we found that the low manure addition treatment exhibited a higher eliminating rate (ER) of abundant genes than the high manure treatment (averaged 88.8 % vs. 79.6 %). Among them, the top 4 genes (including *emrB*, *TolC*, *mexT*, and *rosB*) had an ER exceeding 90 %. This aligns with other studies indicating that aerobic composting is effective at eliminating abundant ARGs [61,62]. After inoculation with SBI, 11 types of ARGs in pig manure compost were all reduced, and the reduction effect of ARGs in the treatment with microbial inoculant was

Table 1
Abundant ARGs (Top 10 in relative abundance) and their elimination rates (ER in %) in composts with various manure addition and *Streptomyces-Bacillus* Inoculant (SBI). ER refers to the calculation of the removal rate of ARGs in mature composts compared with the initial composting stage. Statistics of gene abundance among treatments were included in Table S9.

Treatments	Eliminating rate (ER, in %) of the top 6 abundant ARGs						average ER (%)
Low manure (10 %)	***emrB***	***TolC***	***mexT***	***rosB***	***mdtB***	***acrB***	
Amount	98.4	97.3	95.5	90.6	87.3	63.4	88.8
High manure (20 %)	***emrB***	***TolC***	***mexT***	***rosB***	***mexW***	***vanR***	
Amount	98.1	93	86.7	79.9	74.1	45.7	79.6
SBI inoculant +	***cpxR***	***mexT***	***macB***	***mexW***	***bacA***	***mexF***	
Low manure	97.6	81.5	73.5	51.9	43.2	34.2	63.7
SBI inoculant +	***cpxR***	***mexT***	***macB***	***mexW***	***bacA***	***mexF***	
High manure	83.1	95.8	5.7	88.8	39.5	38	58.5

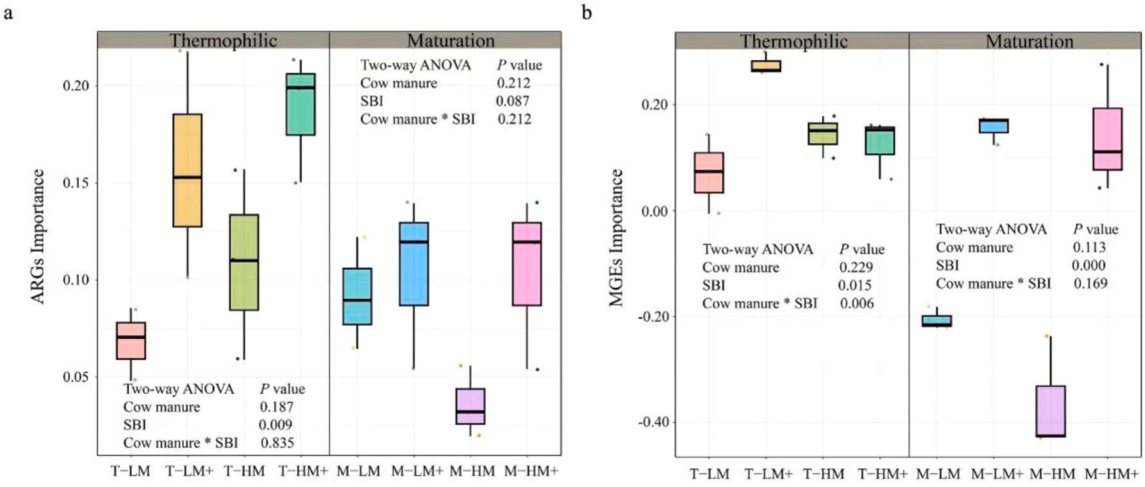

Fig. 4. Importance of antibiotic resistance genes (ARGs; a) and mobile genetic elements (MGEs; b) under the cow manure and *Streptomyces-Bacillus* Inoculants (SBI) addition during composting. Full meaning of abbreviations are the same than those presented in Fig. 1 legend.

significantly higher than that in the no-inoculant group [71]. This reduction may result from the high temperature generated during composting (Table. S9), which degrades antibiotics and hormones, thereby reducing selective pressure on ARGs and decreasing their abundance [72]. Diehl and LaPara [73] found that removal rates and efficiency of tetracycline-related genes (i.e., *tetA, tetO, tetW, tetX*, and *tetL*) increased with temperature, suggesting ARGs degradation may relate to host bacteria succession under temperature changes [74].

After applying the SBI, the average elimination rate of dominant ARGs was higher in the low-manure (averaged 63.7 %) than high-manure (58.5 %) treatments (Table 1), consistent with non-SBI treatments, indicating initial manure amount as the key factor affecting ARGs elimination, given livestock manure's high ARG reservoir [75–77]. In our study, the abundant ARGs detected in mature composts differed from those in non-SBI treatments after applying the SBI. This variation in dominant ARG types induced by the SBI (Table 1) may result from the mechanisms for the selection of ARGs in thermophilic bacteria, as indicated by the difference between the *Bordetella*-dominance of SBI treatments and the *Phenylobacterium*-dominated non-SBI treatments (Fig. 1b).

Mobile genetic elements (MGEs) showed various changes under the application of SBI and/or varied manure amount (Fig. 3b). Insertion Sequences (IS) and transposase constitute the predominant fraction (averaging ~83.7 %) across all treatments, playing indispensable roles in MGE transposition [78,79]. For example, IS, short DNA sequences, are ubiquitous in bacterial and archaeal genomes. Transposase enzymes recognize and cleave DNA around IS, facilitating their insertion into new genomic loci, thereby inducing recombination, repair, and mutation [80,81]. The relative abundance of transposase declined during the thermophilic and maturation stages of composting, consistent with the change observed in the relative abundance of the top 6 ARGs (Fig. 3a). This consistency may stem from the concurrent reduction of ARGs and MGEs during the elevated temperatures of the thermophilic stage [72, 82], as high temperatures diminish MGE abundance (e.g., integrase and transposase), thereby impeding horizontal gene transfer of ARGs. However, at the maturation stage, SBI showed a significant effect on the variation of MGEs (Fig. 4b). The relative abundance of certain MGEs (*IS*, *integrase*, *istA*, *qacEdelta*, *tniA*, and *transposase* in the low cow manure addition group; *IS*, *ISCR*, *integrase*, *qacEdelta*, *tniB*, and *transposase* in the high cow manure addition group) significantly increased under the application of SBI (Table S9, $P < 0.05$). This effect might be attributed to the SBI-induced bacterial community composition change (such as *Sphingobacterium*, *Pseudoxanthomonas*, *Bordetella* etc.; Table S7), and the significant correlationships between MGEs and bacterial community under the SBI application (Fig. S3). Consequently, more potential host cells are created, promoting gene exchange among microorganisms. Given that MGEs typically possess multiple copies at diverse genomic locations, they facilitate sequence exchange among identical or related fragments, particularly horizontal gene transfer [83,84].

Based on the Two-way ANOVA results, we found that the addition of SBI had significant effects on the changes of ARGs and MGEs, particularly during the thermophilic stage (Fig. 4). This effect persisted through the mature stage, as evidence by its significant influence on the MGEs ($P < 0.01$) and its influence on ARGs which approached high significance ($P = 0.087$). In contrast, the amount of cow manure (Low, 10 % vs. High, 20 %) exerts a weak impact on the variation of MGEs and ARGs, at both the thermophilic ($P > 0.05$) and mature stages ($P > 0.05$; Fig. 4). The interactive effect of SBI and manure amount significantly affected the change in MGEs during the thermophilic stage ($P = 0.006$; Fig. 4b).

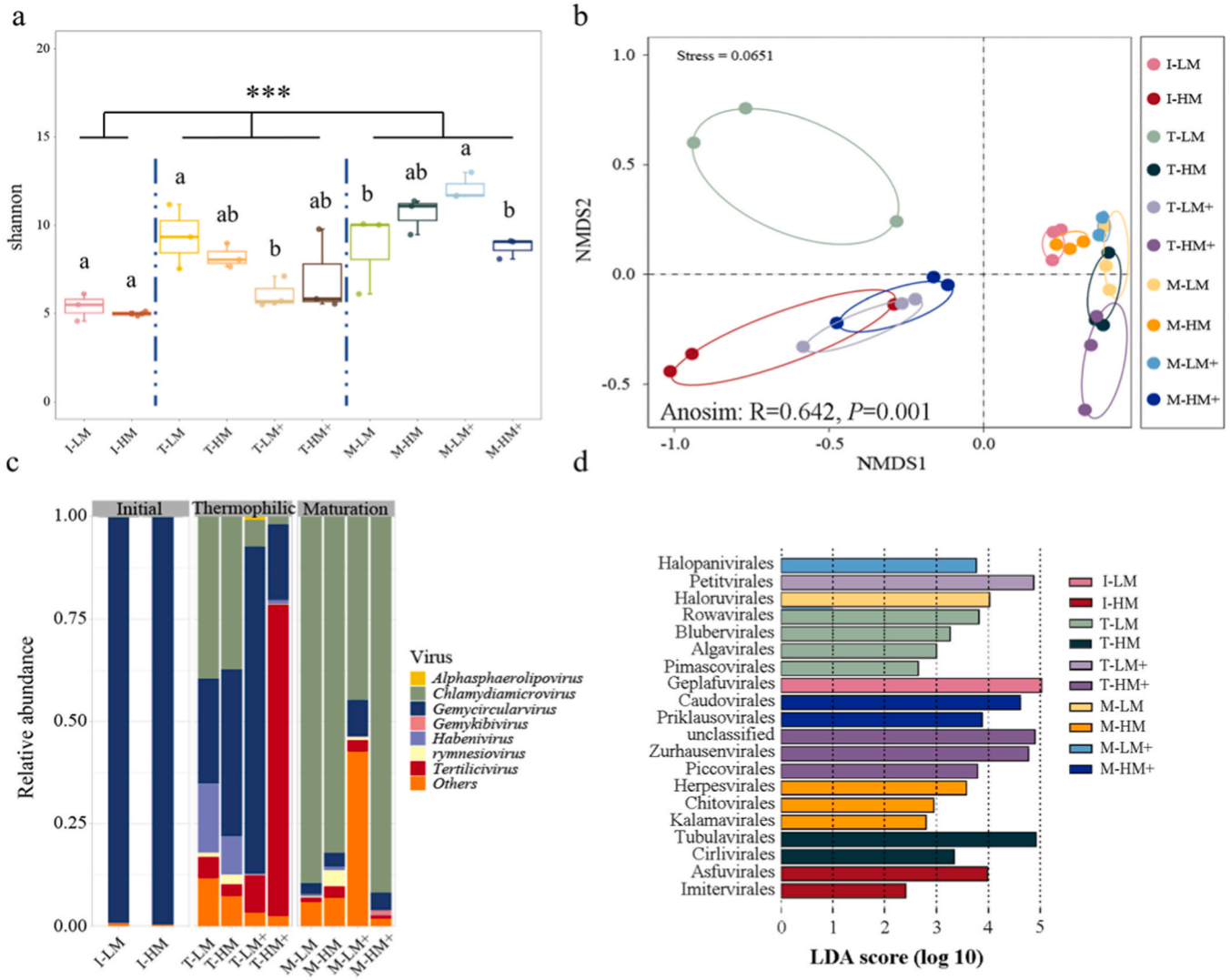

Fig. 5. Variations in virus community during composting. (a) α-diversity indices community structure (b), composition (at the genus level; c), and the biomarkers of various treatments (d). In Fig. a, different lowercase letters among treatments indicate significant differences at $P < 0.05$, with asterisks denoting significance among composting stages (*** $P < 0.001$). For sub-Fig. b, community compositions were depicted by unweighted non-metric multi-dimensional scaling plots (NMDS) of pairwise Bray-Curtis community distance across treatments. Treatment descriptions correspond to those outlined in Table S11. In Fig. d, discriminating virus genera were selected based on the histogram of linear discriminant analysis with an LDA score > 2.0 and $P < 0.05$.

3.4. Changes of virus community during composting

Significant variations were observed in viral Shannon diversity across composting stages (Fig. 5a; $P < 0.001$), accompanied by notable differences in virus community composition (Anosim, R = 0.642, $P = 0.001$), as evidenced by their distinct separation along the NMDS axis 1 (Fig. 5b). Thus, a detailed investigation into viral composition was warranted. During the initial composting stage, the relative abundance of highly prevalent (>95 % in relative abundance) *Gemycircularvirus,* then experienced a significant decrease in the following stages (Table S11). *Gemycircularvirus*, a class of circular DNA viruses, is frequently encountered in environmental fungi, particularly in soil and water environments [85,86]. The high abundance of a fungal virus in the initial stages may support a high environmental fungi proportion available due to the corn straw decomposition abilities of fungal saprotrophs, that are reduced in the thermophilic phase. *Gemycircularvirus* was higher in the high manure addition scenario (I-HM) compared to the low manure addition scenario (I1; Fig. 5c and Table S11). The elevated abundance in 'I-HM' treatment could be attributed to the increased cow manure amount fostering increased virus abundance [87].

At the thermophilic stage, the relative abundance of *Habenivirus* and *Tertilicivirus* increased but significantly declined at the maturation stage, with *Chlamydiamicrovirus* emerging as the dominant genus (Fig. 5c). Both *Habenivirus* and *Tertilicivirus* belong to the filamentous bacteriophage family. While viral diversity remained relatively stable in high manure addition treatments, the application of SBI in low manure conditions led to a significant decrease in viral Shannon index (observed in T-LM+ with SBI compared to T-LM without SBI) during the thermophilic stage. This reduction was consistent with significant alterations in virus community structure ('T-LM+' vs. 'T-LM', Anosim, R = 0.642, $P \leq 0.001$; Fig. 5b).

Conversely, during the maturation stage, viral diversity increased in the SBI treatment compared to the non-SBI treatment, mirroring the change in virus community structure ('M-LM+' vs. 'M-LM', Anosim, R=0.642, $P \leq 0.001$; Fig. 5b). No significant differences in Shannon index were observed between SBI and non-SBI treatments with high manure addition during the thermophilic and maturation stages (Fig. 5a). SBI intervention notably reduced the diversity of the virus community during the high-temperature stage, possibly due to co-evolution between host bacteria and viruses, particularly

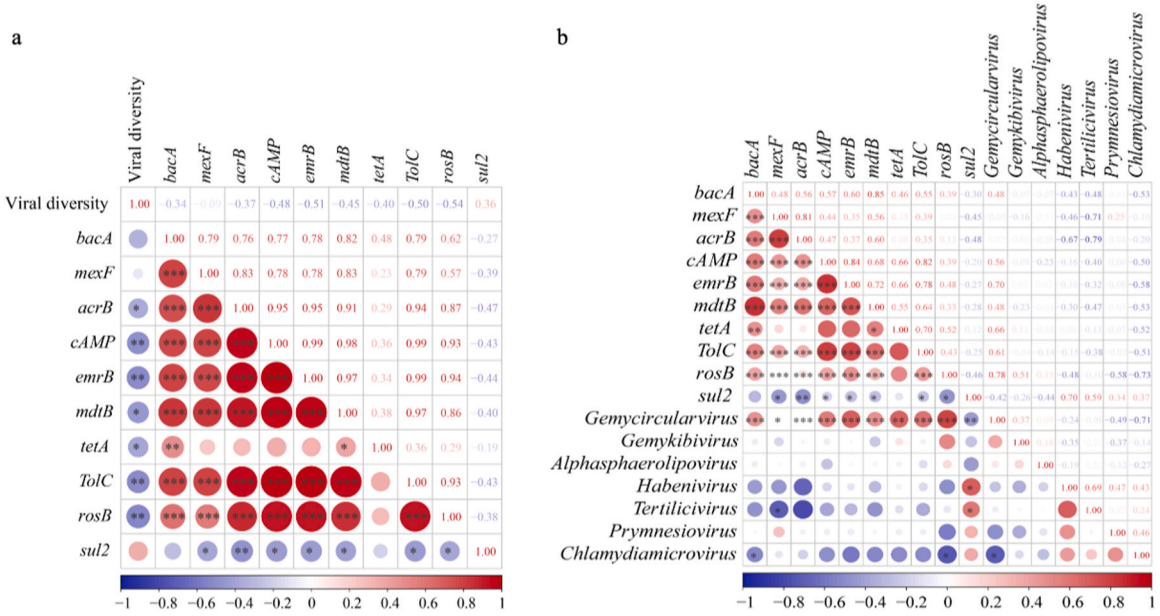

Fig. 6. Correlation analysis between antibiotic resistance genes and virus community (a, viral Shannon diversity; b, dominant viral taxa at the genus level) during composting. Asterisks indicate significant correlations between ARGs and viruses (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$). The red and blue circles indicate positive and negative correlations, respectively. The larger the circle, the stronger the correlation.

bacteriophages, leading to significant bacterial cell death [88,89]. Bacteriophages can alter bacterial competition dynamics, facilitate horizontal gene transfer among bacteria [90], and induce damage or demise of host bacteria [91–93]. We utilized Linear Discriminant Analysis Effect Size (LEfSe) to identify viral biomarkers, and the results revealed 20 virus order biomarkers across all treatments, with *Geplafuvirales* being the most prominent biomarkers, displaying the highest LDA score (LDA score = 5.02).

Viral Shannon diversity showed significant negative relationships with most abundant ARGs (Fig. 6a). Specifically, the viral diversity was negatively correlated with the *acrB* (r = –0.37), *cAMP* (r = –0.48), *emrB* (r = –0.51), *mdtB* (r = –0.45), *tetA* (r = –0.40), *TolC* (r = –0.50), and *rosB* (r = –0.54) (Fig. 6a).

Within ARGs, most of them formulated significant associations, except for the *sul 2*, that was negatively linked with other ARGs (Fig. 6a).

Among viral members, the *Gemycircularvirus* exhibited the most notable positive correlations with major ARGs, including *bacA* (r = 0.48), *cAMP* (r = 0.56), *emrB* (r = 0.70), *mdtB* (r = 0.48), *TolC* (r = 0.61), and *rosB* (r = 0.78) (Fig. 6b). Other three viral taxa also had significant relationships with certain ARGs. For instance, the relative abundance of *Habenivirus* (r = 0.70) and *Tertilicivirus* (r = 0.59) was significantly positively correlated with the relative abundance of *sul2*. *Chlamydiamicrovirus* was negatively correlated with *bacA* (r = –0.53) and *rosB* (r = –0.73), respectively (Fig. 6b).

3.5. The influence of bacteria, virus, and MGEs on ARGs during the co-composting of cow manure and agricultural biowaste

The interplay of bacteria, viruses, and mobile genetic elements (MGEs) during the co-composting of cow manure and agricultural biowaste significantly impacts antibiotic resistance genes (ARGs).

Mantel analysis revealed significant positive correlations within MGEs, and even stronger correlations within ARGs (Fig. 7). With the exception of *sul 2*, the 8 abundant ARGs exhibited negative correlationships with MEGs (Fig. 7). The application of SBI strongly influenced the interactions among bacteria, viruses, MGEs, and ARGs (Fig. 7a). Notably, SBI significantly strengthened the associations between bacteria and MGEs (particularly for *IS*, *ISCR*, and *qacEdelta*; Fig. 7a), while it reduced the impact of viruses on ARGs, as indicated by the non-significant associations with *emrB*, *mdtB*, *TolC*, and *sul 2* (Fig. 7a). In the case of cow manure, an increased initial manure amount accelerates the establishment of significant relationships between bacteria and MGEs (such as *IS* and *ISCR*), as well as between bacteria and ARGs (*emrB* and *mdtB*) (Fig. 7d).

Redundancy analysis (RDA) revealed that variations in bacteria, viruses, and MGEs collectively accounted for two-thirds (67 %) of the total variation in ARGs (Fig. 8a). Specifically, RDA1 and RDA2 explained 66.9 % of the total variation in ARGs. Notably, among MGEs, insertion sequences (IS), short, free-moving DNA sequences prevalent in bacterial genomes, emerged as the primary factors driving ARGs variation during composting (Fig. 8a). Within bacterial communities, Proteobacteria and Firmicutes emerged as key taxa influencing ARGs variation (Fig. 8a). This could be attributed to their roles as primary hosts of potential ARGs and their dominance during the thermophilic stage, a crucial phase for ARGs elimination [94]. The decreased abundance of ARGs host bacteria likely limits the horizontal transfer of ARGs [95].

The relationships among bacteria, viruses, MGEs and ARGs were elucidated using PLS-PM analysis (Fig. 8). The analysis revealed that viruses have a significant positive effect on ARGs (r = 0.43, $P < 0.001$), but a direct negative effect on MGEs (r = –0.17). Conversely, bacteria exert a direct negative effect on MGEs (r = –0.74, $P < 0.001$) and ARGs (r = –0.71, $P < 0.001$) (Fig. 8b). Regarding the individual inoculant effect, it was found that the application of SBI inoculant can: i) transform the effect of viruses on ARG, from positive (r = 0.29, $P < 0.05$; without SBI; Fig. 8d) to negative (r = –0.44; Fig. 8c); and ii) weaken the influence of bacterial community on MGEs (as indicated by the decreased path coefficient form 0.84 to 0.45) and ARG (path coefficient decreased from 0.88 to 0.48) (Fig. 8c, d). For cow manure, it was observed that an increased initial manure amount strongly shifted the direct influence of virus on ARG, form positive (r = 0.33, $P < 0.05$; Fig. 8e) to negative (r = –0.62, $P < 0.01$; Fig. 8f). The negative correlation between ARGs and bacteria (Fig. 8b, r = –0.71, $P < 0.001$), is consistent with the elimination of dominant ARGs (Table 1), and the reduced horizontal ARGs transfer during composting [88]. Furthermore, both bacterial and viral diversities and community structure have shown significant associations with changes of ARGs during composting (Fig. S3), which may be related to the close association between viruses and bacteria [93], and their significant influence on ARGs dynamics during composting

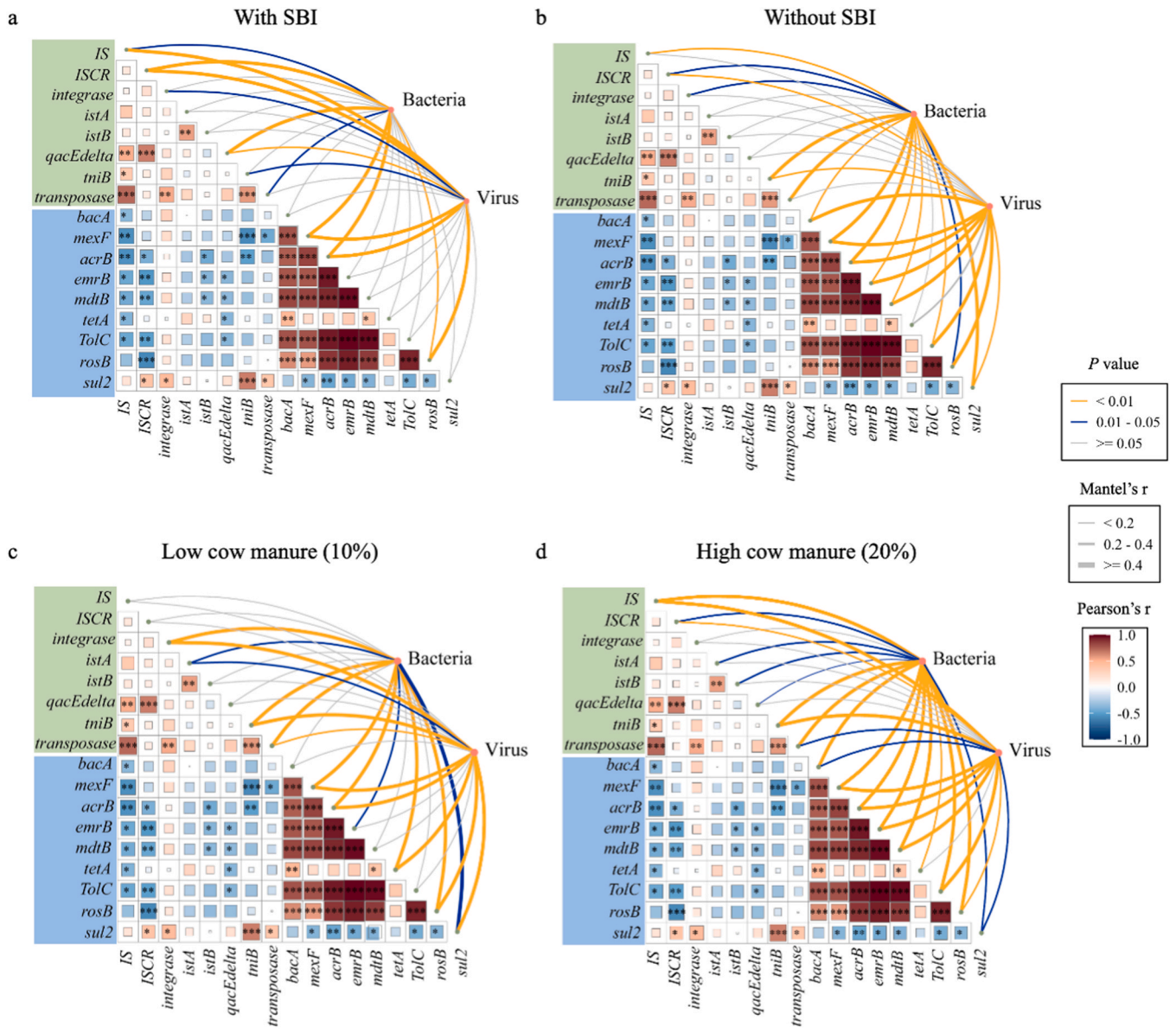

Fig. 7. Mantel test analysis of the bacterial and viral communities, and their associations with antibiotic resistance genes (ARGs, shown in blue shadow) and mobile genetic elements (MGEs, shown in green shadow) in different treatments [with (a) and without (b) SBI (*Streptomyces-Bacillus* 1:1) inoculant], and different cow manure amount [10 % vs. 20 % w/w in c) and d)] during composting. Microbial dataset used for mantel analysis was the first principal component (PC 1) value of community structure at the genus level. Color columns within the squares on the right, denote Pearson's correlation coefficients. Line thickness and color represent correlation coefficient and significance levels, respectively according to squares on the right. Asterisks inside the squares in the figure body indicate significance levels (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

[68].

Viruses now being recognized as important carriers and disseminators of ARGs [95]. Viruses, particularly phages, play crucial roles in specific biochemical reactions within compost by infecting host microorganisms (bacteria and fungi). Viral community exerted a predominantly positive effect on ARGs (Fig. 8b), likely due to the crucial role of viruses, particularly phages, as carriers and transmitters of ARGs. For instance, ARGs-carrying phages were found to proliferate within *Escherichia coli* cells in environmental virome analyses [96]. Moreover, phages can enhance gene exchange between bacterial species and mediate antibiotic delivery, thereby increasing local drug concentrations [97,98]. In our study, *Gemycircularvirus* emerged as a key taxon influencing ARGs variation within the viral community, with its abundance decreasing significantly during the thermophilic stage, likely due to the temperature's role in reducing the abundance of virus-host interactions and inhibiting ARGs-carrying virus amplification [6]. During hype-thermophilic composting, lytic bacteriophages can drive the ecological succession of mild thermophilic bacteria, thereby affecting bacterial diversity [99]. Moreover, viruses can induce host bacteria to resist environmental stress by encoding auxiliary metabolic genes [100, 101]. Phages facilitate the transfer of host genome segments and MGEs through generalized transfer, a widespread mechanism of gene transfer in bacteria [102].

3.6. Main processes involve during the transfer of resistance genes between bacteria

The transfer of resistance genes between bacteria occurs primarily through three mechanisms: transformation, transduction and conjugation [103,104]. These mechanisms provide a variety of pathways for the transmission of resistance genes between bacteria: i) Through transformation (Fig. 9), bacteria can absorb external DNA fragments from the

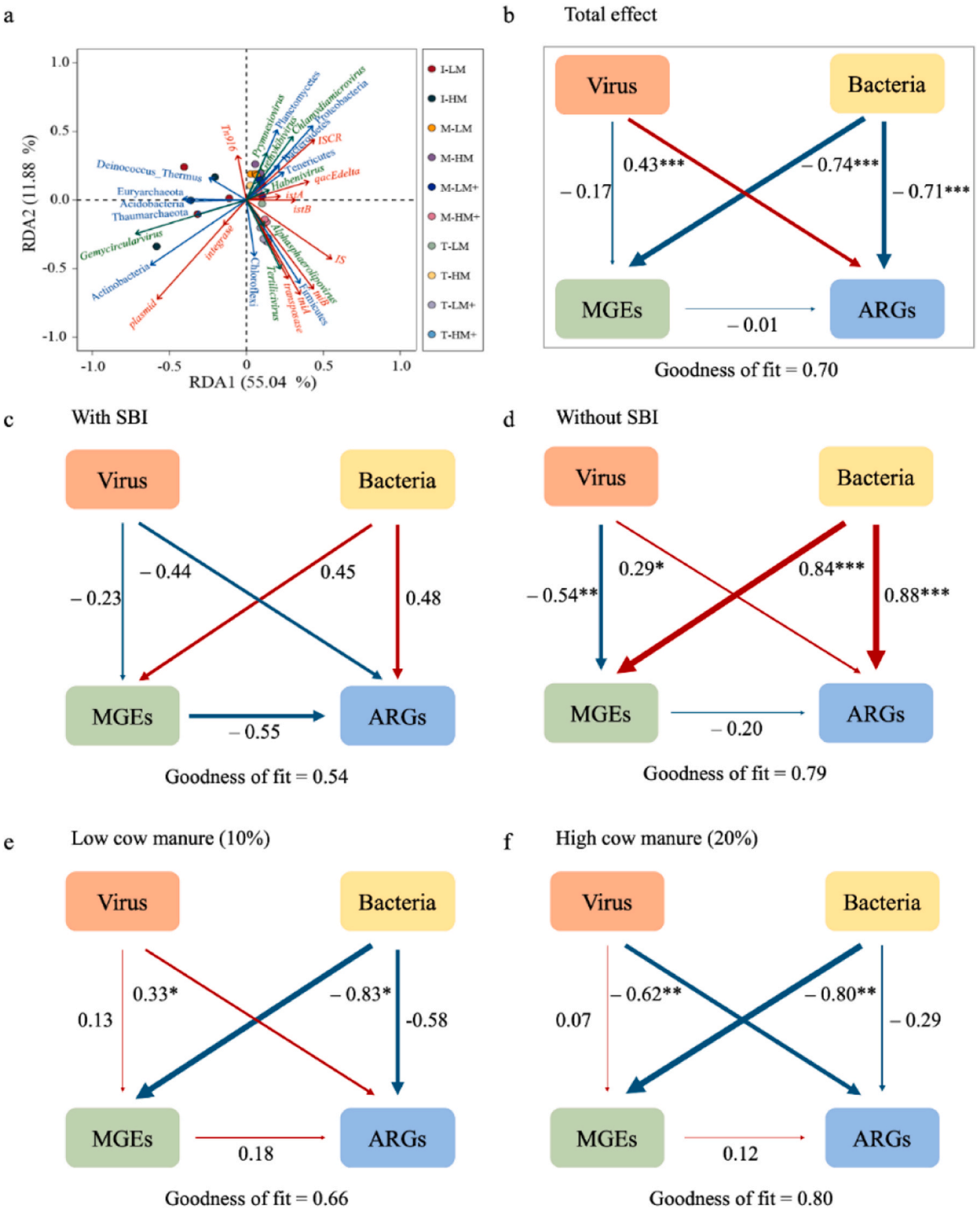

Fig. 8. Redundancy analysis (RDA, a) and overall partial least squares path modelling (PLS-PM) analysis (b) of the relationships between bacteria, virus, MGEs and ARGs in different treatments [with (c) and without (d) SBI (*Streptomyces-Bacillus* 1:1 inoculant)], and different cow manure amount [10 % (e) vs. 20 % (f) w/w] during composting. Bacterial and viral dataset used for mantel analysis was the first principal component (PC 1) value of community structure at the genus level. Red and blue arrows indicate positive and negative effects ($P < 0.05$), respectively. The numbers on the arrows represent standardized path coefficients. The width of the arrows is proportional to the strength of the path coefficients. Models were assessed by a goodness of fit statistic.

environment, which may include resistance genes [105].

Once the external DNA is integrated into its genome, the recipient bacteria acquire new resistant traits; ii) Transduction involves the transfer of DNA fragments mediated by bacteriophages (Fig. 9). Previous studies have demonstrated that *E. coli* can be transformed by plasmid DNA under natural conditions, suggesting that *E. coli* can absorb DNA and that transformation can thus contribute to the ARGs transmission [106,107]; and iii) During bacteriophage infection, the viral genetic material is injected into the host bacterium for replication. In this process, the host bacterial DNA can be mistakenly packaged into the viral protein shell and transferred to other bacteria in subsequent infections. If this DNA contains resistance genes, the recipient bacteria can acquire the corresponding resistance [108–110]. Transduction can occur naturally and ubiquitously, playing a significant role in resistance transfer, potentially beyond our current understanding. Studies have shown that transduction drives the genetic diversity of intestinal colonizing *E. coli*

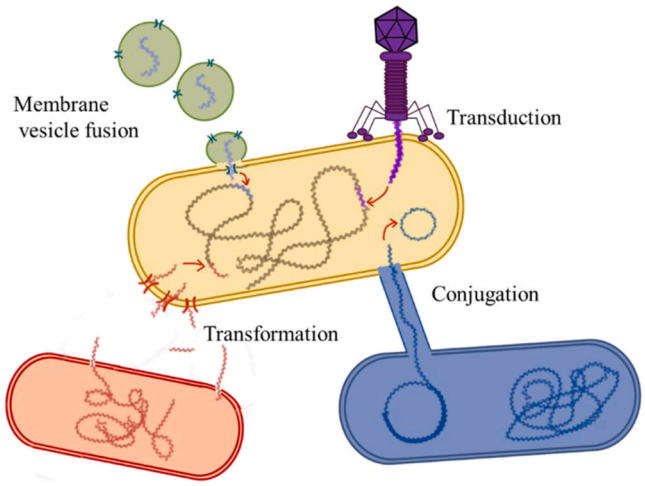

Fig. 9. A conceptual graph showing the main processes involve during the transfer of resistance genes between bacteria.

strains in mice, promoting the emergence of resistance in intestinal bacteria [111]; and iii) Conjugation is a direct method of DNA transfer between bacterial cells [112]. During this process, two bacterial cells are connected through an extracellular connection pipe called a sex connector (Fig. 9), allowing one bacteria (the donor) to transfer its DNA to another (the recipient). This transfer process can include plasmids, which are small DNA molecules carrying resistance genes [113]. These findings emphasize the strong association between the virome and ARGs, highlighting the importance of considering viral activity in future manure composting practices."

4. Conclusion

The utilization of livestock manure in agricultural practices, while beneficial for soil fertility and crop productivity, also presents inherent risks associated with pathogen proliferation and the enrichment and transmission of ARGs. To further explore the composting effects on the fate of ARGs, bacteria and viruses, and to reveal the behavior of the three, in our investigation, the introduction of composite microbial inoculants SBI aimed to enhance decomposition efficiency and expedite the degradation of harmful substances. We observed significant alterations in the bacterial and viral community structures during composting, consequently impacting the distribution of ARGs. Redundancy analysis identified ISCR, Proteobacteria, and qacEdelta as pivotal drivers of ARGs variation, suggesting that changes in bacterial community composition, govern the horizontal transfer of ARGs. Additionally, the initial amount of cow manure influenced the efficacy of SBI on viruses, with a significant reduction observed in viral Shannon index at the thermophilic stage under low manure conditions. Virus diversity and mobile genetic elements exerted positive effects on ARGs, with virus diversity exhibiting a stronger influence. Considering viruses non-negligible role in spreading ARGs, low manure amount in combination with SBI inoculum is an important method for the hygienization of the compost. By trade-offs between pathogens and resistome, aerobic co-composting stands out as an environmentally sustainable approach capable of mitigating these risks by effectively eliminating pathogens and preventing ARGs from entering soils.

Environmental implication

Antibiotic resistance genes (ARGs) and virous-associated ARGs are perceived as globally emerging bio-pollutants. They have been widely concerned in livestock manure recycling. Microbial inoculants are attractive solutions to promote compost mature but their roles in ARGs elimination are not clear. We examined the influence of inoculants on viral community, pathogen, ARGs, and their relationships. The results indicate microbial inoculant can accelerate compost's thermophilic stage, favoring the decrease of viral abundance; and viral diversity exerted a predominant effect on ARGs elimination. These findings provide a new idea for mitigating the risk of ARGs in livestock manure composting.

CRediT authorship contribution statement

Xiaofei Shi: Resources, Investigation, Conceptualization. **Parag Bhople:** Methodology, Investigation, Data curation. **Yimei Huang:** Writing – review & editing, Resources. **Katharina Maria Keiblinger:** Writing – review & editing, Validation. **Ziyan Zhou:** Writing – original draft, Software, Formal analysis, Data curation. **Dong Liu:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Fuqiang Yu:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Shimei Yang:** Validation, Methodology, Investigation, Data curation.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.135355.

References

[1] Qiao, L., Liu, X., Zhang, S., et al., 2021. Distribution of the microbial community and antibiotic resistance genes in farmland surrounding gold tailings: a metagenomics approach. Sci Total Environ 779, 146502.
[2] Debroas, D., Siguret, C., 2019. Viruses as key reservoirs of antibiotic resistance genes in the environment. ISME J 13 (11), 2856–2867.
[3] Yue, Z., Zhang, J., Zhang, J., et al., 2023. Combined virome analysis and metagenomic sequencing to reveal the viral communities and risk of virus–associated antibiotic resistance genes during composting. J Hazard Mater 459.
[4] Sarmah, A.K., Meyer, M.T., Boxall, A.B., 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.
[5] Liao, H., Lu, X., Rensing, C., et al., 2018. Hyperthermophilic composting accelerates the removal of antibiotic resistance genes and mobile genetic elements in sewage sludge. Environ Sci Technol 52 (1), 266–276.

[6] Zhang, M., He, L.Y., Liu, Y.S., et al., 2020. Variation of antibiotic resistome during commercial livestock manure composting. Environ Int 136, 105458.
[7] Wang, J., Ben, W., Zhang, Y., et al., 2015. Effects of thermophilic composting on oxytetracycline, sulfamethazine, and their corresponding resistance genes in swine manure. Environ Sci-Proc IMP 17 (9), 1654–1660.
[8] Duan, M., Zhang, Y., Zhou, B., et al., 2019. Changes in antibiotic resistance genes and mobile genetic elements during cattle manure composting after inoculation with Bacillus subtilis. Bioresour Technol 292, 122011.
[9] Hu, T., Wang, X., Zhen, L., et al., 2019. Effects of inoculation with lignocellulose-degrading microorganisms on antibiotic resistance genes and the bacterial community during co-composting of swine manure with spent mushroom substrate. Environ Pollut 252 (Pt A), 110–118.
[10] Liu, Y., Zheng, L., Cai, Q., et al., 2021. Simultaneous reduction of antibiotics and antibiotic resistance genes in pig manure using a composting process with a novel microbial agent. Ecotoxicol Environ Saf 208, 111724.
[11] Zhou, Z., Shi, X., Bhople, P., et al., 2024. Enhancing C and N turnover, functional bacteria abundance, and the efficiency of biowaste conversion using Streptomyces-Bacillus inoculation. J Environ Manag 358, 120895.
[12] Liao, H., Liu, C., Ai, C., et al., 2023. Mesophilic and thermophilic viruses are associated with nutrient cycling during hyperthermophilic composting. ISME J 17 (6), 916–930.
[13] Debroas, D., Siguret, C., 2019. Viruses as key reservoirs of antibiotic resistance genes in the environment. ISME J 13, 2856–2867.
[14] Ma, B., Wang, Y., Zhao, K., et al., 2024. Biogeographic patterns and drivers of soil viromes. Nat Ecol Evol.
[15] Cai, L., Feng, C., Xie, L., et al., 2022. Ecological dynamics and impacts of viruses in Chinese and global estuaries. Water Res 226.
[16] Liang, X., Wagner, R.E., Zhuang, J., et al., 2019. Viral abundance and diversity vary with depth in a southeastern United States agricultural ultisol. Soil Biol Biochem 137.
[17] Yue, Z., Zhang, J., Zhang, J., et al., 2023. Combined virome analysis and metagenomic sequencing to reveal the viral communities and risk of virus–associated antibiotic resistance genes during composting. J Hazard Mater 459.
[18] Zhang, W., Yu, C., Yin, S., et al., 2023. Transmission and retention of antibiotic resistance genes (ARGs) in chicken and sheep manure composting. Bioresour Technol 382, 129190.
[19] Xu, Z., Qi, C., Zhang, L., et al., 2021. Regulating bacterial dynamics by lime addition to enhance kitchen waste composting. Bioresour Technol 341.
[20] Urban, M., Cuzick, A., Rutherford, K., et al., 2017. PHI-base: a new interface and further additions for the multi-species pathogen–host interactions database. Nucleic Acids Res 45 (D1), D604–D610.
[21] Yang, Y., Jiang, X., Chai, B., et al., 2016. ARGs-OAP: online analysis pipeline for antibiotic resistance genes detection from metagenomic data using an integrated structured ARG-database. Bioinformatics 32 (15), 2346–2351.
[22] Li, B., Yang, Y., Ma, L., et al., 2015. Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. ISME J 9 (11), 2490–2502.
[23] Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30 (15), 2114–2120.
[24] Li, D., Luo, R., Liu, C.M., et al., 2016. MEGAHIT v1.0: A fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods 102, 3–11.
[25] Li, H., Durbin, R., 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25 (14), 1754–1760.
[26] Nayfach, S., Camargo, A.P., Schulz, F., et al., 2021. CheckV assesses the quality and completeness of metagenome-assembled viral genomes. Nat Biotechnol 39 (5), 578–585.
[27] Guo, J., Bolduc, B., Zayed, A.A., et al., 2021. VirSorter2: a multi-classifier, expert-guided approach to detect diverse DNA and RNA viruses. Microbiome 9 (1), 37.
[28] Zhang, L., Niu, J., Lu, X., et al., 2023. Dosage effects of organic manure on bacterial community assemblage and phosphorus transformation profiles in greenhouse soil. Front Microbiol 14.
[29] Neher, D.A., Weicht, T.R., Bates, S.T., et al., 2013. Changes in bacterial and fungal communities across compost recipes, preparation methods, and composting times. PLOS ONE 8 (11), e79512.
[30] Li, H., Li, X., Zhang, D., et al., 2023. Addition of exogenous microbial agents increases hydrogen sulfide emissions during aerobic composting of kitchen waste by improving bio-synergistic effects. Bioresour Technol 384, 129334.
[31] Zhou, L., Yang, X., Wang, X., et al., 2023. Effects of bacterial inoculation on lignocellulose degradation and microbial properties during cow dung composting. Bioengineered 14 (1), 2185945.
[32] Zhou, Z., Shi, X., Bhople, P., et al., 2024. Enhancing C and N turnover, functional bacteria abundance, and the efficiency of biowaste conversion using Streptomyces-Bacillus inoculation. J Environ Manag 358.
[33] Guo, X., Guo, W., Yang, M., et al., 2022. Effect of Bacillus additives on fermentation quality and bacterial community during the ensiling process of whole-plant corn silage. Processes 10 (5).
[34] Lu, L., Zhang, J., Peng, C., 2019. Shift of soil polycyclic aromatic hydrocarbons (PAHs) dissipation pattern and microbial community composition due to rhamnolipid supplementation. Water Air Soil Pollut 230 (5).
[35] Mehmood, M.A., Fu, Y., Zhao, H., et al., 2022. Enrichment of bacteria involved in the nitrogen cycle and plant growth promotion in soil by sclerotia of rice sheath blight fungus. Stress Biol 2 (1).
[36] Liu, C., Yao, H., Chapman, S.J., et al., 2020. Changes in gut bacterial communities and the incidence of antibiotic resistance genes during degradation of antibiotics by black soldier fly larvae. Environ Int 142.
[37] Huy, H., Jin, L., Lee, Y.-K., et al., 2013. *Arenimonas daechungensis* sp. nov., isolated from the sediment of a eutrophic reservoir. Int J Syst Evol Microbiol 63 (Pt_2), 484–489.
[38] Young, C.-C., Kämpfer, P., Ho, M.-J., et al., 2007. Arenimonas malthae sp. nov., a gammaproteobacterium isolated from an oil-contaminated site. Int J Syst Evol Microbiol 57 (12), 2790–2793.
[39] Jin, L., Kim, K.K., Im, W.-T., et al., 2007. Aspromonas composti gen. nov., sp. nov., a novel member of the family Xanthomonadaceae. Int J Syst Evol Microbiol 57 (8), 1876–1880.
[40] Zhu, J., Wang, H.-M., Zhang, Q., et al., 2017. Arenimonas alkanexedens sp. nov., isolated from a frozen soil sample. Antonie Van Leeuwenhoek 110 (8), 1027–1034.
[41] Nakayama, T., Homma, Y., Hashidoko, Y., et al., 1999. Possible role of xanthobaccins produced by Stenotrophomonas sp. strain SB-K88 in suppression of sugar beet damping-off disease. Appl Environ Microbiol 65 (10), 4334–4339.
[42] Sharma, M., Khurana, H., Singh, D.N., et al., 2021. The genus Sphingopyxis: Systematics, ecology, and bioremediation potential - a review. J Environ Manag 280.
[43] Verma, H., Dhingra, G.G., Sharma, M., et al., 2020. Comparative genomics of Sphingopyxis spp. unravelled functional attributes. Genomics 112 (2), 1956–1969.
[44] Yang, F., Feng, H., Massey, I.Y., et al., 2020. Genome-wide analysis reveals genetic potential for aromatic compounds biodegradation of sphingopyxis. BioMed Res Int 2020, 1–12.
[45] He, Y., Liu, D., He, X., et al., 2022. Characteristics of bacterial and fungal communities and their impact during cow manure and agroforestry biowaste co-composting. J Environ Manag 324, 116377.
[46] Mao, L., Kang, J., Sun, R., et al., 2024. Ecological succession of abundant and rare subcommunities during aerobic composting in the presence of residual amoxicillin. J Hazard Mater 465.
[47] Meng, L., Xu, C., Wu, F., et al., 2022. Microbial co-occurrence networks driven by low-abundance microbial taxa during composting dominate lignocellulose degradation. Sci Total Environ 845.
[48] Cao, M.-K., Guo, H.-T., Zheng, G.-D., et al., 2021. Microbial succession and degradation during kitchen waste biodrying, highlighting the thermophilic phase. Bioresour Technol 326, 124762.
[49] Muhammad, T., Jiang, C., Li, Y., et al., 2024. Impacts and mechanism of coal fly ash on kitchen waste composting performance: The perspective of microbial community. Chemosphere 350.
[50] Li, G., Zhu, Q., Niu, Q., et al., 2021. The degradation of organic matter coupled with the functional characteristics of microbial community during composting with different surfactants. Bioresour Technol 321.
[51] Juliusz, Perkowski, Jerzy, et al., 1991. Cumulation of mycotoxins in maize cobs infected withFusarium gramihearum. Mycotoxin Res.
[52] Rampersad, S., 2020. Pathogenomics and management of fusarium diseases in plants. Pathogens 9 (5).
[53] Wilson, R.A., Talbot, N.J., 2009. Under pressure: investigating the biology of plant infection by Magnaporthe oryzae. Nat Rev Microbiol 7 (3), 185–195.
[54] Baker, B., Zambryski, P., Staskawicz, B., et al., 1997. Signaling in plant-microbe interactions. Science 276 (5313), 726–733.
[55] Dean, R., Van Kan, J.A.L., Pretorius, Z.A., et al., 2012. The Top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol 13 (4), 414–430.
[56] Esperon, F., Albero, B., Ugarte-Ruiz, M., et al., 2020. Assessing the benefits of composting poultry manure in reducing antimicrobial residues, pathogenic bacteria, and antimicrobial resistance genes: a field-scale study. Environ Sci Pollut Res Int 27 (22), 27738–27749.
[57] El Hayany, B., El Fels, L., Ouhdouch, Y., et al., 2021. Fate of pathogenic microorganisms during lagooning sludge composting and exploration of bacteriophages as indicator of hygienization. Environ Technol Innov 21.
[58] Manyi-Loh, C.E., Mamphweli, S.N., Meyer, E.L., et al., 2016. An overview of the control of bacterial pathogens in cattle manure. Int J Environ Res Public Health 13 (9).
[59] Gurtler, J.B., Doyle, M.P., Erickson, M.C., et al., 2018. Composting to inactivate foodborne pathogens for crop soil application: a review. J Food Prot 81 (11), 1821–1837.
[60] Perreten, V., Boerlin, P., 2003. A new sulfonamide resistance gene (*sul3*) in *Escherichia coli* is widespread in the pig population of Switzerland. Antimicrob Agents Chemother 47 (3), 1169–1172.
[61] Chang, J., Jiang, T., Zhao, M., et al., 2019. Variation pattern of antibiotic resistance genes and microbial community succession during swine manure composting under different aeration strategies. J Chem Technol Biotechnol 95 (2), 466–473.
[62] Pan, Y., Zeng, J., Li, L., et al., 2020. Coexistence of antibiotic resistance genes and virulence factors deciphered by large-scale complete genome analysis. mSystems 5 (3).
[63] Sardar, M.F., Zhu, C., Geng, B., et al., 2021. The fate of antibiotic resistance genes in cow manure composting: shaped by temperature-controlled composting stages. Bioresour Technol 320 (Pt B), 124403.
[64] Wang, J., Ben, W., Zhang, Y., et al., 2015. Effects of thermophilic composting on oxytetracycline, sulfamethazine, and their corresponding resistance genes in swine manure. Environ Sci Process Impacts 17 (9), 1654–1660.

[65] Heuer, H., Smalla, K., 2006. Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. Enviro Microbiol 9 (3), 657–666.
[66] Flores-Orozco, D., Patidar, R., Levin, D.B., et al., 2020. Effect of mesophilic anaerobic digestion on the resistome profile of dairy manure. Bioresour Technol 315, 123889.
[67] Wang, G., Li, G., Chang, J., et al., 2021. Enrichment of antibiotic resistance genes after sheep manure aerobic heap composting. Bioresour Technol 323, 124620.
[68] Qiu, T., Wu, D., Zhang, L., et al., 2021. A comparison of antibiotics, antibiotic resistance genes, and bacterial community in broiler and layer manure following composting. Environ Sci Pollut Res Int 28 (12), 14707–14719.
[69] Zhang, Y., Zhou, J., Wu, J., et al., 2022. Distribution and transfer of antibiotic resistance genes in different soil–plant systems. Environ Sci Pollut Res 29 (39), 59159–59172.
[70] Pärnänen, K.M.M., Narciso-Da-Rocha, C., Kneis, D., et al., 2019. Antibiotic resistance in European wastewater treatment plants mirrors the pattern of clinical antibiotic resistance prevalence. Sci Adv 5 (3), eaau9124.
[71] Li, K., Cao, R., Mo, S., et al., 2020. Swine manure composting with compound microbial inoculants: removal of antibiotic resistance genes and their associations with microbial community. Front Microbiol 11, 592592.
[72] Ho, Y.B., Zakaria, M.P., Latif, P.A., et al., 2013. Degradation of veterinary antibiotics and hormone during broiler manure composting. Bioresour Technol 131, 476–484.
[73] Diehl, D.L., Lapara, T.M., 2010. Effect of temperature on the fate of genes encoding tetracycline resistance and the integrase of class 1 integrons within anaerobic and aerobic digesters treating municipal wastewater solids. Environ Sci Technol 44 (23), 9128–9133.
[74] Nakasaki, K., Hirai, H., Mimoto, H., et al., 2019. Succession of microbial community during vigorous organic matter degradation in the primary fermentation stage of food waste composting. Sci Total Environ 671, 1237–1244.
[75] Lipszyc, A., Szuplewska, M., Bartosik, D., 2022. How do transposable elements activate expression of transcriptionally silent antibiotic resistance genes? Int J Mol Sci 23 (15).
[76] Wang, C., Dong, D., Strong, P.J., et al., 2017. Microbial phylogeny determines transcriptional response of resistome to dynamic composting processes. Microbiome 5 (1), 103.
[77] Wang, K., Yin, D., Sun, Z., et al., 2022. Distribution, horizontal transfer and influencing factors of antibiotic resistance genes and antimicrobial mechanism of compost tea. J Hazard Mater 438, 129395.
[78] Khedkar, S., Smyshlyaev, G., Letunic, I., et al., 2022. Landscape of mobile genetic elements and their antibiotic resistance cargo in prokaryotic genomes. Nucleic Acids Res 50 (6), 3155–3168.
[79] Lipszyc, A., Szuplewska, M., Bartosik, D., 2022. How do transposable elements activate expression of transcriptionally silent antibiotic resistance genes? Int J Mol Sci 23 (15).
[80] Qiu, T., Huo, L., Guo, Y., et al., 2022. Metagenomic assembly reveals hosts and mobility of common antibiotic resistome in animal manure and commercial compost. Environ Micro 17 (1), 42.
[81] Shintani, M., Vestergaard, G., Milakovic, M., et al., 2023. Integrons, transposons and IS elements promote diversification of multidrug resistance plasmids and adaptation of their hosts to antibiotic pollutants from pharmaceutical companies. Environ Microbiol 25 (12), 3035–3051.
[82] Miller, J.H., Novak, J.T., Knocke, W.R., et al., 2016. Survival of antibiotic resistant bacteria and horizontal gene transfer control antibiotic resistance gene content in anaerobic digesters. Front Microbiol 7, 263.
[83] Partridge, S.R., Kwong, S.M., Firth, N., et al., 2018. Mobile genetic elements associated with antimicrobial resistance. Clin Microbiol Rev 31 (4).
[84] Zhang, Y., Xu, R., Xiang, Y., et al., 2021. Addition of nanoparticles increases the abundance of mobile genetic elements and changes microbial community in the sludge anaerobic digestion system. J Hazard Mater 405, 124206.
[85] Krupovic, M., Ghabrial, S.A., Jiang, D., et al., 2016. Genomoviridae: a new family of widespread single-stranded DNA viruses. Arch Virol 161 (9), 2633–2643.
[86] Orton, J.P., Morales, M., Fontenele, R.S., et al., 2020. Virus discovery in desert tortoise fecal samples: novel circular single-stranded DNA viruses. Viruses 12 (2).
[87] Chen, M.-L., An, X.-L., Liao, H., et al., 2021. Viral Community and Virus-Associated Antibiotic Resistance Genes in Soils Amended with Organic Fertilizers [J]. Environ Sci Technol 55 (20), 13881–13890.
[88] Engelhardt, T., Kallmeyer, J., Cypionka, H., et al., 2014. High virus-to-cell ratios indicate ongoing production of viruses in deep subsurface sediments. ISME J 8 (7), 1503–1509.
[89] Williamson, K.E., Corzo, K.A., Drissi, C.L., et al., 2013. Estimates of viral abundance in soils are strongly influenced by extraction and enumeration methods. Biol Fertil Soils 49 (7), 857–869.
[90] Canchaya, C., Fournous, G., Chibani-Chennoufi, S., et al., 2003. Phage as agents of lateral gene transfer. Curr Opin Microbiol 6 (4), 417–424.
[91] Puxty, R.J., Millard, A.D., 2023. Functional ecology of bacteriophages in the environment. Curr Opin Microbiol 71, 102245.
[92] Clokie, M.R., Millard, A.D., Letarov, A.V., et al., 2011. Phages in nature. Bacteriophage 1 (1), 31–45.
[93] Koskella, B., Hernandez, C.A., Wheatley, R.M., 2022. Understanding the impacts of bacteriophage viruses: from laboratory evolution to natural ecosystems. Annu Rev Virol 9 (1), 57–78.
[94] Liu, Q., He, X., Luo, G., et al., 2022. Deciphering the dominant components and functions of bacterial communities for lignocellulose degradation at the composting thermophilic phase. Bioresour Technol 348.
[95] Qiu, X., Zhou, G., Wang, H., 2022. Nanoscale zero-valent iron inhibits the horizontal gene transfer of antibiotic resistance genes in chicken manure compost. J Hazard Mater 422, 126883.
[96] Blanco-Picazo, P., Morales-Cortes, S., Ramos-Barbero, M.D., et al., 2023. Dominance of phage particles carrying antibiotic resistance genes in the viromes of retail food sources. ISME J 17 (2), 195–203.
[97] Yacoby, I., Benhar, I., 2008. Targeted filamentous bacteriophages as therapeutic agents [J]. Expert Opin Drug Deliv 5 (3), 321–329.
[98] Modi, S.R., Lee, H.H., Spina, C.S., et al., 2013. Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome. Nature 499 (7457), 219–222.
[99] Knowles, B., Silveira, C.B., Bailey, B.A., et al., 2016. Lytic to temperate switching of viral communities. Nature 531 (7595), 466–470.
[100] Zheng, X., Jahn, M.T., Sun, M., et al., 2022. Organochlorine contamination enriches virus-encoded metabolism and pesticide degradation associated auxiliary genes in soil microbiomes. ISME J 16 (5), 1397–1408.
[101] Brum, J.R., Hurwitz, B.L., Schofield, O., et al., 2016. Seasonal time bombs: dominant temperate viruses affect Southern Ocean microbial dynamics. ISME J 10 (2), 437–449.
[102] Penades, J.R., Chen, J., Quiles-Puchalt, N., et al., 2015. Bacteriophage-mediated spread of bacterial virulence genes. Curr Opin Microbiol 23, 171–178.
[103] Vrancianu, C.O., Popa, L.I., Bleotu, C., et al., 2020. Targeting plasmids to limit acquisition and transmission of antimicrobial resistance. Front Microbiol 11.
[104] Tao, S., Chen, H., Li, N., et al., 2022. The spread of antibiotic resistance genes in vivo model. Can J Infect Dis Med Microbiol 2022, 3348695.
[105] Yu, Z., Wang, Y., Henderson, I.R., et al., 2022. Artificial sweeteners stimulate horizontal transfer of extracellular antibiotic resistance genes through natural transformation. ISME J 16 (2), 543–554.
[106] Sun, D., 2018. Pull in and push out: mechanisms of horizontal gene transfer in bacteria. Front Microbiol 9, 2154.
[107] Hasegawa, H., Suzuki, E., Maeda, S., 2018. Horizontal plasmid transfer by transformation in Escherichia coli: environmental factors and possible mechanisms. Front Microbiol 9.
[108] Kline, K.A., Chiang, Y.N., Penadés, J.R., et al., 2019. Genetic transduction by phages and chromosomal islands: the new and noncanonical. PLOS Pathog 15, 8.
[109] Voigt, E., Rall, B.C., Chatzinotas, A., et al., 2021. Phage strategies facilitate bacterial coexistence under environmental variability. PeerJ 9.
[110] Torres-Barceló, C., 2018. The disparate effects of bacteriophages on antibiotic-resistant bacteria. Emerg Microbes Infect 7 (1), 168.
[111] Frazão, N., Sousa, A., Lässig, M., et al., 2019. Horizontal gene transfer overrides mutation in Escherichia coli colonizing the mammalian gut. Proc Natl Acad Sci U S A 116 (36), 17906–17915.
[112] Virolle, C., Goldlust, K., Djermoun, S., et al., 2020. Plasmid transfer by conjugation in gram-negative bacteria: from the cellular to the community level. Genes (Basel) 11 (11).
[113] Partridge, S.R., Kwong, S.M., Firth, N., et al., 2018. Mobile genetic elements associated with antimicrobial resistance. Clin Microbiol Rev 31 (4).